

**Quadriceps arthrogenic muscle inhibition:
Neurophysiological mechanisms and possible therapeutic
interventions**

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Attestation of Authorship

I hereby declare that this submission is my own work and that, to the best of my knowledge and belief, it contains no material previously published or written by another person (except where explicitly defined in the awards and publications section), nor material which to a substantial extent has been submitted for the award of any other degree or diploma of a university or other institution of higher learning.

Signed

Dated.....

Awards and Publications

Work from this thesis has led to the following publications and an international research award:

1. Rice D.A., McNair P. J. and Lewis G.N. (2011). Mechanisms of quadriceps muscle weakness in knee joint osteoarthritis: The effects of prolonged vibration on torque and muscle activation in osteoarthritic and healthy control subjects". Arthritis Research and Therapy, 13(5):R151
2. Rice D.A. and McNair P.J. (2010). Quadriceps arthrogenic muscle inhibition: Neural mechanisms and treatment perspectives". Seminars in Arthritis and Rheumatism, 40(3): 250-266.
3. Rice D., McNair P. J., and Dalbeth N. (2009) Effects of cryotherapy on arthrogenic muscle inhibition using an experimental model of knee swelling. Arthritis & Rheumatism, 61(1), 78-83.
4. Basmajian Award from the International Society of Electrophysiology and Kinesiology, for the paper: Rice D.A., McNair P. J. and Lewis G.N. A loss of Ia afferent feedback may contribute to quadriceps inhibition in patients with knee joint osteoarthritis. Presented in Aalborg, Denmark, June 2010.

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Abstract

Quadriceps arthrogenic muscle inhibition (AMI) is a near universal finding after knee joint injury, surgery and pathology; leading to marked quadriceps weakness that impairs physical function and may hasten joint degeneration. While AMI has been linked to a number of factors such as articular swelling, inflammation, pain, joint laxity and sensory receptor damage, its underlying neural mechanisms are poorly understood. Furthermore, interventions aimed at reversing AMI have been underdeveloped. As such, the aims of this thesis were to enhance our current understanding of AMI's neurophysiological mechanisms and investigate the efficacy of three different interventions that may counteract AMI's deleterious effects.

The first study examined the efficacy of cryotherapy in reversing quadriceps AMI caused by experimental knee joint swelling. Healthy participants were randomly assigned to a cryotherapy or a control group. Quadriceps maximum effort voluntary contractions were performed at baseline, after the experimental infusion of saline dextrose into the knee joint and following 20 minutes of cryotherapy or a no intervention control period. Experimental joint swelling led to a significant reduction in knee extensor peak torque, electromyography amplitude and muscle fibre conduction velocity. Compared to the control group, cryotherapy led to a significant increase in knee extensor peak torque and muscle fibre conduction velocity. These

findings suggest that cryotherapy may provide a useful adjunct to traditional resistance training in individuals with AML.

Study two explored the effects of experimental joint swelling and cryotherapy on quadriceps corticomotor excitability, short interval intracortical inhibition, intracortical facilitation and intermuscular coherence. Both swelling and cryotherapy led to a significant increase in the area of the quadriceps motor evoked potential but had no significant effect on short interval intracortical inhibition, intracortical facilitation or intermuscular coherence in the β -band. These findings suggest that joint swelling and cryotherapy lead to an increase in quadriceps corticomotor excitability that does not appear to be associated with increased excitability in the primary motor cortex.

Study three examined the effects of knee joint aspiration and intraarticular corticosteroid injection on flexion reflex excitability and knee extensor peak torque in individuals with chronic arthritis. Flexion reflex threshold and knee extensor peak torque were measured at baseline, immediately after knee joint aspiration alone and 5 ± 2 and 15 ± 2 days after aspiration and corticosteroid injection. Both flexion reflex threshold and knee extensor peak torque increased immediately after joint aspiration and, to a greater degree, 5 ± 2 and 15 ± 2 days following aspiration and corticosteroid injection, suggesting that swelling and inflammation may increase flexion reflex

excitability in humans, as has previously been shown in animals. The notable increase in knee extensor peak torque 15 ± 2 days after aspiration and corticosteroid injection indicates that this may be a clinically useful intervention to reverse AML in individuals with chronic knee joint arthritis.

Study four examined the effects of infrapatellar tendon vibration on knee extensor peak torque and electromyography amplitude in healthy controls, individuals with knee joint osteoarthritis and individuals who had recently undergone anterior cruciate ligament reconstruction. Following prolonged tendon vibration, knee extensor peak torque and electromyography amplitude decreased significantly in healthy controls, but did not change in the osteoarthritis or anterior cruciate ligament reconstruction groups, indicating a dysfunction in the gamma (γ)-loop pathway that may contribute to quadriceps AML. All participants then attended a second testing session 6 ± 3 days later, where the effects of short duration tendon vibration on knee extensor peak torque and electromyography amplitude were investigated. A series of 12 maximum effort quadriceps contractions was performed under 4 different vibration conditions: 0 Hz, 5 Hz, 80 Hz and 200 Hz. The order of conditions was randomised for each participant and both the participant and tester were blinded to the vibration condition. There was no significant difference in knee extensor peak torque or electromyography amplitude across vibration conditions. As short duration tendon vibration failed to enhance quadriceps muscle activation in populations with an established γ -

loop dysfunction, this may indicate an impairment in the afferent portion of the γ -loop or a failure of Ia afferent input to facilitate the quadriceps α -motoneuron pool in the presence of ongoing inhibition from other neural pathways.

Findings from this thesis provide important new insights into the neurophysiological mechanisms underlying quadriceps AML and potential therapeutic interventions that may negate its influence on rehabilitation. These observations will assist in the design of rehabilitation programmes that target AML directly, helping to minimise muscle atrophy and enhance quadriceps strength gains after knee joint injury, knee surgery and in individuals with chronic knee joint pathology.

Chapter One: Introduction

THE PROBLEM

The knee is one of the most frequently injured joints in the human body. In New Zealand, the incidence of knee injury is second only to back injury with an average of 132,949 new knee injuries reported each year for the last 5 years [1]. This is equivalent to approximately 3,079 new knee injuries per 100,000 population [2]. Over the same time period, the average cost of treating these injuries was almost NZ\$ 200 million a year [1]. Furthermore, the knee is commonly affected by chronic joint pathology such as arthritis, one of the leading causes of morbidity and disability internationally and a condition afflicting 1 in 6 New Zealanders aged 15 years or over [3]. Surgical management of both acute knee injury and chronic joint pathology is common. For example, meniscectomy and anterior cruciate ligament (ACL) reconstruction are often performed after traumatic knee injury, and many cases of arthritis progress to total knee arthroplasty (TKA). The number of TKAs performed is on the rise in the western world, largely due to the pressures of an ageing, and increasingly overweight, population. In the United States of America, it is projected that the number of TKAs conducted each year will increase by an astonishing 673% from 2005 to 2030 [4]. Similar increases are already occurring in New Zealand, with the number of TKAs performed each year having risen by 111% from 2002 to 2010 [5].

A common finding after knee injury, surgery and pathology is severe and long lasting quadriceps muscle weakness. This pattern of weakness was first described in depth by Charcot as early as 1889, a problem he termed 'atrophic articular paralysis' [6]. Quadriceps weakness after joint damage is partly due to muscle atrophy and partly due to ongoing neural inhibition that prevents the quadriceps from being fully activated, a process known as arthrogenic muscle inhibition (AMI). Quadriceps AMI occurs across a wide range of knee joint pathologies, with significant neural activation deficits observed in patients with osteoarthritis (OA) [7], rheumatoid arthritis (RA) [8], anterior knee pain [9], after patella contusion [10], following ACL injury [11, 12] and ACL reconstruction [13], after meniscal damage [14] and meniscectomy [15, 16] and in patients who have undergone knee replacement surgery [17-19].

AMI has long been of concern to clinicians as it may lead to ongoing quadriceps muscle weakness for months or even years after the initial knee injury or surgery [12, 15, 20] and appears to be an ever present cause of muscle weakness in patients with arthritis [8, 21, 22]. Furthermore, AMI can delay or even prevent effective muscle strengthening; hindering rehabilitation considerably. While mild AMI does not preclude quadriceps strength gains entirely [8, 11, 23], it almost certainly restricts their magnitude as a portion of the muscle cannot be activated [24, 25]. In the early stages after trauma or when joint damage is extensive, AMI can be severe and

quadriceps strengthening protocols are often ineffective. Despite heavy resistance training, quadriceps strength remains unchanged or even declines significantly [19, 20, 26-29], an effect attributed to AMI [19, 20].

Thus, as well as being a direct cause of quadriceps weakness, AMI can prevent effective strengthening, leading to persistent and often severe muscle weakness. This is consistently observed in the literature. For example, 4 months to 5 years after acute knee injury, quadriceps strength deficits of 22-47% have been observed compared to matched controls with no history of knee injury [12, 30-32]. Similarly, compared to healthy controls, quadriceps strength deficits of between 10-76% have been observed in individuals with knee joint osteoarthritis (OA) (reviewed by [33]), while those with rheumatoid arthritis (RA) of the knee were reported to have a mean quadriceps strength deficit of 37% [8]. Moreover, in a systematic review of strength deficits after ACL reconstruction, Palmeiri-Smith et al. [34] reported that side to side quadriceps deficits of >20% were frequently observed 6 months postoperatively, with ~60% of studies reporting ongoing quadriceps deficits of $\geq 10\%$ in the operated limb 1-7 years after surgery. Similarly, side to side quadriceps strength deficits of 15-40% have been shown in the first 4 months after arthroscopic meniscectomy, with deficits of 10-18% remaining 6 months to 4 years after surgery (reviewed by [35]). Finally, 6-33 months after TKA, the quadriceps on the operated limb remains 11-29% weaker than the

unoperated limb [36-40] and 20-35% weaker than the quadriceps of healthy, age matched controls [36, 40-42].

Persistent quadriceps weakness is clinically important for a number of reasons. Firstly, adequate quadriceps strength is needed to optimally perform sporting activities [26, 27] and essential activities of daily living such as walking, standing up from sitting and climbing stairs [43]. As such, quadriceps weakness has frequently been associated with impaired physical function following knee injury [26, 27, 44], knee surgery [18, 27, 39, 42, 45] and in patients with chronic knee joint pathology [46-48]. Furthermore, quadriceps weakness has been linked to the knee "giving way" after ACL injury [44, 49], in individuals with OA [50] and in a large sample of community dwelling older adults [51]. An unstable knee is an important source of disability and has been linked to an increased rate of falling [51], placing the individual at risk of secondary injury such as fracture. Finally, the quadriceps have an important protective function at the knee joint, working eccentrically during the early stance phase of gait to "cushion" the knee and acting to decelerate the limb prior to heel strike, reducing impulsive loading [52, 53]. Weaker quadriceps have been associated with an increased rate of loading at the knee joint [53, 54] and recent longitudinal data has shown that greater baseline quadriceps strength may protect against incident knee pain [55, 56], patellofemoral cartilage loss [55] and tibiofemoral joint space narrowing

[57]. Thus, persistent quadriceps weakness may increase the risk of further joint damage and/or degeneration.

For these reasons, restoring quadriceps muscle strength can be considered a vital component of effective rehabilitation after knee injury, knee surgery and in patients with chronic knee joint pathologies [58-60]. Unfortunately, the evidence presented above clearly demonstrates that current rehabilitation protocols regularly fail to return quadriceps strength to levels seen in healthy individuals. A number of authors have recognised this problem [34, 61-67] and argued that in order to effectively reverse quadriceps muscle weakness it is necessary to target AML specifically, as this may be the major underlying cause of ongoing weakness. In order to effectively reverse AML, it is important to first understand its underlying neurophysiological mechanisms. In this regard, it is generally accepted that AML is caused by a change in the discharge of sensory receptors from in and around the damaged knee joint [61, 62, 66, 68-71]. However, the specific spinal reflex and/or supraspinal pathways mediating AML remain poorly understood. Better understanding AML's neurophysiological mechanisms is important as it may allow the development of more effective interventions to reverse quadriceps muscle weakness while also permitting preventative measures to be put in place to reduce the severity of muscle weakness and atrophy after planned knee trauma such as surgery.

Furthermore, at this time, evidence concerning the use of interventions to address AML can be considered preliminary [64]. Many of the treatments that have been investigated are either clinically impractical (e.g. regular intraarticular injection of local anaesthetic) [66] or do not appear to be universally effective (e.g. neuromuscular electrical stimulation) [72, 73]. As such, there is an urgent need for further investigation of interventions that may help clinicians to overcome AML, thus enhancing recovery after knee joint trauma and pathology.

STATEMENT OF THE PROBLEM

Quadriceps AML remains a significant barrier to effective rehabilitation following knee injury, knee surgery and in patients with chronic knee joint pathology. The underlying neural mechanisms that lead to AML are incompletely understood and there is a lack of evidence regarding the best interventions that may help clinicians to reverse AML. Thus, the aims of this thesis were to gain a better understanding of the neurophysiological mechanisms contributing to quadriceps AML and to assess the effectiveness of three different interventions in reducing AML, thus enhancing quadriceps muscle activation.

To address these aims the following studies were undertaken:

- Study 1 An investigation into the effects of cryotherapy on knee extensor peak torque, electromyography amplitude and muscle fibre conduction velocity following experimental knee joint swelling (Chapter 3)
- Study 2 An investigation into the effects of experimental knee joint swelling and cryotherapy on quadriceps corticomotor excitability, intracortical excitability and intermuscular coherence in the β -band in healthy control subjects (Chapter 4)
- Study 3 An investigation into the effects of knee joint aspiration and intraarticular corticosteroid injection on flexion reflex excitability and knee extensor peak torque in individuals with chronic arthritis of the knee joint (Chapter 5)
- Study 4 An investigation into the effects of infrapatellar tendon vibration on knee extensor peak torque and electromyography amplitude in healthy control subjects, individuals with osteoarthritis of the knee and individuals who had recently undergone anterior cruciate ligament reconstruction (Chapter 6)

SIGNIFICANCE OF THE STUDY

Findings from this study have significance for health professionals involved in the rehabilitation of individuals following knee joint trauma or pathology. The study strengthens our understanding of AMI's underlying neurophysiological mechanisms and provides further evidence for the use of therapeutic strategies such as cryotherapy and corticosteroid injection in the treatment of this problem. These findings may help clinicians to enhance their current rehabilitation practices; minimising muscle atrophy and increasing quadriceps strength gains after joint damage. In turn, this may enhance joint stability, hasten the recovery of physical function after acute knee injury and surgery and slow the deterioration in function that is often observed in individuals with arthritis. Finally, preventing or attenuating ongoing quadriceps weakness may have a protective function at the knee joint, reducing the risk of further joint degeneration.

Chapter Two: Literature Review

INTRODUCTION

The objective of this chapter is to provide the reader with a deeper understanding of AML, with a focus on its potential neural mechanisms and therapeutic interventions that may help to reduce its impact on rehabilitation. The first section of this chapter will outline the clinical presentation of AML. Following this, the sensory innervation of the knee joint will be reviewed and an outline provided of factors that may alter joint afferent discharge in the presence of knee damage. Thereafter, spinal reflex pathways that have been implicated in AML are examined and the potential influence of supraspinal centres on AML is discussed. Finally, an overview is presented of some of the most promising therapeutic interventions that may help clinicians to overcome AML.

LITERATURE SEARCH

To implement the review, an initial search of the literature was undertaken using a variety of sources including experimental papers, review papers and conference proceedings, as well as a general internet search. From this initial search an extensive keyword list was developed (e.g. quadriceps, knee extensors, muscle inhibition, voluntary activation, arthrogenic, arthrogenous, knee injury, knee trauma, osteoarthritis, gonarthrosis, rheumatoid arthritis,

knee surgery, joint receptors, articular receptors, afferent, sensory, neuromuscular, reflex inhibition, interneuron, motoneuron, supraspinal, swelling, effusion, inflammation, pain, instability, cryotherapy, corticosteroid, intraarticular, vibration, spindle, Ia afferent). An initial check of the keyword list was made against a number of databases (AMED, CINAHL, MEDLINE, OVID, SPORTDiscus and Scopus). Where appropriate, additional keywords were added and modifications to the keyword list made. This was supplemented with a review of the bibliographies of past review papers on AMI. Only peer-reviewed papers published in the English language were included in this review.

THE CLINICAL PRESENTATION OF AMI

Quadriceps AMI appears to be ubiquitous in the presence of damage to the knee joint, occurring across a wide range of acute knee injuries, following knee surgery and in the case of chronic knee joint pathology [9-11, 13, 16-21, 23, 47, 74-87]. AMI has been quantified using electromyography (EMG), interpolated twitch or burst superimposition. Interpolated twitch and burst superimposition are the most commonly used methods and rely on electrical stimulation augmenting quadriceps force production during maximum effort contractions, thereby revealing incomplete muscle activation. Interpolated twitch superimposes a supramaximal percutaneous electrical stimulation to the femoral nerve or quadriceps muscle during a maximal effort contraction and again at rest, calculating activation failure using the formula:

1- (superimposed twitch force at maximum effort/superimposed twitch force at rest)

Burst superimposition involves electrical stimulation of the quadriceps only during maximum effort contraction and calculates voluntary activation using the formula:

$$\text{Maximum effort force} / (\text{maximum effort force} + \text{superimposed stimulus force})$$

Unfortunately, researchers have used a number of different stimulation parameters (e.g. single electrical stimulus vs. trains of stimuli, different joint angles, estimated vs. measured resting twitch force) to quantify AMI, all of which can alter estimates of voluntary muscle activation [88]. Furthermore, in healthy subjects quadriceps activation has been found to be 8-16% higher using burst superimposition compared to interpolated twitch [88] while even interpolated twitch has been suggested to overestimate true activation [89]. The heterogeneity and limitations of the methods used to assess AMI makes it difficult to compare the absolute magnitude of inhibition across studies and suggests that, in some cases, the magnitude of AMI may have been underestimated¹. Nevertheless, repeated measures of interpolated twitch and burst superimposition within single studies (i.e. using the same stimulus parameters) provide valuable information concerning the time course of AMI and how its severity may vary across different patient groups.

¹ For the purposes of this literature review, the magnitude of AMI as assessed by burst superimposition and interpolated twitch was calculated under the assumption that normal quadriceps activation (in healthy, uninjured participants) equals 95%. In studies that used the same stimulus parameters to compare quadriceps activation in healthy controls and patients with joint pathology, the difference between the two groups is presented.

AMI is most severe in the acute stages after joint trauma. To investigate the early progression of AMI, Shakespeare et al. [16] asked patients to perform maximum effort isometric quadriceps contractions and compared the amplitude of presurgery quadriceps EMG to that recorded at various times in the first 2 weeks after menisectomy. These authors found that EMG amplitude was typically reduced by 50-70% in the first few hours after surgery. Over the next 24 hours, inhibition tended to become more severe (80-90%) and by 3-4 days was still 70-80%. After 10-15 days, inhibition had subsided somewhat but was still 30-50%. Similarly, AMI is enhanced in the first 3-4 weeks after TKA. Using burst superimposition, researchers [18, 19] have shown that AMI increases substantially from presurgery central activation deficits of approximately 10% to almost 30% when assessed 3-4 weeks after surgery. During the same time period, quadriceps strength decreased by an average of 60% (range 30-85%) [18], with changes in AMI accounting for ~65% of the variability in the change in quadriceps strength.

There is evidence that with time the severity of AMI lessens. For instance, Snyder-Mackler et al. [84] found that 9 out of 12 patients with a sub-acute, isolated ACL tear (average of 3 months postinjury) had significant quadriceps inhibition but that no inhibition was detectable in patients with a chronic ACL rupture (average of 2 years postinjury). Furthermore, Urbach et al. [13] have shown that the magnitude of AMI is reduced in the long term following ACL reconstruction. Prior to surgery (average of 13 months postinjury) patients

demonstrated mean quadriceps activation deficits 16% greater than matched controls with no history of knee injury. Eighteen months postsurgery, quadriceps activation had improved significantly to be 6% lower than controls. Similarly, 18 months after unicompartmental knee arthroplasty, Machner et al. [17] observed a reduction in AMI to 18% from presurgery quadriceps activation deficits that averaged 28%. Over a longer time frame, Berth et al. [41] found that central activation deficits improved from ~15% presurgery to ~6% by 33 months after TKA.

However, in the medium term (up to 6 months after joint damage) clear reductions in AMI do not always occur with time. Following knee arthroscopy, Suter et al. [14] found no significant change in the magnitude of AMI when patients were assessed presurgery, 6 weeks and 6 months postsurgery. More recently, Berth et al. [90] compared the recovery from two different surgical approaches for TKA (subvastus vs. midvastus approach). Across both groups, the magnitude of AMI remained similar (15-20% central activation deficits) when test before surgery and 3 and 6 months after surgery.

Thus, based on the available evidence, it appears as if AMI is most severe in the first few days after joint damage before reducing somewhat, plateauing in the medium term (up to 6 months) and then slowly declining in the longer term (18-33 months). However, it is apparent that notable levels of AMI may

still be present months and in many cases years after joint damage. To further highlight this point, Becker et al. [15] have shown that residual levels of AMI (~8% central activation deficits compared to healthy, age matched controls) remain a mean of four years after arthroscopic meniscectomy, despite no radiological or clinical evidence of further joint degeneration.

Following acute injury, the severity of AMI varies according to the extent of joint damage [12, 62, 80]. Amongst patients with isolated ACL ruptures, relatively small quadriceps activation deficits may be seen following injury, ranging from 3-8% when tested a mean of 6 weeks to 31 months postinjury [11, 12, 87, 91]. In contrast, ACL injured patients with additional joint damage (ligamentous, capsular, meniscal and/or bony) demonstrate quadriceps activation deficits of 15-41% several months or in some cases years after the initial trauma [12, 20]. The relationship between joint damage and AMI is less clear in patients with chronic joint disease. In patients with OA, Pap et al. [21] assessed the magnitude of quadriceps AMI in relation to joint damage, scored retrospectively according to the extent of cartilage degeneration observed during articular surgery. Quadriceps activation deficits were found to be higher in subjects with moderate (stage II) joint damage (19%) compared to those with greater (stage IV) deterioration (12%). Interestingly, in OA patients with severe radiographic degeneration (grade IV on the Kellgren-Lawrence scale), AMI appears to be the major underlying cause of

weakness, accounting for more of the variance in quadriceps strength than muscle atrophy [75].

In patients with OA, researchers [92, 93] have reported a significant relationship between gender and the magnitude of AMI, with inhibition tending to be more severe in women. In contrast, amongst ACL injured subjects no such relationship has been found [12, 91]. There does not appear to be a significant relationship between age and the severity of quadriceps inhibition in patients with ACL injuries or OA [12, 92].

AMI often occurs bilaterally after unilateral knee injury or pathology. Bilateral inhibition has been observed in patients with isolated ACL ruptures [11-13, 87, 91], extensive traumatic knee injuries [12, 20], OA [14, 17, 23], anterior knee pain [9] and following ACL reconstruction [13], partial meniscectomy [15] and knee arthroplasty [17]. AMI in the contralateral limb is typically less severe than that in the injured limb. However, quadriceps activation deficits as high as 16-24% have been documented in the uninjured limb amongst patients with extensive traumatic knee injuries [12, 20], anterior knee pain [9] and after knee arthroplasty [17]. Similar to the injured side, contralateral AMI has been shown to persist for up to four years after joint damage [15].

Clinically, it has been claimed that AMI preferentially affects the vastus medialis portion of the quadriceps [59]. Following the intraarticular injection of 60 ml of saline, Kennedy et al. [94] reported that the most dramatic inhibition of Hoffmann reflex (H-reflex) amplitude was consistently observed in the vastus medialis portion of the quadriceps. Similarly, Spencer et al. [71] observed that the infusion of 20 ml of saline into the knee joint was sufficient to inhibit the vastus medialis H-reflex, while 50-60 ml of fluid was required to inhibit the vastus lateralis and rectus femoris H-reflexes. Moreover, at the maximum level of effusion (60 ml) the greatest magnitude of inhibition was observed in the vastus medialis H-reflex (~45%) when compared to the vastus lateralis (~31%) and rectus femoris (~35%) muscles [71]. However, these findings may be at least partially explained by the larger amplitude of the vastus medialis H-reflex recordings at baseline, as it has since been demonstrated that the magnitude of H-reflex inhibition varies as a function of its initial size [95]. Furthermore, in contrast to these earlier observations, experimental knee joint swelling has been shown to decrease vastus medialis and vastus lateralis EMG activity to a similar degree during the stance phase of both walking and jogging activities [96, 97]. Similarly, the infusion of saline into arthritic knee joints has been shown to produce virtually identical reductions in EMG amplitude in vastus medialis and vastus lateralis during maximal effort isometric quadriceps contractions [98].

SENSORY INNERVATION OF THE KNEE JOINT

AMI is thought to be caused by a change in afferent discharge from the damaged knee joint [61, 62, 99]. To better understand the neural mechanisms explaining AMI it is therefore important to appreciate the range of sensory receptors within the knee joint, and their function. Articular sensory receptors can be divided into two major classes, those that are innervated by large, myelinated afferent fibres (Group II afferents), and those that are innervated by thinly myelinated or unmyelinated afferents (Group III and IV afferents) [100].

Group II afferents

The proportion of thickly myelinated, group II afferent fibres in the knee joint is relatively small. In the knee joint of the cat, as few as 16% of afferent fibres are thought to be of group II origin [101]. Group II afferents terminate in corpuscular nerve endings, which are activated by mechanical stimuli [100, 101]. These nerve endings are highly sensitive, with low firing thresholds, and include ruffini endings, lamellar (paciniform) corpuscles, and golgi-tendon organ-like endings [100, 101].

Ruffini endings

The majority of corpuscular endings in the knee joint are slowly adapting ruffini endings [101]. These receptors are found within ligaments and within and on the outer surface of the fibrous layer of the joint capsule [102-104]. A greater density of ruffini endings is found within the posterior and anterior sections of the fibrous capsule than the medial and lateral portions [105]. In fact, in a series of posterior sections of the cat's knee joint capsule, Grigg and Hoffman [106] classified 94 sensory neurons as ruffini endings from a total of 96 neurons identified, suggesting the posterior section of the fibrous capsule may almost exclusively contain this type of sensory ending. Each ruffini corpuscle consists of a set of 2 to 6 long, cylindrical processes [102]. These cylinders contain collagen fibrils that are anchored to the collagen matrix of the fibrous joint capsule [102, 103, 107]. The longitudinal axes of these cylindrical processes have been observed to vary according to the direction of collagen fibrils within the matrix of the capsule [102]. As such, it is generally assumed that ruffini endings function as stretch receptors, responding to multidirectional mechanical loading of the fibrous capsule [102, 103, 107].

Lamellar (Paciniform) corpuscles

Lamellar (Paciniform) or golgi-mazzoni receptors are thin, lamellar, bulb-like structures that have been found between the synovial and fibrous layers of the joint capsule, within the fibrous layer of the capsule, ligaments and

menisci [101-103, 108]. They have a greater density in the medial and lateral portions of the fibrous capsule and those areas that are somewhat shielded from high tensile loads, such as the sides of the patellar tendon [100, 105, 106]. In a histological examination of sections from the medial joint capsule of the cat's knee, 59 of 61 identifiable neural endings were found to be golgi-mazzoni in nature [106]. These were all located on the inner surface of the fibrous joint capsule.

Lamellar corpuscles are widely considered to be rapidly adapting, dynamic mechanoreceptors, thereby encoding the velocity of joint movement [102, 103, 108-110]. However, Grigg et al. [106] found that these endings often function as both rapidly adapting and slowly adapting receptors. Many golgi-mazzoni type endings were initially observed to have a rapidly adapting response to mechanical loading. However, by manipulating the location and magnitude of the applied stimulus, these authors were able to show that almost all of these sensory endings were also able to function as slowly adapting receptors. Lamellar corpuscles are poorly activated by joint rotations and are relatively insensitive to tensile loading [100, 106]. Instead, they are sensitive to compressive forces such as indentation of the surface of the fibrous capsule and increases in hydrostatic pressure [100, 106]. Following the injection of saline into the joint capsule, the activity of these receptors has been shown to progressively increase with corresponding increases in intraarticular pressure [106]. Due to these observations, and their location, it

has been hypothesised that these receptors primarily function as sensors of deep pressure within the knee joint [106].

Golgi tendon organ-like endings

Golgi tendon organ-like endings are the largest articular mechanoreceptors, and are usually thinly encapsulated, fusiform corpuscles [101, 104, 111]. These receptors are sparsely located in joint ligaments and menisci and do not occur within the joint capsule itself [101, 104, 112, 113]. Within the ligaments of the knee, the long axis of the sensory organ often runs parallel to the long axis of the ligament [104, 111]. Golgi tendon organ-like receptors are slowly adapting, with high mechanical thresholds [111]. The primary function of Golgi tendon organ-like receptors is thought to be the measurement of ligament tension, particularly at the extremes of joint motion [113, 114].

Group III and IV afferents

The vast majority of afferent fibres innervating the knee joint are high threshold, lightly myelinated (group III) or unmyelinated (group IV) fibres [100, 101]. Data from the cat [101] suggests that group III and IV afferents make up approximately 74% of the posterior articular nerve and 91% of the medial articular nerve, the two major articular nerves supplying the knee joint.

Non-corporcular (free nerve) endings

Group III and IV afferents commonly receive input from polymodal receptors known as free nerve endings. Free nerve endings are widespread, being found throughout the knee joint, most commonly situated near blood vessels [101, 103]. These sensory organs have been identified between the synovial and fibrous layers of the capsule as well as within the fibrous capsule itself, the fibro-adipose tissue surrounding the capsule, ligaments, menisci and the periosteum [101, 103, 108]. Free nerve endings have a high threshold and respond to strong mechanical stimuli and inflammatory chemicals such as histamine, bradykinin and prostaglandin [100, 113, 115, 116]. Their major function appears to be as nociceptors, signalling excessive joint loading or tissue damage. However, a sizeable proportion of free nerve endings may also function as high threshold mechanoreceptors, with as many as 43% of group III and IV afferents from the medial articular nerve of the cat being activated by non-painful, passive movements of the knee joint [117].

CHANGES IN AFFERENT DISCHARGE DUE TO JOINT DAMAGE

A number of factors have been identified that may alter afferent discharge from the knee joint in patients with arthritis or following knee injury and surgery (Figure 2.1). These include swelling, inflammation, joint laxity and a loss of output from articular sensory receptors due to structural damage.

Swelling

Swelling is often perennial in arthritic joint conditions and can also continue long after the acute phase of knee injury and surgery. Despite aspiration of acute haemarthrosis, swelling has been shown to persist for an average of 3 months after ACL injury and for 12 months following ACL reconstruction [118]. Swelling causes significant quadriceps AML, even in the absence of factors such as inflammation, pain and structural damage. This has been repeatedly demonstrated by infusing fluid into undamaged knee joints. Direct recordings from articular nerves in animals have shown that swelling significantly increases both the firing frequency and recruitment of group II afferents [107, 119-122]. Moderate levels of joint infusion rarely evoke pain [68, 97, 123-125], making it unlikely that a significant number of group III and IV afferents are stimulated by swelling alone. However, as some of these fibres can be activated by mechanical stimulation [117, 126], a portion may increase their discharge in response to swelling, particularly at higher intraarticular pressures or in the presence of inflammation [126, 127].

By infusing fluid into human knee joints, researchers have shown that swelling reduces quadriceps EMG activity [68, 96, 97, 128, 129], H-reflex amplitude [70, 71, 94, 123, 130, 131] and muscle force production [98, 124, 125, 132]. The potency of swelling's effect is revealed by the finding that as little as 10 ml of fluid may cause notable inhibition [60, 68, 125], while infusions between 20-60 ml are capable of reducing maximum isokinetic knee extensor torque by 30-

40% [124, 125]. Several lines of evidence suggest that swelling's inhibitory effect is mediated by joint afferents. Injecting local anaesthetic into swollen joints largely abolishes AMI [71, 125] and an infusion as large as 300 ml failed to provoke inhibition in a patient with Charcot neuropathy of the knee [68]. Astonishingly, a recent case study [82] has reported absolute increases in knee extensor torque of approximately 400% 1-2 hours after aspirating 150 ml of synovial fluid from an acutely injured knee joint. From the results presented, this appears to represent almost a 50% (from ~13% to ~60%) increase in the quadriceps strength ratio between the injured and uninjured limbs.

There is a close relationship between intraarticular pressure and the discharge of articular afferents, particularly in the swollen knee. In the presence of swelling, intraarticular pressure is raised across all joint angles [132-134]. Even in the resting position, an effusion as small as 5 ml is sufficient to lift intraarticular pressure above atmospheric pressure [125]. In the swollen knee, passive movement of the joint produces a characteristic U shaped curve, with peaks in intraarticular pressure occurring in full extension and at end range flexion, and a decrease in mid-range [132, 133, 135, 136]. The modulation of intraarticular pressure with joint angle becomes progressively more pronounced with greater volumes of effusion [120, 125, 132]. Similarly, direct recordings from animals have shown that as the knee is moved towards the extremes of motion, in both extension and flexion, joint afferent

discharge increases significantly [117, 137, 138], a pattern that becomes exaggerated in the presence of an effusion [120].

Given the relationships presented above, it is perhaps not surprising that the magnitude of AMI has been found to vary with joint angle. Greater inhibition occurs towards the extremes of joint motion, where intraarticular pressure and afferent discharge are greatest [82, 85, 132, 139-141]. In acutely injured knee joints, quadriceps inhibition is significantly greater in full extension [85] and towards end range flexion [82] than in mid-range. In patients with chronic, perennial effusions, it has been demonstrated that AMI is greater in full extension than in 90° of flexion [139]. Even in the absence of a clinically detectable effusion, patients may exhibit more than double the amount of AMI in full extension when compared to 30-40° of knee flexion in the first few days following menisectomy [140, 141].

In summary, swelling raises intraarticular pressure and increases the discharge of group II afferents from the knee. Swelling has a strong inhibitory effect on the quadriceps and even small, clinically undetectable effusions may cause significant AMI. Furthermore, the magnitude of AMI is modulated according to joint angle and the greater the level of effusion, the stronger the relationship between joint angle and inhibition is likely to be.

Inflammation

While swelling clearly has the potential to cause severe AML, it is not solely responsible for this process. In patients with RA, the combination of aspiration and intraarticular corticosteroid injection has been found to increase knee extensor peak torque and EMG amplitude by approximately 30% after 14 days, an effect attributed to a reduction in AML [142]. Torque increased by 8.8 Nm immediately after aspiration but by a much larger 21 Nm 14 days after corticosteroid injection, suggesting that decreased inflammation due to the corticosteroid may have played an important role in reducing AML. Similarly, Fahrer et al. [143] showed that after aspirating OA knee joints, subsequent infusion of local anaesthetic led to further increases in quadriceps activation. These findings suggest that other, non-pressure mediated, afferent impulses also contribute to AML.

In support of this conjecture, several studies involving animals have examined the effects of inflammation on joint afferent discharge using experimental models of arthritis. These investigations have shown that the induction of inflammation produces potent, long lasting changes in the sensitivity of articular free nerve endings supplied by group III and IV joint afferents, a process known as peripheral sensitisation [126, 144, 145]. The activation threshold of these receptors is lowered so that normal joint movement or non-noxious mechanical stimulation of articular structures results in notable group III and IV afferent discharge [126, 144, 145]. In addition, these sensory

receptors demonstrate increased responsiveness to noxious mechanical stimuli and an augmented spontaneous discharge when the knee joint is held in a static position [115, 126, 144]. Finally, the inflammatory process may activate a number of silent free nerve endings [116, 144, 145]. Usually insensitive to both innocuous and noxious stimuli, the release of inflammatory mediators “awakens” these receptors, substantially lowering their threshold and allowing them to respond to a wide range of mechanical stimuli [145, 146]. Collectively, these phenomena greatly enhance the output from group III and IV joint afferents to the central nervous system after joint damage.

As most group III and IV joint afferents are considered to be nociceptive, inflammation can be expected to increase pain in conjunction with afferent discharge [147]. However, it is important to remember that AMI can occur in the absence of pain. Furthermore, nociceptive afferent output is modulated at multiple spinal and supraspinal sites, all of which can influence pain perception [148]. Thus, consciously perceived pain may not closely reflect the motor effects of nociceptive afferent output (e.g. muscle inhibition), which may be largely mediated at the spinal level and are subject to their own modulatory influences.

This is reflected in the literature, where the relationship between pain and AMI is inconsistent. In uninjured healthy controls, experimental knee pain has

recently been shown to reduce both isometric and isokinetic quadriceps peak torque during subsequent maximum voluntary contractions [149]. Moreover, a significant positive association was found between pain intensity ratings and the change in quadriceps peak torque. Similarly, amongst subjects with anterior knee pain, those who rated their knee pain higher on a visual analogue scale (VAS) tended to have higher levels of quadriceps AMI [14]. Furthermore, reductions in knee pain have been associated with an increase in quadriceps activation postsurgery [150] and in patients with RA [142] and OA [151, 152]. In contrast, other studies have found a weak relationship between pain and AMI [9, 16, 18, 19, 80, 92, 153]. After knee surgery, a 15 ml intraarticular injection of local anaesthetic was found to significantly reduce both pain and AMI [16, 153]. However, if only 10 ml of anaesthetic was infused, pain was largely eradicated while AMI remained unchanged. Shakespeare et al. [16] further demonstrated that in the first 24 hours after meniscectomy, quadriceps activation during a maximum voluntary contraction was typically reduced by 80-90% compared to presurgery measures and patients reported severe pain with muscle contraction. However, 3-4 days postsurgery pain had decreased to 7/100 on a visual analogue scale, yet quadriceps inhibition was still between 70 and 80%. Two weeks after the operation, when pain was largely absent, AMI was commonly 30-50%. Similarly, weak correlations ($r^2 = 0.09-0.22$) have been found between pain and AMI in patients with OA [92] and after TKA [18, 19]. Finally, in patients with anterior knee pain, non-steroidal anti-inflammatory

drugs have been shown to significantly reduce pain compared to placebo but have no effect on the magnitude of AML [9].

To summarise, the release of inflammatory mediators due to arthritis, injury or surgery substantially increases joint afferent discharge by sensitising free nerve endings innervated by group III and IV afferents. In humans, the intraarticular injection of local anaesthetic or corticosteroid reduces quadriceps AML over and above aspiration, probably by silencing some of these sensory endings. Many of the group III and IV joint afferents influenced by peripheral sensitisation are involved in nociceptive signalling. While the presence of knee pain may be associated with quadriceps inhibition, it appears to be a poor indicator of the magnitude of AML. Importantly, substantial inhibition occurs in the absence of pain and reducing pain does not necessarily lessen the severity of AML.

Joint laxity

Joint laxity may alter the activation of sensory receptors in the knee joint. Structural damage or degeneration of soft tissues structures associated with the knee (e.g. ligaments, joint capsule) leads to greater translation of the joint surfaces during movement that is likely to increase the activation of mechanoreceptors and nociceptors involved in signalling the limits of joint motion [62]. This has been demonstrated in animals by surgically transecting

the ACL and directly measuring afferent activity from the major nerves supplying the knee joint. Following ACL transection, Gomez-Barrena and colleagues [154, 155] noted significant increases in the transmission of afferent impulses during a range of standardised movements of the knee joint. Immediately after transection, Gomez-Barrena et al. [154] surgically reconstructed the ACL and repeated the articular nerve recordings. Reconstruction was found to partially reverse these changes, with overall articular discharge decreasing towards baseline values. However, differences in afferent discharge were still noted between normal and ACL reconstructed knee joints. A recent study by the same researchers [156] (using the same ACL reconstruction model) suggests that despite afferent discharge tending to normalise over time, some differences still persist 9-18 months after ACL reconstruction. While direct comparison to humans cannot be made, these studies provide evidence that joint laxity causes anomalous firing of sensory receptors during joint movement. Surgical stabilisation of the knee reduces joint laxity and can perhaps normalise afferent activity to a degree. However, abnormalities in joint afferent discharge may still be apparent compared to the uninjured knee, even in the absence of damage to other joint structures.

Damage to articular sensory receptors

Joint damage does not unequivocally lead to increased firing of articular sensory receptors. Whether due to acute knee injury, surgery, or pathology, trauma and/or degeneration of joint structures (e.g. ligaments, joint capsule)

may simultaneously damage the sensory endings located within these tissues, thus reducing the normal afferent output from this population of receptors [62, 80, 113, 157]. An anomalous increase in joint afferent discharge (as with swelling) is strongly associated with AML. However, different populations of joint afferents may have opposing effects on α -motoneuron excitability. Experiments involving cats suggest that background joint afferent discharge has competing excitatory and inhibitory influences on the quadriceps α -motoneuron pool and that in the normal, undamaged knee the net effect may be excitatory [158, 159]. In support of this premise, Konishi et al. [157, 160] have shown that injecting undamaged human knee joints with 5 ml of local anaesthetic reduces knee extensor force output ($-8.8 \pm 7.3\%$) and integrated EMG ($-17.1 \pm 11\%$) during maximum voluntary contractions. Repeating the procedure in a population with chronic ACL injury had no such effect, with knee extensor torque and EMG remaining unchanged. These observations led Konishi et al. to reiterate previous authors' suggestions [62, 80, 113] that a loss of normal excitatory output from a population of knee joint receptors may also contribute to AML.

SPINAL REFLEX PATHWAYS IMPLICATED IN AML

Abnormal afferent discharge from the knee may alter the excitability of reflex pathways within the spinal cord, which in turn reduce the excitability of the quadriceps α -motoneuron pool and prevent supraspinal centres from fully activating the muscle [62, 66, 70, 157, 161]. Joint afferents project widely to

many classes of spinal neurons [162, 163] and thus have the potential to influence quadriceps α -motoneuron excitability via multiple, independent pathways. At this time, three spinal pathways have been identified that may contribute to AML (Figure 2.1). These are the:

- Group I non-reciprocal (Ib) inhibitory pathway
- Flexion reflex
- Gamma (γ)-loop

Although independent, these pathways should not be thought of as mutually exclusive [66]. Instead, it is likely that they are simultaneously affected by joint pathology, with the sum of their actions governing the overall magnitude of AML. While other spinal pathways (e.g. recurrent inhibition, lumbar propriospinal pathways) may well be involved in AML, their role has not yet been explored in any detail.

Group I non-reciprocal (Ib) inhibition

Anatomically, afferent fibres from the major articular nerves of the cat's knee are known to project to two major areas within the spinal cord [164].

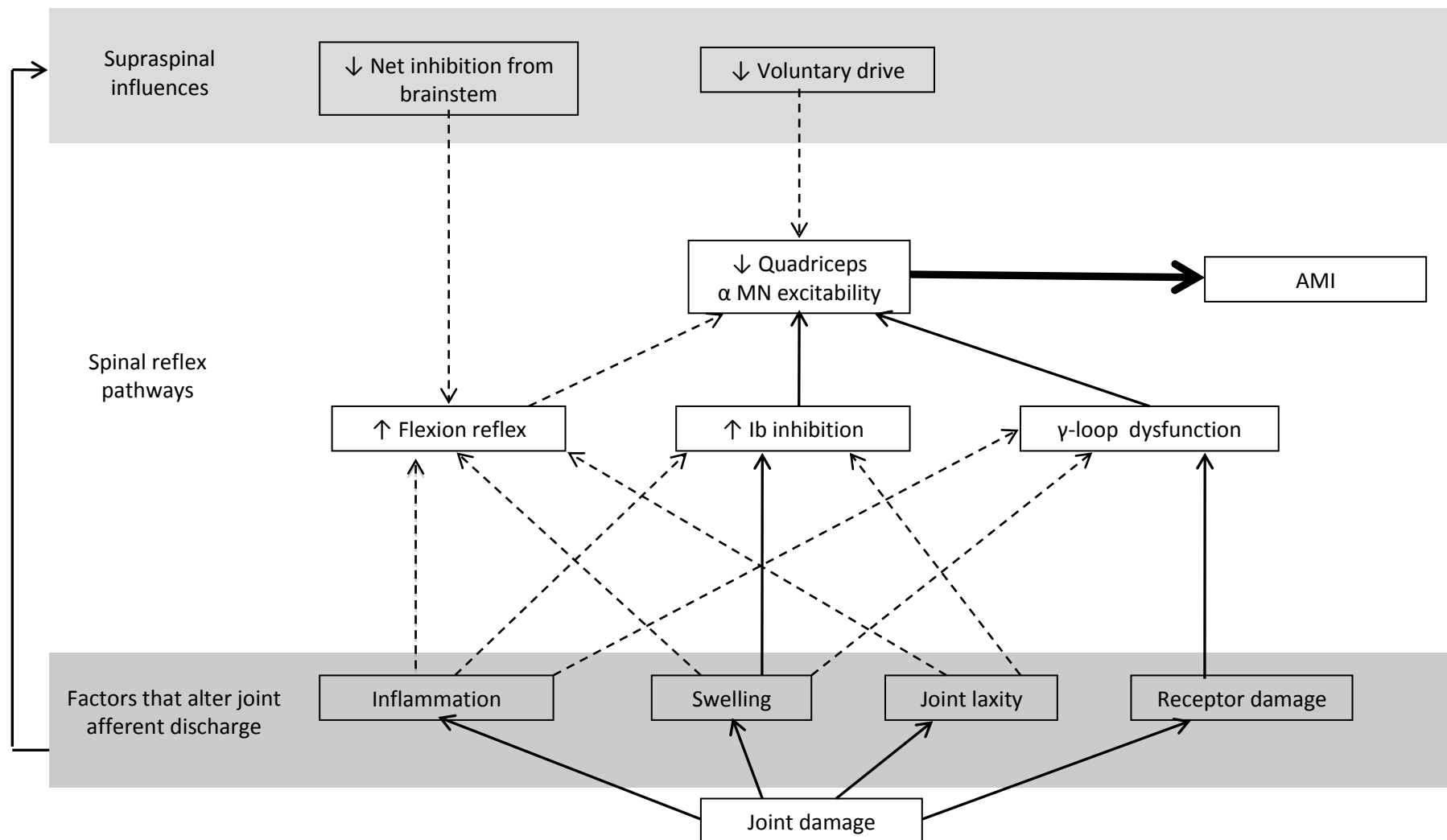


Figure 2.1. Schematic diagram summarising the proposed mechanisms contributing to quadriceps AMI. Solid lines represent pathways with greater evidence supporting their existence.

One of these is the superficial part of the lumbar dorsal horn and the other is a region spanning laminae V, VI and VII, the known location of group I non-reciprocal (Ib) interneurons [165]. The dominant input to these interneurons is from Ib afferent fibres innervating Golgi tendon organs located near the musculotendinous junction. However, Ib interneurons receive widespread convergent input from a number of peripheral sensory receptors, including joint afferents [165].

Lundberg et al. [166] investigated the link between joint afferent discharge and Ib interneuron activity by electrically stimulating the posterior articular nerve of the cat's knee joint at low stimulus intensities. Ib inhibition of extensor α -motoneurons was facilitated at two distinct latencies, suggesting the existence of both disynaptic and trisynaptic excitatory pathways from group II knee joint afferents to Ib inhibitory interneurons. These findings were later confirmed by Harrison and Jankowska [165] using direct, intracellular recordings from Ib interneurons in the lumbosacral spinal cord of the cat.

As swelling is known to significantly enhance the discharge of group II afferents, joint effusion may contribute to AMI by facilitating Ib inhibition of the quadriceps α -motoneuron pool. This is supported by the findings of Iles et al. [70], who infused uninjured human knee joints with saline and used the spatial facilitation technique to show that swelling enhances Ib inhibition of

the quadriceps H-reflex both at rest and during voluntary muscle contraction. It is unknown whether an increase in group III and IV joint afferent discharge also facilitates the Ib inhibitory pathway. However, this remains a possibility as electrical stimulation of group III and IV joint afferents has been shown to excite Ib interneurons in the cat, probably via polysynaptic pathways [165].

Flexion reflex

The flexion reflex is a polysynaptic pathway activated by group III and IV afferents that typically produces a pattern of flexor facilitation and extensor inhibition [167, 168]. As such, it has been suggested [66, 169] that enhanced flexion reflex excitability may be partially responsible for quadriceps AML. The interneurons involved in the flexion reflex have not yet been clearly identified. However, recent evidence from studies involving animals suggests that wide dynamic range neurons play a major role in mediating the flexion reflex [170, 171]. These interneurons are predominantly located in lamina V of the dorsal horn and receive convergent input from a number of peripheral afferent sources, including knee joint sensory receptors [163, 172]. A consequence of articular inflammation and the resulting barrage of group III and IV afferent input is that wide dynamic range neurons become hyper-excitable [173]. This process is known as central sensitisation and is characterised by long lasting changes in synaptic efficacy (for a review see [174]). As a result, after the onset of knee joint inflammation, the activation threshold of wide dynamic range neurons is progressively reduced and they demonstrate enhanced

activity in response to innocuous and noxious stimuli applied to the knee [173]. Additionally, as inflammation develops, there is an expansion of their receptive fields, with neurons showing a heightened response to mechanical stimuli from adjacent areas such as the thigh, or even remote, non-inflamed tissue as far afield as the contralateral limb [173].

In several studies involving animals, the experimental induction of knee joint arthritis has been shown to markedly increase flexion reflex excitability [127, 161, 175]. For instance, following joint inflammation, significantly increased activity in knee flexor α -motoneurons was shown in response to standardised pinching of both the ipsilateral and contralateral toes [175]. Importantly, flexor α -motoneurons did not show an increase in resting discharge, indicating an enhanced excitability of the interneurons mediating the flexion reflex response, rather than a change in the excitability of the α -motoneurons themselves [175]. Remarkably, at the peak of knee joint inflammation, the amplitude of the electrically induced flexion reflex has been reported to increase by an average of 545% (SEM \pm 174%) [161], while the number of flexor α -motoneurons responding to local pressure and/or gentle flexion and extension of the knee increased from 14% to 41%, suggesting a parallel reduction in flexion reflex threshold [127]. Ferrell et al. [161] demonstrated that injecting a local anaesthetic into the inflamed knee returned flexion reflex amplitude back to control values, confirming the role of articular sensory receptors in this response.

While evidence from studies involving humans is less cogent, it seems likely that the flexion reflex contributes to quadriceps AML. Le Roux et al. [79] examined the relationship between knee joint pathology and flexion reflex excitability. Compared with healthy controls, significantly lower flexion reflex thresholds were found in patients with anterior knee pain; probably inferring an amplified excitability of this pathway. Importantly, these authors showed that activation of the flexion reflex produced concomitant inhibition of the ongoing quadriceps EMG during isometric contraction of the knee extensors. Recently, it has been shown that flexion reflex thresholds are lower in patients with knee OA [176] and ACL injury [177] compared to age and gender matched controls. However, in patients with OA [176], no significant relationship was found between flexion reflex threshold and the magnitude of AML, assessed using burst superimposition. This may be partly due to the insensitivity of burst superimposition to lower levels of AML as surprisingly, only 4 out of 20 subjects with OA were found to have quadriceps activation deficits in this study [176]. Alternatively, the lack of a relationship between flexion reflex threshold and the severity of AML could relate to the cross-sectional nature of these measurements and the marked heterogeneity of the flexion reflex threshold, which is known to vary widely, even in healthy pain-free individuals [178]. Further research is warranted in patients with established joint pathology examining changes in the excitability of the flexion reflex pathway over time. In Chapter 5 of this thesis, we will explore longitudinal

changes in flexion reflex excitability following knee joint aspiration and corticosteroid injection in individuals with chronic arthritis.

Gamma (γ)-loop

The γ -loop is a spinal reflex circuit formed when γ motoneurons innervate primary muscle spindles that in turn transmit excitatory impulses to the homonymous α -motoneuron pool via Ia afferent fibres (Figure 2.2). Normal function of the γ -loop is necessary to achieve full muscle activation during voluntary contractions. Thus, impaired transmission along this pathway may contribute to AMI [80, 113, 157]. To investigate the importance of the γ -loop to muscle activation, a number of authors have used prolonged vibration to experimentally attenuate the excitability of Ia afferent fibres. A vibratory stimulus, applied to the muscle or its tendon, temporarily blocks transmission in Ia afferent fibres by increasing presynaptic inhibition, raising the activation threshold of Ia fibres and/or causing neurotransmitter depletion at the Ia afferent terminal ending [179]. In healthy subjects, prolonged vibration (20-30 minutes) causes a reduction in EMG activity [157, 180, 181], motor unit firing rates [180] and muscle force output [157, 180-182] during subsequent maximum voluntary contractions.

However, in patients who have ruptured their ACL, prolonged vibration has no effect on quadriceps force output or EMG activity [157, 160, 183]. This

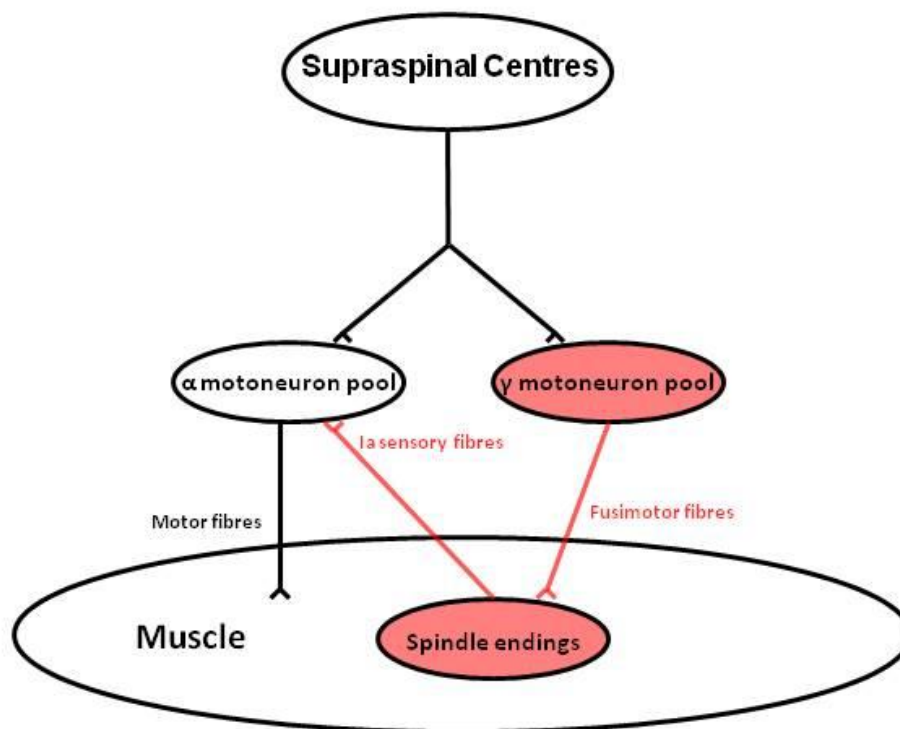


Figure 2.2. Schematic diagram of the γ -loop. During voluntary muscle contraction, supraspinal centres co-activate the α -motoneuron and γ -motoneuron pools. The γ -motoneuron pool in turn innervates muscle spindle endings via fusimotor nerve fibres, enhancing their firing. Muscle spindles provide a tonic excitatory input to the homonymous α -motoneuron pool via Ia sensory nerve fibres. This Ia sensory input is necessary for full muscle activation to occur.

suggests a deficit in the transmission of Ia input to the α -motoneuron pool and has been termed γ -loop dysfunction [157]. Similar findings have been confirmed in patients after ACL reconstruction up to 20 months postsurgery

[77, 83, 183]. Interestingly, it has been demonstrated that γ -loop dysfunction occurs bilaterally in ACL injured and reconstructed patients [77, 183] but that transmission in the contralateral γ -loop may be (at least partially) restored 18 months after surgery [77]. It is currently unknown whether γ -loop dysfunction contributes to AML in other knee joint pathologies. In Chapter 6 of this thesis, we will examine the function of the γ -loop pathway in individuals with OA of the knee joint.

A number of potential neural mechanisms can be considered to explain γ -loop dysfunction. Researchers have suggested that structural damage to the ACL results in a loss of excitatory feedback from ligamentous mechanoreceptors to quadriceps γ -motoneurons and/or supraspinal centres that diminishes α - γ co-activation during strong muscle contractions [66, 80, 113, 157]. In support of this conjecture, Konishi and colleagues [157, 160] have shown that injecting undamaged knee joints with 5 ml of local anaesthetic reduced maximum isometric knee extensor torque and integrated EMG. However, the same infusion of local anaesthetic into knee joints with an isolated ACL rupture had no effect on knee extensor torque or EMG. Furthermore, prolonged vibration of the infrapatellar tendon in subjects with uninjured but anaesthetised knee joints did not diminish maximum quadriceps force output or EMG amplitude. Taken together, these observations led Konishi et al. [157] to conclude that excitatory output from mechanoreceptors within the ACL may be critical to the maintenance of

normal γ -loop function. Given the relatively sparse innervation of the ACL compared to other structures in the knee joint [101, 103], this seems unusual. It remains to be determined if other sensory receptors in the knee joint could also be involved.

Alternatively, or perhaps concurrently, an increase in the discharge of nociceptive joint afferents may contribute to γ -loop dysfunction. Scott et al. [184] have shown that low intensity stimulation of the posterior articular nerve in the cat, sufficient to activate group II and III knee joint afferents, has a net excitatory effect on extensor γ -motoneurons. However, if a second, high intensity stimulus (activating group IV joint afferents) was applied beforehand, the excitatory effect of group II and III afferents was abolished or reduced. Thus, the discharge of group IV afferents may suppress the excitatory effects of low threshold joint receptors on extensor γ -motoneurons [184]. Whether this occurs in humans is not known.

In addition, quadriceps γ -loop dysfunction could be at least partially explained by impaired transmission along the afferent portion of the pathway, either due to an increase in Ia afferent presynaptic inhibition or a change in the sensitivity of the muscle spindles themselves. Presynaptic inhibition involves spinal inhibitory interneurons that project to the synaptic terminals of Ia afferent fibres, adjusting the quantity of neurotransmitter

released in response to an afferent volley; thus modulating synaptic efficacy (for a review see [185]). As activity from a wide range of peripheral sensory receptors, including joint receptors [186], can modify the excitability of presynaptic inhibitory interneurons, a change in articular afferent discharge could theoretically impair quadriceps γ -loop function via this mechanism [187]. However, the evidence supporting this theory is limited and findings to date are conflicting. Poststimulus time histograms from single quadriceps motor units have shown that electrical stimulation of knee joint afferents prior to femoral nerve stimulation does not change the amplitude of the initial, purely monosynaptic component of the resulting H-reflex response [188]. This suggests that joint afferent discharge does not alter presynaptic inhibition of quadriceps Ia afferents. In contrast, using a modified H-reflex protocol, Palmieri et al. [187] found that quadriceps paired reflex depression increased after experimental knee joint infusion. This finding led Palmieri et al. to conclude that an increase in presynaptic inhibition may contribute to AML. However, this interpretation can be challenged on methodological grounds and should be considered with caution.

Finally, it is possible that a change in the sensitivity of the muscle spindles themselves contributes to quadriceps γ -loop dysfunction. In this respect, independent to their γ -efferent drive, muscle spindles have been shown to receive sympathetic input via adrenergic receptors [189, 190]. Animal studies have shown that the resting discharge and responsiveness of muscle spindles

to stretch can be reduced by an increase in sympathetic activity [191-193]. As the sympathetic nervous system may be overactive in chronic joint pathology [194-196], it is possible that spindle sensitivity is affected via this mechanism. Furthermore, muscle disuse due to hind limb unloading has been shown to suppress spindle sensitivity in rats [197]. It is unknown whether a reduction in muscle spindle sensitivity occurs in humans and could partially explain quadriceps γ -loop dysfunction.

In summary, quadriceps γ -loop dysfunction contributes to AMI after ACL injury and ACL reconstruction. There is evidence to suggest that ACL injury disrupts the flow of excitatory joint afferent output to the quadriceps γ -motoneuron pool and/or supraspinal centres, attenuating γ -motoneuron discharge and ultimately, Ia afferent facilitation of the quadriceps α -motoneuron pool. A change in joint afferent discharge could theoretically enhance quadriceps Ia presynaptic inhibition, contributing to γ -loop dysfunction. However, this has yet to be clearly determined. Finally, it is possible that increased sympathetic nervous system activity or muscle disuse could decrease the sensitivity of the muscle spindles themselves, making them less responsive to γ -efferent drive. Future research should investigate the presence of γ -loop dysfunction in other knee joint pathologies, aim to achieve a stronger understanding of its underlying neurophysiological causes and investigate possible interventions that may help to reverse it. In this regard, Chapter 6 of this thesis will examine the effects of short duration tendon vibration on knee extensor peak

torque and EMG amplitude in individuals with OA and following ACL reconstruction. It is our premise that short duration vibration may be a useful adjunct to temporarily restore Ia afferent drive to the quadriceps α -motoneuron pool during strong voluntary contractions, thus enhancing muscle activation.

SUPRASPINAL INFLUENCES ON AML

Joint afferents are known to have extensive supraspinal as well as spinal projections [198-202]. Research to date has largely focused on the spinal mechanisms behind AML. However, supraspinal centres are highly likely to be affected by changes in joint afferent discharge. Importantly, descending pathways have widespread projections to interneurons and motoneurons at the spinal level (for reviews see [148, 162, 163]), and thus have the potential to strongly influence AML.

Changes in corticomotor excitability

Transcranial magnetic stimulation (TMS) of the motor cortex has recently been used to quantify changes in corticomotor excitability associated with chronic knee joint pathology [203, 204]. Fascinatingly, it was found that quadriceps corticomotor excitability was higher in patients with chronic anterior knee pain (average duration 3.5 years) than in healthy control subjects [204]. This was despite lower quadriceps EMG amplitude during

maximal contractions and diminished patellar tendon reflexes in subjects with joint pathology. Furthermore, corticomotor excitability of a muscle distant from the knee joint, extensor digitorum brevis, was not different between groups. This suggests a localised increase in quadriceps corticomotor excitability in patients with chronic anterior knee pain. Similarly, Heroux and Tremblay [203] investigated quadriceps corticomotor excitability in chronic ACL injured subjects (median time since injury 22 months) and found that resting motor threshold was significantly lower in the injured compared to the uninjured limb. No significant differences were found between limbs in healthy control subjects. While the above findings suggest that quadriceps corticomotor excitability is increased, the location of the observed changes (i.e. cortical vs. subcortical level) is not easily determined using single pulse TMS. However, as there is strong evidence that the quadriceps α -motoneuron pool is likely to be inhibited [70, 71, 94, 123, 205] it may be that chronic knee joint pathology paradoxically increases excitability in the area of the primary motor cortex projecting to the quadriceps α -motoneuron pool. While speculative, it is possible that enhanced cortical excitability allows the central nervous system to increase corticospinal drive to the quadriceps in order to counteract α -motoneuron inhibition by spinal reflex pathways. It should be emphasised that findings to date relate to chronic knee joint pathology. The effect of acute knee injury on corticomotor excitability has not been examined and it is unclear whether changes in excitability occur at a cortical or a subcortical level. In Chapter 4 of this thesis we will investigate the effects of an acute model of knee injury on quadriceps corticomotor

excitability, utilise paired pulse TMS to examine changes in the excitability of local interneurons within the primary motor cortex and explore changes in quadriceps intermuscular coherence, an indirect measure of common cortical drive to the quadriceps muscle.

Brainstem modulation of the flexion reflex

Descending brainstem pathways typically exert a tonic inhibitory control over spinal neurons involved in pain processing and the flexion reflex, including wide dynamic range neurons [148, 186, 206]. Joint injury or inflammation greatly enhances descending input from brainstem pathways that has been shown to have both inhibitory and facilitatory components [148, 206-210]. Thus, it is possible that joint damage leads to a reduction in the effectiveness of descending inhibition [66] and/or enhanced descending facilitation to wide dynamic range neurons, increasing excitability in the flexion reflex pathway and amplifying AML.

Investigations in animals have shown that acute arthritis (3-48 hours) results in a net increase in descending inhibition to wide dynamic range neurons [206, 208-210] that may help to limit central sensitisation of wide dynamic range neurons and suppress flexion reflex excitability. However, with time, descending inhibition returns to baseline levels [208, 211], subsiding as early as one week after inflammation commences despite continued hyperalgesia

[211]. Likewise, time dependent changes in the efficacy of diffuse noxious inhibitory controls (DNICs) have been observed following the induction of experimental arthritis in the rat [207]. DNICs are considered an endogenous form of pain control and refer to the widespread, brainstem mediated inhibition of spinal and trigeminal wide dynamic range neurons that is triggered by the stimulation of peripheral nociceptors. Danziger and his colleagues [207] showed that in the acute stages of arthritis (24-28 hours) DNIC-mediated inhibition of convergent trigeminal neurons was enhanced compared to control conditions. However, in animals with ongoing arthritis (3-4 weeks), DNIC-mediated inhibition decreased to normal levels despite continued hyperalgesia. Similarly, a reduction in the efficacy of DNIC-mediated inhibition has been noted in humans with chronic OA of the hip [212] and knee joints [213]. Both studies followed similar protocols, with a pressure algometer used to induce graded mechanical stimulation of the soft tissue overlying the joint. The threshold for pressure-mediated pain was found to be significantly lower in arthritic patients compared to a healthy control group [212, 213]. Ischaemic arm pain was then induced, a procedure commonly used in laboratory studies to evoke DNIC-mediated inhibition of wide dynamic range neurons. As expected, the threshold for pressure-mediated pain increased significantly in healthy control subjects [212, 213]. However, in patients with chronic arthritis, ischaemia had little effect on pressure-mediated pain; suggesting a possible dysfunction in the descending inhibition of wide dynamic range neurons [212, 213].

However, in a similar study, Leffler et al. [214] found no evidence for DNIC dysfunction amongst subjects with RA. As expected, RA patients had significantly lower pressure pain thresholds over their thigh compared to healthy, age and gender matched controls. In this study, DNIC-mediated inhibition was evoked by immersing the contralateral hand in a bath of ice cold water (the cold-pressor test), after which pressure pain thresholds were reassessed in both groups. After cold water immersion, pressure pain thresholds increased significantly in both RA and healthy control subjects, suggesting preserved function of DNIC-mediated inhibition in patients with RA. These findings are at odds with the previous observations in OA patients [212, 213] and experimental arthritis [207] described above. As suggested by Leffler et al. [214], this discrepancy may relate to the populations tested (OA vs. RA vs. animal models of experimental arthritis), the duration and location of joint disease, or differences between methods of inducing pressure pain and DNIC-mediated inhibition. Nevertheless, the balance of evidence suggests that chronic joint pathology may be associated with dysfunction in the brainstem modulation of wide dynamic range neurons involved in pain perception and the flexion reflex pathway. The net effect of brainstem regulation appears to be influenced by the stage of joint injury, suggesting a possible role for brainstem pathways in the maintenance of flexion reflex hyper-excitability after articular damage (Figure 2.1). In turn, this may contribute to the long lasting AML that is often observed after knee injury, surgery and in patients with arthritis.

Reduced voluntary effort

Studies investigating changes in quadriceps activation rely on the motivation of their participants. It has been suggested that reductions in quadriceps strength and activation may be partly due to a subconscious adjustment in voluntary effort, perhaps for fear of damaging or eliciting pain from the injured joint [66, 99, 169]. Intuitively, this seems reasonable and a decrease in voluntary effort may well contribute to reduced quadriceps activation, particularly in the presence of significant joint pain. However, it should be remembered that a strong reflex component to AMI has been established by a number of studies [70, 71, 94, 123]. Moreover, Wood et al. [125] found no evidence that a reduction in voluntary effort contributes to AMI when utilising an experimental model of joint infusion. In this study, the knee joint was distended with different volumes of saline dextran solution or local anaesthetic. Subjects were blindfolded throughout the testing procedure and were kept unaware of the volume of fluid injected. Both the subjects and the tester were unaware of the nature of fluid injected. The presence of saline dextran within the knee joint caused marked reductions in maximal isokinetic torque at all velocities tested. However, subsequent injection of anaesthetic almost completely restored force to preeffusion values. In addition, when anaesthetic was infused before saline solution, knee extensor torque remained stable over time. As subjects were unaware of the nature of fluid injected, the authors concluded that the observed reductions in

quadriceps activation were due to reflex actions of articular afferents, not due to changes in volition.

THERAPEUTIC INTERVENTIONS THAT MAY COUNTER AMI

The following section will provide a brief overview of the current state of evidence for therapeutic interventions that may help to counter AMI. For more detailed information on the specific interventions utilised in this thesis the reader is referred to Chapter 3, pgs. 61-76 (cryotherapy); Chapter 5, pgs. 182-190 (aspiration and corticosteroid injection); and Chapter 6, pgs. 215-231 (tendon vibration).

Joint aspiration

In the first 3-5 days after menisectomy, aspiration of fluid from the knee (range 36-85 ml) has been found to consistently reduce (although rarely abolish) quadriceps AMI [99]. Similarly, a recent case study showed that aspirating 150 ml of fluid from the knee a week after sustaining an acute injury produced large increases in quadriceps strength and activation [82]. However, in patients with chronic inflammatory arthropathies, aspiration may have no significant effect on AMI [139] or produce moderate (14-18%) increases in quadriceps strength [98, 143]. This may relate to the volume of fluid aspirated in these studies, which was typically lower than in studies involving acute injury. Alternatively, it may be that chronic joint pathology

leads to changes in capsular compliance [119, 139] and/or damage to articular receptors that reduces the afferent response to swelling. Thus, while aspiration appears to be an effective way to reduce AMI in patients with an acutely swollen knee joint, its clinical benefit may be questionable in patients with chronic arthritic joint disease, particularly where effusion is expected to reoccur within a short time frame.

Intraarticular corticosteroid injection

In patients with RA, intraarticular corticosteroid injection has been shown to increase knee extensor peak torque and EMG by ~30% after 14 days, an effect attributed to a reduction in AMI [142]. In OA patients, Jones and Doherty [215] reported that aspiration and corticosteroid injection significantly increased knee extensor peak torque when analysed on an intention to treat basis, but not when only patients with complete data sets were analysed. There was no change in muscle strength in patients who received a placebo injection [215]. Failure to observe a clear increase in quadriceps strength in OA patients may be related to a lack of notable joint inflammation. Corticosteroid injection may either be more effective in patients with advanced OA, when inflammation is more prevalent [216] or in the case of an acute flare in joint pain, which is often accompanied by overt signs and symptoms of inflammation [217].

Non-steroidal anti-inflammatory drugs (NSAIDS)

There is conflicting evidence regarding the use of NSAIDS to reduce AML.

Suter et al. [9] found that 7 days of NSAIDs (naproxen sodium, 550 mg) taken twice daily reduced pain but failed to diminish AML in a group of patients with anterior knee pain. In contrast, there is indirect evidence that NSAIDS may help to reduce AML after knee surgery. Ogilvie Harris et al. [218] investigated the effects of twice daily doses of a NSAID (naproxen sodium, 550 mg) for 6 weeks compared to placebo after arthroscopic menisectomy. Patients in the NSAID group had significantly less pain ($p < 0.001$), swelling ($p < 0.001$) and quadriceps atrophy ($p = 0.01$) compared to the placebo group and returned to work or sport quicker ($p \leq 0.002$). Similarly, in a double blind, placebo controlled study, Arvidsson and Eriksson [219] showed that daily doses of an NSAID (piroxicam, 20 mg) led to significantly increased isokinetic knee extensor torque values compared to placebo across a range of joint angular velocities at 3, 7, 11 and 21 days after open menisectomy. While strength was only measured semi-quantitatively, 10 days of twice daily NSAIDs (naproxen sodium, 550 mg) was found to significantly improve quadriceps strength after arthroscopy compared to placebo ($p < 0.05$) [220]. Furthermore, in a recent randomised controlled trial in OA patients, Petersen et al. [24] showed that 12 weeks of progressive resistance training led to significantly greater gains in knee extensor peak torque when combined with NSAIDs (600mg of ibuprofen) taken twice daily compared to placebo tablets.

Glucosamine sulphate

There is evidence that combining glucosamine sulphate with resistance training may lead to greater gains in quadriceps strength. Petersen et al. [24] randomised OA patients to a glucosamine sulphate or placebo group and showed that 12 weeks of progressive resistance training with glucosamine sulphate supplementation (500mg, 3 times daily) led to significantly greater gains in knee extensor peak torque compared to resistance training with placebo tablets. There was no difference in quadriceps muscle hypertrophy between groups, suggesting that the additional gain in quadriceps strength may relate to a greater reduction in AML in the glucosamine sulphate group.

Local anaesthetic

Following experimental joint infusion [71, 125], menisectomy [16] and in patients with OA [143] the intraarticular injection of local anaesthetic has been used to partially silence afferent impulses from the joint, effectively reducing AML. However, a more recent study found that while local anaesthetic reduced AML in patients with OA, the improvements were not statistically different from placebo [151]. Furthermore, the invasive and short-lasting nature of this treatment (a few hours) makes it clinically impractical. A number of injections would have to be administered in order to achieve an appropriate therapeutic effect, increasing the risk of sequelae such as joint infection.

Cryotherapy

Like local anaesthetic, cryotherapy may temporarily reduce afferent input from the joint (and thus AML) but has the added benefit of being non-invasive. Thirty minutes of cryotherapy has been shown to reverse the decline in quadriceps H-reflex amplitude that is seen after swelling, an effect that lasts for at least 30 minutes after the ice is removed from the joint [131].

Furthermore, Hopkins [128] showed that 30 minutes of cryotherapy negated the reductions in peak torque, power and quadriceps EMG caused by swelling during a semi-recumbent stepping task performed at 36% of maximum intensity. While these observations are highly promising, these studies have important methodological limitations that hamper the ability to draw definitive conclusions regarding the clinical utility of cryotherapy in reversing AML. As such, further investigation is warranted. In this regard, Chapter 3 of this thesis examines the effects of cryotherapy on AML during maximum voluntary contractions and Chapter 4 explores the effects of cryotherapy on quadriceps corticomotor and intracortical excitability.

Transcutaneous electrical nerve stimulation (TENS)

Following open menisectomy [99] and ACL reconstruction [150], high frequency TENS has been shown to increase quadriceps activation during subsequent maximal voluntary contractions. Furthermore, Hopkins et al. [205] have found that high frequency TENS (120 Hz, pulse width 0.1 seconds)

prevents the decline in quadriceps H-reflex amplitude seen after the infusion of fluid into the knee joint. Recently, the application of high-frequency (150 Hz, pulse width 0.15 seconds) TENS to OA knee joints has been shown to significantly improve quadriceps activation during maximal voluntary contractions ($p < 0.05$) [81]. The improvement in quadriceps activation with TENS (~11%) was greater compared to a matched control group of OA patients (~1%) who did not receive an intervention ($p < 0.05$). More recently, Pietrosimone et al. have extended these findings to 4 week a period of resistance training. Compared to a sham TENS and resistance training group, significantly larger gains in knee extensor peak torque were observed after 2 weeks and 4 weeks in a group of OA patients that underwent the same volume of resistance training (12 sessions) combined with high frequency TENS (150 Hz, pulse width 0.15 seconds) for up to 8 hours per day. Furthermore, compared to baseline, there was a significant increase in the quadriceps central activation ratio after 2 weeks ($p = 0.001$) and 4 weeks ($p = 0.002$) of the combined TENS and resistance training group.

Low frequency (4 Hz, pulse width 1 second), acupuncture-like TENS has been reported to increase quadriceps force output by 71% in OA patients after 2 weeks of treatment (20 minutes per day, 5 days per week) [152]. Such large changes in quadriceps strength in just 2 weeks suggest a substantial improvement in voluntary activation. It remains unknown whether low

frequency TENS may be effective in reducing AMI in patients with other knee joint pathologies.

Altering fluid distribution or capsular compliance

McNair et al. [124] showed that infusing 60 ml of saline and dextrose into undamaged knee joints reduced quadriceps isokinetic peak torque by approximately 30%. However, peak torque returned to preinfusion levels after a 3-4 minute period of submaximal flexion and extension movements of the knee. MRI scans of the knee joint at each measurement interval showed that the volume of fluid within the joint capsule was largely unchanged, suggesting that submaximal exercise may modulate mechanoreceptor discharge by increasing the compliance of the joint capsule and/or by redistributing fluid throughout the knee joint, reducing local capsular strain [119, 124]. Thus, in patients with an effused knee, a series of non-weight bearing, submaximal movements of the joint may serve to reduce AMI prior to quadriceps strengthening.

Neuromuscular electrical stimulation (NMES)

The therapeutic advantage of NMES is that it activates the muscle directly, circumventing the inhibited α -motoneuron pool [66]. Thus, NMES may help to minimise quadriceps atrophy after joint damage, thereby reducing quadriceps weakness. It should be noted that in many cases, voluntary

exercise is as effective, if not more effective, than NMES in improving quadriceps strength (for a review see [221]). However, there is some evidence [84, 222-225] that after knee injury and surgery the combination of NMES and volitional training may achieve greater gains in quadriceps strength when compared to volitional training alone. If isometric protocols are used, NMES may obtain superior results when performed with the knee partially flexed [223] compared to full extension [222]. Additionally, the benefits of NMES appear to be dose dependant, with high intensity, maximally tolerated stimulations proving more effective than those performed at lower intensities [84, 223]. However, other studies have failed to show important benefits of using NMES. A large randomised controlled trial recently reported no difference in the change in quadriceps strength and central activation 3-12 months after TKA in a group who underwent resistance training alone compared to a group who underwent resistance training combined with NMES [72].

Transcranial magnetic stimulation (TMS)

Urbach et al. [226] have shown that TMS improves quadriceps activation following TKA when it is applied during maximum voluntary quadriceps contractions. Statistically significant improvements in knee extensor peak torque and a trend towards increased voluntary activation were found to persist up to 60 minutes after 3 single pulses of TMS applied to the motor cortex. Similarly, in patients who had undergone meniscectomy, Gibbons et al.

[227] showed that TMS applied during quadriceps maximum voluntary contractions led to a significant improvement in quadriceps central activation ratio 10 and 60 minutes after stimulation, while no such changes were apparent in the control group, who only performed maximum voluntary contractions. While the improvements were modest ($\leq 10\%$ increase in knee extensor torque; $\sim 6\%$ improvement in central activation), the dose of TMS used in these studies (single treatment session, 3 single pulses, 60-100% of maximum stimulator output) was low. These findings indicate a need for further research, investigating the effect of different stimulation parameters on AMI in subjects with knee joint pathology and at different stages after joint damage. The major disadvantage of transcranial magnetic stimulators is their cost, which may prohibit the widespread use of this technique in clinical settings.

Chapter Three: The effects of cryotherapy on quadriceps arthrogenic muscle inhibition due to experimental swelling in the knee joint

INTRODUCTION

Knee joint swelling is a potent cause of quadriceps AML, with the injection of fluid into uninjured knee joints notably reducing knee extensor peak torque [98, 124, 125], quadriceps EMG activity [97, 98, 128] and quadriceps H-reflex amplitude [71, 94, 123, 205]. It has been reported that as little as 10 ml of fluid induces inhibition [68, 125], with infusions between 20 ml and 60 ml capable of reducing maximum isokinetic knee extensor torque by 30-40% [124, 125]. Aspirating or injecting a local anaesthetic into the infused joint largely abolishes AML, confirming the role of articular sensory receptors in this process [71, 125].

Interventions that reduce AML should enhance rehabilitation by allowing earlier and more effective quadriceps strengthening to take place in patients with knee damage. Recently, some evidence [205] has emerged which suggests that cooling the knee joint may temporarily reduce AML. Icing the knee joint for 30 minutes reversed the decline in quadriceps H-reflex amplitude observed after joint infusion, an effect that lasted for at least 30 minutes after the ice was removed from the knee [205]. More recently,

Hopkins [128] showed that 30 minutes of cryotherapy may negate the reductions in lower limb peak torque, power and quadriceps EMG amplitude caused by knee infusion during a semi-recumbent stepping task.

While these observations are highly promising, they should be interpreted with some caution. Firstly, when measuring quadriceps H-reflex amplitude, Hopkins et al. [205] failed to monitor the stability of the M-wave across time. As a result, it could be that part of the change in H-reflex amplitude was due to an alteration in the peripheral recording and/or stimulating conditions, rather than a change in spinal reflex excitability [228, 229]. Secondly, H-reflex amplitude is influenced by a number of factors that can be independent of α -motoneuron excitability such as Ia afferent presynaptic inhibition and the oligosynaptic components of the H-reflex response [188, 228, 229]. As such, we cannot be sure that the observed increase in H-reflex amplitude truly reflects a change in α -motoneuron excitability. For example, cryotherapy could lead to a disproportionate reduction in Ia afferent presynaptic inhibition with little to no change in quadriceps α -motoneuron excitability. If this were the case, quadriceps activation may be unaffected despite a large increase in H-reflex amplitude [229]. Finally, the semi-recumbent stepping task in Hopkins's study [128] relied on the activation of many muscles in the lower limb (not just the quadriceps) and was performed at a low level of muscle activation (~36% of maximum voluntary contraction), making its relevance to quadriceps strengthening questionable. This is important as, from a clinical

perspective, AML is most problematic during strong voluntary contractions, where it can prevent effective strengthening of the quadriceps muscle [20].

Hence, the purpose of this study was to clarify the effects of cryotherapy on AML during maximum effort quadriceps contractions. Alterations in knee extensor peak torque, muscle fibre conduction velocity (MFCV) and EMG amplitude were examined in response to experimental joint infusion and cryotherapy. We hypothesised that each of these variables would be reduced by joint swelling and that cryotherapy would partially reverse these changes; returning knee extensor torque, MFCV and EMG amplitude back towards their baseline values.

BACKGROUND AND METHODOLOGICAL CONSIDERATIONS

Before moving onto the methods section of this chapter, an overview is warranted concerning the background and key methodological considerations to be taken into account when using cryotherapy as an intervention and in the measurement of isometric torque, EMG amplitude and MFCV.

Cryotherapy

Background

Since the time of the ancient Greeks, cryotherapy has been used as a therapeutic aid in the treatment of musculoskeletal injury and disease [230-232]. Cryotherapy is known to produce a number of neurophysiological effects that have the potential to influence both AML and muscle force output. These are discussed in more detail below.

Sufficient cooling of the knee joint may reduce the sensitivity of articular sensory receptors, preventing their discharge in response to mechanical and nociceptive stimuli [205, 233]. In this regard, several authors have shown that cryotherapy is capable of reducing intraarticular temperature [234-238].

Bocobo et al. [239] iced the knee joints of dogs and reported that the intraarticular temperature was decreased by 4.1 °C after 15 minutes and 6.5 °C after 30 minutes. In healthy human subjects, a 15 minute application of ice chips to the knee has been shown to reduce skin temperature by ~13 °C and intraarticular temperature by ~6 °C [237]. When the ice chips were left in place for 30 minutes reductions of 16.6 °C and ~8 °C in were observed for skin temperature and intraarticular temperature respectively [237]. Similarly, in patients with chronic arthritis, Oosterveld and Rasker [240] showed that 30 minutes of icing led to a 16.6 °C decrease in skin temperature and a corresponding 6.4 °C decrease in intraarticular temperature.

The decrease in intraarticular temperature persists after the ice is removed from the joint. Oosterveld et al. [237] reported that after 30 minutes of knee joint cryotherapy, intraarticular temperature continued to decline for a further 15 minutes after the ice was removed, followed by gradual return towards baseline values. Similarly, following 60 minutes of icing, Warren et al. [238] reported that intraarticular temperature continued to decrease for 30 minutes after cryotherapy ceased. Notably, Warren et al. [238] showed that intraarticular temperature remained 11.2 °C cooler than baseline values 60 minutes after the removal of the ice, while Oosterveld et al. [237] reported that intraarticular temperature was still 2.7 °C colder than baseline two and a half hours after removal.

A drop in intraarticular temperature has been shown to reduce the discharge rate of articular sensory receptors. In the cat's knee joint, reducing the absolute temperature of the articular tissue to ~25 °C has been shown to effectively halve the discharge rate of ligamentous sensory receptors in response to a standardised mechanical stress [233]. The significance of this observation is highlighted by the finding that 30 minutes of icing reduced intraarticular temperature in the uninjured human knee to ~22.5 °C [237]. While temperature effects on articular receptor sensitivity have not been directly assessed in humans, cryotherapy has been shown to markedly reduce the sensitivity of sensory receptors in both muscle and cutaneous tissue [241, 242].

In addition to its effects on intraarticular temperature and sensory receptor sensitivity, cryotherapy is known to slow the conduction velocity of peripheral nerves [243, 244]. It is thought that cooling the nerve alters ion channel permeability in the axonal membrane, interfering with the normal propagation of action potentials along the sensory fibre [245]. More specifically, cooling the nerve increases the absolute and relative refractory periods of the afferent fibre [246], preventing the propagation of high frequency impulses [247]. In fact, there appears to be a near linear relationship between tissue temperature and nerve conduction velocity [243, 248]. Furthermore, at the moderate levels of cooling expected with joint cryotherapy, the rate of change in nerve conduction velocity is similar for both myelinated and unmyelinated fibres [243, 248].

Importantly, the depth of the nerve relative to the icing modality appears to have an influence on tissue cooling and therefore the decrease in nerve conduction velocity. For example, Lee et al. [244] showed that applying ice packs to the elbow for 20 minutes (where the nerve is located superficially) led to a 29.4% decrease in ulnar nerve conduction velocity when compared to applying ice packs to the muscle bulk of the flexor carpi ulnaris for 20 minutes, which led to a 13.4% decrease in conduction velocity. Thus, in order to fully appreciate the potential effects of knee joint cryotherapy on nerve conduction velocity, the anatomy of the nerves supplying the knee joint needs to be considered. Studies of human cadavers have shown that the

knee joint is innervated by branches of the tibial, obturator, femoral, common peroneal and saphenous nerves [94, 249]. Importantly, nearly all of these articular nerve branches are superficial at the level of the knee joint. For instance, the articular branches of the tibial and obturator nerves run through the popliteal fossa; the branches of the common peroneal nerve run around the head of the fibular and along the anterolateral joint line; the major articular branch of the femoral nerve emerges distal to the vastus medialis and passes under the medial retinaculum; and the saphenous branch runs along the medial tibia, just inferior to the joint line [94, 249]. Thus, it seems highly likely that icing the knee will reduce the conduction velocity of the major articular nerves supplying the knee joint. Furthermore, as a cold-induced block of high frequency nerve impulses depends on the temperature of the coldest part of the nerve rather than the average conduction along the length of the nerve [247], the propagation of afferent output from the joint to the spinal cord will almost certainly be attenuated by cryotherapy.

In terms of the magnitude of this effect, a number of different studies have reported remarkably similar findings of a 1.5-3.2% decrease in nerve conduction velocity per 1 °C change in temperature (typically measured from the skin or subcutaneous tissue close to the cooled nerve) [244, 248, 250-253]. The importance of such a change can be appreciated when it is considered that reductions in joint line skin temperature of ~15-21 °C have

been observed after 20-30 minutes of knee joint cryotherapy [205, 237, 238]. Using these values, we can estimate that articular nerve conduction velocity may be decreased by 23-67% compared to baseline values. Together with the likely loss of sensory end organ sensitivity, a reduction in articular nerve conduction velocity should result in dorsal horn neurons receiving notably less joint afferent input over a given period of time [205, 254]. As a consequence, a decreased peak to peak amplitude of depolarisation and diminished firing rate of the interneurons interposed in the pathways mediating AMI would be expected [205].

In support of this assertion, Sluka et al. [254] demonstrated that icing an inflamed knee joint reduces the excitability of the flexion reflex. These authors induced an artificial inflammation in the knee joints of rats and assessed the excitability of the flexion reflex via the withdrawal latency in response to noxious stimuli applied to the ipsilateral paw. While inflammation significantly reduced paw withdrawal latency, icing the knee joint for 20 minutes reversed these changes, indicating a reduction in the excitability of the flexion reflex pathway. Control animals, without inflammation of the knee, showed no significant changes to paw withdrawal latency after cryotherapy. The authors suggested that the increase in paw withdrawal latency following cryotherapy may reflect a reduction in sensory output from the inflamed joint due to the intervention [254].

Alternatively, a cryotherapy induced decrease in spinal reflex excitability may be partially mediated by an increase in descending inhibition from brainstem pathways. Cold stimuli are known to activate non-noxious and noxious thermoreceptors in the skin, increasing sensory output from these populations of afferents [255, 256]. Noxious cold stimulation has been shown to act as a counterirritant, enhancing descending inhibition of wide dynamic range neurons involved in mediating the flexion reflex [257-260]. In this way, cryotherapy may disinhibit the quadriceps a-motoneuron pool by two mechanisms; the first a direct reduction in the articular sensory output responsible for causing AML and the second an indirect effect via the recruitment of descending inhibitory pathways that suppress the excitability of spinal cord interneurons involved in mediating AML.

In addition to these disinhibitory effects, there is a growing body of evidence that cryotherapy can have a facilitatory effect on central nervous system excitability, enhancing muscle force output. This has been demonstrated by icing the joints of healthy uninjured controls and examining changes in variables such as H-reflex amplitude and peak torque production. For instance, Krause et al. [261] showed that icing the uninjured ankle joint for 30 minutes led to a significant facilitation of soleus H-reflex amplitude, with a strong inverse correlation between ankle joint temperature and H-reflex amplitude ($r^2 = -0.94$). Similar findings have been reported when cryotherapy is applied to the knee joint. Hopkins et al. [205] showed that icing the knee

joint for 30 minutes reversed the decline in H-reflex amplitude observed in response to experimental joint swelling. Moreover, at its peak, the H-reflex amplitude not only returned to its baseline value but increased ~20% beyond preeffusion measures, an effect that lasted for an additional 30 minutes after the ice was removed. These authors concluded that cryotherapy not only reduces AML, but facilitates the quadriceps a-motoneuron pool beyond normal levels. Regrettably, these conclusions are hampered by methodological issues. Firstly, it is well established that H-reflex amplitude may be altered due to factors that are independent of changes in a-motoneuron excitability [188, 228, 229]. Thus, we cannot be sure that the observed increase in H-reflex amplitude truly reflects a facilitation of the quadriceps a-motoneuron pool beyond preeffusion values. Furthermore, both of these studies failed to monitor M-wave stability, making it impossible to ascertain whether or not their findings were confounded by a change in peripheral stimulating and/or recording conditions.

More robust evidence for an excitatory effect of cryotherapy on the central nervous system was provided by Hopkins and Stencil [130], who demonstrated that soleus H-reflex: M-wave ratios and peak isokinetic plantar flexor torque increased by ~15% following the 30 minute application of crushed ice to the ankle joint. This facilitation continued for a further 60 minutes after the removal of the ice. There were no significant differences in H-reflex or peak torque measures in the control group, who did not receive

cryotherapy but underwent an identical testing procedure. These findings have since been confirmed by Palmieri-Smith et al. [262] who showed that icing the uninjured ankle for 20 minutes facilitated the soleus H-reflex: M-wave ratios immediately postintervention and for a further 20 minutes after the ice was removed. Thus, in addition to its disinhibitory effects, cryotherapy appears to have a facilitatory effect on the central nervous system that increases H-reflex excitability and may enhance maximal force production of the muscles surrounding the cooled joint.

The mechanisms by which cryotherapy achieves these effects remain poorly understood. In this regard, noxious cold stimuli have been shown to activate the sympathetic nervous system [263] and enhance descending input from monoaminergic brainstem pathways to the spinal cord [264]. For example, the inhibitory effects of noxious cold stimulation on flexion reflex excitability are known to be partly mediated by the activation of serotonergic brainstem pathways [264]. Furthermore, Palmieri-Smith et al. [262] showed that icing the ankle joint for 20 minutes led to a significant increase in serum norepinephrine levels that remained elevated for at least 10 minutes after the ice was removed from the joint.

An increase in circulating monoamines may have facilitatory effects locally (at the level of the muscle fibre) and centrally, within the ventral horn. With

respect to local effects, extrafusal muscles fibres are known to be under the direct influence of the sympathetic nervous system via adrenergic receptors located on the muscle cell membrane [265]. Theoretically, activation of the sympathetic nervous system may have different effects on the contractile machinery of type I and II muscle fibres. Force production in type I fibres tends to be weakened, with shortening of the duration of their twitch torque due to an increased relaxation rate [266]. In contrast, it is thought that sympathetic activation enhances the contractility of type II fibres by enhancing calcium ion release from the sarcoplasmic reticulum, thus increasing twitch torque amplitude [267, 268]. If the facilitatory effect on the force output of type II fibres is greater than the weakening effect on type I fibres then maximum force production may be enhanced by sympathetic activity. However, recent evidence [263] suggests that this is not the case, with sympathetic activation due to cold water immersion of the hand failing to augment electrically evoked twitch force production of the tibialis anterior. This finding argues against local muscle effects explaining the increase in force production after cryotherapy. Furthermore, such a mechanism fails to explain the observed increase in H-reflex: M-wave ratios following cryotherapy [130, 262].

Instead, it may be that increased monoamine release has a central excitatory effect, at the level of the ventral horn. In this regard, it has been shown that norepinephrine and other monoamines such as serotonin have

strong neuromodulatory effects on α -motoneurons, directly activating receptors on the α -motoneuron cell membrane that enhance their intrinsic excitability. This can lead to the generation of persistent inward currents that allow self-sustained motoneuron firing while at the same time, amplifying the effects of excitatory synaptic input from supraspinal and peripheral sources (for a review see [269]). It may be through this mechanism that cryotherapy enhances α -motoneuron excitability, facilitating H-reflex amplitude and increasing maximum force production.

Finally, cryotherapy's facilitatory effects on maximum force production may be partially explained by cortical mechanisms. Researchers have shown that cutaneous afferent input can directly enhance motor cortex excitability [270, 271], while strong cooling of the skin is known to increase regional blood flow and electroencephalography signals in many different regions of the cortex [272, 273]. Given these findings, the effects of cryotherapy on cortical excitability deserve further investigation.

Methodological considerations

Location of cryotherapy

In order for cryotherapy to effectively enhance quadriceps muscle activation, it is important that local cooling of the muscle is minimised. A recent systematic review of 25 studies [274] examining muscle strength after

cryotherapy reported that ~75% of studies showed a significant decline in maximum force production following local muscle cooling. Cooling the muscle has been shown to strongly reduce spindle sensitivity [275], which is likely to impair quadriceps activation by attenuating normal transmission in the γ -loop [276]. Furthermore, reducing intramuscular temperature has peripheral effects on the contractile processes of the muscle, slowing the rate of adenosine triphosphate hydrolysis, muscle fibre conduction velocity and calcium release from the sarcoplasmic reticulum (for a review see [277]). To counter the potentially detrimental effects of cryotherapy on muscle force production, previous researchers [128, 205] have ensured that when icing the knee joint, the ice bags are secured below the superior border of the patella. Using this positioning, they have found no evidence of local quadriceps muscle cooling.

Duration of cryotherapy

Both the sensitivity of articular sensory receptors [233] and nerve conduction velocity [244, 248] decrease in a near linear manner with a reduction in temperature. Thus, in order to maximise cryotherapy's efficacy, it is important that the joint is cooled sufficiently. As a general rule, the longer the ice is left in place the greater the cooling effect, both superficially and within the joint. For example, Oosterveld et al. [237] monitored changes in skin temperature and intraarticular temperature during the 30 minute application of ice chips to the knee joint. After 10, 20 and 30 minutes, skin temperature had reduced

to approximately 16 °C, 12 °C and 11.5 °C respectively. After 15 minutes and 30 minutes, intraarticular temperature had decreased by ~5.5 °C and 8 °C. Similarly, in individuals with arthritis, Oosterveld and Rasker [240] reported skin temperatures of 18 °C, 16.5 °C and 16 °C after 10, 20 and 30 minutes of knee joint icing, with decreases in intraarticular temperature of ~3.5 °C and 6 °C after 15 minutes and 30 minutes respectively. For both healthy controls [237] and patients with chronic arthritis [240] a significant correlation (Spearman's $\rho = 0.75-0.87$) has been found between the maximal change in skin temperature and the maximal change in intraarticular temperature. Importantly, in order for a meaningful reduction in intraarticular temperature to occur, it appears necessary to achieve a minimum level of skin cooling. For example, Dahlstedt et al. [234] explored the effects of cryotherapy on skin temperature and intraarticular temperature in the first 24 hours after ACL reconstruction. These authors reported that intraarticular temperature only began to decrease once skin temperature at the joint dropped below 20 °C. Thus, it is important that the ice is left in place long enough for a notable drop in skin temperature and intraarticular temperature to take place. In this regard, reductions in joint line skin temperature of ~15-21°C have been observed after 20-30 minutes of cryotherapy [205, 237, 238], with intraarticular temperature reducing by as much as 9.4 °C [237]. In contrast, the excitatory effects of cryotherapy appear to occur within a shorter time frame. Palmieri-Smith et al. [262] demonstrated that the soleus H-reflex: M-wave ratio increased significantly after 10 minutes of icing the ankle joint and did not

increase further after 20 minutes of icing or during the 20 minutes after the ice was removed.

Method of cryotherapy

Various cryotherapy modalities are used to achieve a cooling effect, including ice chips, air splints filled with cold water (e.g. cryocuff), frozen peas, frozen gel packs, chemical packs and topical coolants [230]. A number of comparative studies have been undertaken examining their relative cooling effects. McMaster et al. [278] examined the effects of four different modalities on the skin temperature of the thigh. Of these, ice chips in a plastic bag achieved the greatest rate of cooling and largest absolute drop in skin temperature. Similarly, Oosterveld et al. [237] compared a 30 minute application of ice chips in a plastic bag to the application of (liquid nitrogen produced) cold air to the knee joint. Skin temperature was found to decrease by 16.4 °C in the group receiving ice chips and 15°C in the cold air group. However, ice chips were notably more effective in reducing intraarticular temperature, with a 9.4 °C decrease observed compared to a 4 °C decrease in the cold air group. Similar results were observed in a group of patients with chronic arthritis, where ice chips were shown to lead to a greater reduction in intraarticular temperature than the application of cold air [240]. Warren et al. [238] compared the effects of crushed ice in a plastic bag to an air splint filled with iced water on skin temperature and intraarticular temperature at the knee. While both modalities led to significant

decreases in skin temperature and intraarticular temperature, the magnitude of the reduction in intraarticular temperature was greater for the ice chips at all time points. Chesterton et al. [279] compared the skin cooling effects of a frozen gel pack and a bag of frozen peas. After 20 minutes, frozen peas produced the lowest skin temperature of 10.8 °C compared with 14.4 °C for the gel pack. Finally, Karlayanaphotporn and Janwantanakul [280] compared the relative cooling effects of ice chips in a plastic bag, frozen peas and a cold gel pack. After 20 minutes, the ice chips achieved the greatest cooling effect, reducing skin temperature to 10.2 °C compared to 13.9 °C, 14.4 °C for the frozen peas and gel pack respectively.

Contact with the skin

Clinically, it is common for a barrier such a cloth or bandage to be applied between the skin and the icing modality, in order to reduce the risk of skin damage [230]. The obvious disadvantage of this is that it may reduce the magnitude of cooling, thus negating cryotherapy's neurophysiological effects. Ibrahim et al. [281] examined the effects of different skin dressings on the cooling effect of a cryocuff device. After 60 minutes of icing, skin temperature decreased by ~15 °C in a group with no dressing and a group who had a thin tegaderm dressing applied between the skin and cryocuff. In contrast, when a thicker wool and crepe bandage was applied between the skin and cryocuff, skin temperature only dropped by 4 °C. Tsang et al. [282] compared the effects of a 20 minute application of ice cubes in a plastic

bag when applied directly to the skin, through a dry elastic bandage and through a dry towel. Both the bandage and towel notably attenuated the cooling effect, with final skin temperatures of 27 °C and 23 °C compared to 12.5 °C when the ice bag was applied directly to the skin. LaVelle and Snyder [283] observed skin temperatures of 30.5 °C when a plastic bag of chipped ice was applied through a padded bandage, 20.5 °C with a bandage alone, 17.8 °C with a dry cloth, 9.9 °C with a damp cloth and 10.8 °C with no barrier. Finally, Janwantanakul [284] observed a significantly greater rate of cooling and absolute drop in skin temperature when a bag of chipped ice was applied directly to the skin compared to when it was wrapped in a damp cloth.

Summary

Cryotherapy has been shown to have both disinhibitory and facilitatory effects that suggest it may be a useful therapeutic intervention to address quadriceps AML. Firstly, cryotherapy lowers intraarticular temperature and cools the nerves that supply the joint. In turn, this is likely to reduce the sensitivity of articular sensory receptors and impair the propagation of afferent impulses from the joint to the spinal cord. In this way, cryotherapy may have a direct disinhibitory effect, attenuating the aberrant sensory output from the damaged joint that is responsible for causing AML. Secondly, noxious cold stimulation is known to enhance descending inhibition of the interneurons involved in the flexion reflex. As this pathway may be involved in

mediating AML, it is possible that cryotherapy attenuates inhibition via this mechanism. Finally, there is evidence that cryotherapy may have a direct facilitatory effect on central nervous system excitability, counteracting ongoing inhibition of the quadriceps α -motoneuron pool and enhancing muscle force output.

The available evidence suggests that chipped or crushed ice in a plastic bag provides the greatest cooling effect, both at the skin and within the knee joint itself. If a barrier has to be used between the skin and the ice, it is best to be wet rather than dry and as thin as possible. However, applying ice bags directly to the skin may achieve the best cooling effect. Furthermore, it is important to avoid cooling the quadriceps muscle itself. Positioning the ice bags so that they do not extend beyond the superior border of the patella appears to prevent this. Finally, while the excitatory effects of cryotherapy on the central nervous system may manifest within 10 minutes, 20 to 30 minutes of icing may be required to appreciably reduce skin temperature and intraarticular temperature. Importantly, both the disinhibitory and excitatory effects of cryotherapy have been shown to last for 10-60 minutes after the ice is removed from the joint. This suggests that cryotherapy may reduce quadriceps AML for a clinically meaningful period of time, allowing subsequent resistance training to take place.

Isometric torque

Background

Isometric torque is a commonly used measure of muscle strength that is assessed by performing maximum effort voluntary contractions with the joint held in a static position. A variety of devices have been used to measure isometric strength. These include cable tensiometers, hand held dynamometers, linear strain gauges and isokinetic dynamometers. The advantages of testing muscle strength isometrically are that it is quick and easy to perform, can be tested without the necessity for expensive equipment and is highly reliable [285].

Using a variety of isokinetic dynamometers, a number of researchers have demonstrated the excellent repeatability of isometric torque measurements, with reported intraclass correlation coefficients (ICCs) ranging from 0.83 to 0.98 [286-290]. Recently, Drouin et al. [291] have specifically examined the reliability and validity of the Biodex system 3 dynamometer when measuring isometric torque. These authors reported excellent trial-to-trial reliability, with an ICC of 0.99. The standard error of the measurement (SEM) between trials was 0.39 Nm. Additionally, the largest coefficient of variation observed between the criterion measured torque and software measured torque was 1%, suggesting the Biodex System 3 dynamometer provides a valid measure of isometric torque [291].

Similarly, the reliability of measuring isometric strength using a linear strain gauge has been shown to be excellent [292]. These authors tested healthy controls in 90 degrees of knee flexion and demonstrated intrasession ICCs of 0.89-0.92 and SEMs of 2-4 N for knee extension force, with ICCs of 0.85-0.92 and SEMs of 1-2 N for knee flexion. A recent comparison [293] of isokinetic dynamometry and a strain gauge system demonstrated comparable intersession reliability, with ICCs of 0.93 and 0.92 respectively for the knee extensors and 0.89 and 0.96 for the knee flexors. For both knee extension and knee flexion, the SEM of the strain gauge measurements across sessions was less than 0.2 Nm. There was no significant difference in any of the peak torque values derived from the isokinetic dynamometer compared to the strain gauge across any of the testing sessions [293].

The reliability of isometric strength measurements has also been assessed in populations with knee joint pathology. Using a strain gauge system, Fransen et al. [294] demonstrated good intersession reliability (7 day test-retest interval) of strength measures in a sample of 113 individuals with knee OA. The ICCs and SEMs for knee extensor force ranged from 0.79-0.92 and 4-15 N respectively, while the equivalent values for the knee flexors were 0.81-0.90 and 4-11 N. Kean et al. [295] demonstrated an intersession ICC of 0.98 and a SEM of 11Nm for knee extension isometric torque in OA participants measured 1 day to 1 week apart on a Biodex system 3 isokinetic dynamometer. Finally, Staehli et al. [296] tested isometric knee extensor

torque retest reliability in 10 participants with end stage OA and 20 participants who had undergone TKA 5-12 months previously. These authors reported an intersession ICC of 0.95 and a coefficient of variation of 5.3% (~7 Nm) for knee extensor torque measured 3-10 days apart.

Methodological considerations

Stabilisation and Isolation

In order to obtain valid measures of isometric muscle strength, it is important that movement at other joints is minimised as much as possible [297]. This is particularly important for muscles that cross two joints, such as the rectus femoris and hamstrings, as any change in the angle of hip flexion or pelvic rotation will change the length of these muscles, and therefore their mechanical advantage, during isometric testing [298]. In this regard, Weir et al. [299] showed that peak torque production of the knee extensors and flexors differed by 7-31% when the trunk and thigh were securely stabilised compared to when they were not. Furthermore, extraneous movement at other joints may mean that muscle groups other than those being tested erroneously contribute to torque measurements [285]. For these reasons, it is common practice for the trunk, pelvis and ipsilateral thigh to be strapped down during isometric testing at the knee [285, 297, 299]. The position of the upper limb may also be important during strength testing at the knee. Many isokinetic dynamometers have handle bars attached to sides of the chair

which the participant can grip onto and use to splint their upper body. Compared to a condition where participants were asked to fold their arms across their chest, Stumbo et al. [300] found that knee extensor peak torque was significantly higher in males when hand grip stabilisation was allowed. Despite the increase in torque production, there was no corresponding increase in quadriceps EMG amplitude, suggesting that the additional torque production may not have been due to increased activation of the tested muscle group.

Gravity correction

Without correcting for the effects of gravity, the weight of the leg and dynamometer arm can bias isometric torque measurements, increasing torque values during knee flexion and decreasing values during knee extension. As such, it has been argued that gravity corrected values provide a better estimation of the actual torque generated [301]. Gravity correction is commonly achieved by weighing the mass of the leg and dynamometer arm at a single point in the joint range of motion. This is then used by the dynamometer to estimate the influence of gravity at the specific angle of interest for isometric testing, which is added or subtracted from the resulting torque values. When weighing the leg, it is important not to have the limb too close to the horizontal as the tension in the hamstrings and posterior structures of the knee joint may create a passive resistance that is independent of gravity and will confound the measurement [302]. Gravity correction is

particularly appropriate as the knee moves further into extension, where gravity is more likely to bias torque measurements. Conversely, it has been argued [301] that gravity correction is not necessary during isometric contractions at 90 degrees of knee flexion as the lower leg is essentially vertical and gravity will have a minimal effect on peak torque values.

Warm up

The aims of participant warm up prior to strength testing are to minimise the risk of injury and enhance the performance and stability of peak torque production during subsequent maximum effort strength testing [285, 301]. It has been recommended that warm up activities should include both a general and specific warm up [285]. The general warm up is designed to increase muscle temperature and typically involves low intensity repetitive muscle activity such as cycling [26, 303, 304]. An increase in muscle temperature has been shown to increase contractile velocity and may increase maximum torque production [305-307], although the effects do not appear to be as great on isometric torque production [308, 309]. The specific component of the warm up usually involves submaximal performance of the actual movements to be tested, in order to enhance neuromuscular facilitation in a task specific manner [285]. Various submaximal testing protocols have been adopted in the literature, with between 2 and 10 repetitions commonly undertaken, progressing in intensity towards a maximal effort [303, 310-313]. Some authors have combined submaximal and maximal

contractions in the warm up [314, 315]. There is limited experimental evidence to support the use of submaximal contractions or the use of one particular protocol over another. Mawdsley and Croft [316] examined the effects of submaximal contractions on knee extensor peak torque values and participant comfort during subsequent maximum effort strength testing. There was no difference in the mean peak torque values between the group undertaking a three submaximal contraction warm up prior to maximal testing and those who had no warm up. However, it was reported that the group performing a warm up experienced less discomfort during the subsequent maximum effort tests. These authors suggested that submaximal warm up may therefore be useful in preventing injury, but not necessarily in enhancing maximal torque production [316]. Similar findings have been reported with isometric grip strength [317], where submaximal warm up failed to significantly enhance maximal force production.

Feedback given to participants

Strength testing is strongly influenced by the motivation of participants. There is evidence that providing verbal encouragement and visual feedback of performance increases peak torque production. Peak isokinetic knee extensor torque has been shown to increase by 8-12% when participants are given real time visual feedback of their torque production on a screen placed in front of them [318, 319]. Similarly, McNair et al. [320] showed that providing participants with strong verbal encouragement during strength

testing increased peak isometric elbow flexion torque by ~5% compared to identical procedures without any encouragement. There is evidence that combining visual feedback with verbal encouragement enhances peak torque production more than either on their own [321, 322], with O'Sullivan and O'Sullivan [323] reporting that both knee extensor and knee flexor peak torque were enhanced by 14-26% when combined visual feedback and verbal encouragement was given to participants.

Time of day

It is well established that circadian rhythms influence muscle performance (for review see Drust et al. [324]). Independent of the muscle group or speed of contraction tested, muscle force production typically peaks in the early evening [324]. At the knee joint specifically, there is consistent evidence [325] that isometric knee extensor and knee flexor torque measurements exhibit time of day variations of 5-10%, with the largest values always recorded in the early evening and the smallest values in the early morning. Thus, circadian rhythms need to be taken into account with repeated strength testing, which should be performed at the same time of day whenever possible.

Duration of contraction

A number of different contraction durations have been recommended for the assessment of isometric strength. Caldwell et al. [326] suggested a

contraction duration of 4 seconds with a rise time of 1 second from rest to peak torque. Similarly, Chaffin [327] recommended testing isometric strength using a 4-6 second contraction. Sale [328] suggests that isometric contractions of 5 seconds duration are sufficient for most participants to generate their peak torque. There does not appear to be any experimental data to validate these recommendations. However, observations from our own laboratory are that participants generally reach their peak torque within 3 seconds and are able to maintain torque at this level for a maximum of 2 seconds. Therefore, a contraction duration of 5-6 seconds seems appropriate in order to ensure maximum torque development while minimising muscle fatigue and the risk of injury.

Rest interval

The rest interval separating consecutive maximum effort voluntary contractions may influence peak torque production. An adequate rest period is essential for the reperfusion and reoxygenation of the contracting muscle; leading to the replenishment of phosphocreatine stores, elimination of metabolic waste products, rebalancing of intramuscular pH, and stabilisation of muscle membrane potential [329-333]. A variety of rest intervals have been proposed in the literature, ranging from 30 seconds to 10 minutes [326-328]. Experimental evidence supporting these recommendations is sparse. Weir et al. [334] asked participants to perform two 1 repetition maximum (1 RM) bench presses, separated by 1, 3, 5 or 10

minutes rest. There were no appreciable differences in the number of participants in each group who successfully completed the second attempt, suggesting that 1 minutes rest may allow for sufficient neuromuscular recovery. Matuszak et al. [335] asked participants to perform two 1RM back squats, separated by 1, 3 or 5 minutes' rest. The number of participants who successfully completed the second attempt was 75%, 94.1% and 88.2% for the 1, 3- and 5-minute rest conditions respectively. These findings suggest that rest periods longer than 1 minute may be necessary for full recovery in some instances and are in agreement with the observations of Trossman and Li [336], who compared peak isometric grip force across 3 trials with rest periods of 15 seconds, 30 seconds and 60 seconds. They found the decline in force was smallest in the 60 second condition. However, when they compared their findings to pretrial testing in the same participants (where a 2 minute rest interval was given) the difference in force production between consecutive trials was less again with a 2 minute rest period. Thus, inter-trial rest periods of at least 1 minute are necessary, with a 2-3 minute rest period preferable to ensure maximum strength recovery.

Number of repetitions needed to achieve maximum voluntary contraction

The number of maximum voluntary contractions performed may also influence the measurement of isometric torque. Edwards et al. [337] used three maximum voluntary contractions and reported that the first usually produced less torque with the second and third very similar. Murray et al.

[338] found that peak torque tended to be higher on the second of two consecutive isometric strength tests of the knee extensors and knee flexors, although this only reached statistical significance for the knee extensors. In recent recommendations for strength testing, Brown and Weir [285] suggest that three test repetitions are sufficient to elicit a maximal peak torque value. However, additional trials may be necessary in individuals with knee joint pathology [339] who may initially be more cautious in performing strong voluntary contractions, perhaps for fear of eliciting pain or damaging their joint further.

Summary

Isometric torque measurements of both the knee flexors and knee extensors demonstrate good to excellent intrasession and intersession reliability when measured on an isokinetic dynamometer or with a strain gauge system. However, there are a number of important methodological factors to consider when performing these measurements. Firstly, it is important to stabilise the thigh, pelvis and trunk in order to minimise extraneous movement that could bias torque recordings and allow changes in the length tension relationship of the muscles acting at the knee. Secondly, when strength testing is undertaken towards the horizontal, the weight of the limb and dynamometer arm should be taken into account to offset the effects of gravity on peak torque. With respect to warm up, it is generally recommended that both a general and specific warm up are undertaken

prior to maximal effort strength testing. There is some evidence that warm up enhances peak torque production and that the performance of submaximal contractions improves participant comfort during subsequent maximum effort strength testing. To facilitate maximum effort contractions, both verbal encouragement and visual feedback of torque production should be provided to the individual being tested. Furthermore, repeated testing of isometric torque should be undertaken at a similar time of day as significant circadian variations in muscle strength have been observed. Finally, while experimental data supporting the optimum testing parameters is severely limited, three to five maximum effort voluntary contractions of 5-6 seconds duration, with a 2 minute rest period between contractions appears appropriate in the measurement of peak isometric torque.

Surface EMG

Background

When a skeletal muscle is activated, action potentials travel from the α -motoneuron pool via the motor axon to the neuromuscular junction where the muscle fibre cell membranes depolarise, causing a wave of action potentials to course along the length of the muscle fibres as the muscle contracts. The depolarisation of the muscle fibres creates voltage shifts that can be measured using electrodes placed on the surface of the skin – a technique known as surface EMG. In this way, surface EMG can be thought

of as a record of the compound action potential due to the summed activation of several motor units [340]. The primary advantage that surface EMG has over invasive alternatives like needle EMG is that surface electrodes record electrical signals from a larger proportion of the muscle, providing a better representation of global motor unit activity [341, 342]. Surface EMG has been used extensively in the study of muscle function after knee joint damage, both during voluntary contractions [68, 85, 98, 141, 343] and in the collection of evoked responses such the H-reflex [71, 94, 123], the flexion reflex [79, 176, 177] and motor evoked potential [203, 204]. Two important variables that can be extracted from the surface EMG signal during voluntary muscle contractions are the EMG amplitude and MFCV.

An aspect of surface EMG that is qualitatively appealing is that an increase in the strength of a voluntary muscle contraction is typically met with a corresponding increase in the size and intensity of the EMG signal. In other words, as the number of active motor units and their firing frequency increases, so does the amplitude of the EMG signal [344, 345]. Accordingly, EMG amplitude is often considered an index of muscle activation [16, 68, 85, 96, 141, 143, 157, 340]. However, the relationship between voluntary activation and surface EMG amplitude is not strictly linear and may vary between muscles and across measurement times. This is partly because the EMG amplitude largely reflects the activation of superficial motor units and depends on the distribution of motor units within the muscle and their

distance from the electrode [344, 346]. Furthermore, overlapping positive and negative phases of the EMG signal can cancel each other out; affecting amplitude estimates disproportionately at different levels of muscle activation [340]. Nevertheless, EMG amplitude provides a reasonable estimate of muscle activation and, as such, has been used to quantify quadriceps inhibition after knee damage. Compared to baseline values, Shakespeare et al. [16] found that quadriceps EMG amplitude was reduced by up to 90% in the first 24 hours after open meniscectomy. Furthermore, Stratford [85] showed that individuals with an acute knee effusion demonstrated markedly reduced EMG amplitudes during maximum effort quadriceps contractions in full knee extension compared to contractions performed in 30° of flexion. In contrast, EMG amplitude was quantitatively similar at both knee joint angles in individuals without swelling. Similarly, experimental knee joint swelling has been shown to decrease quadriceps EMG amplitude during subsequent maximum voluntary contractions in both healthy controls and individuals with knee joint arthritis [68, 98].

Another variable that can be extracted from surface EMG signals is MFCV. MFCV is estimated by measuring the delay in the propagation of the EMG signal between two electrode pairs a known distance apart. Importantly, MFCV is also dependent on the level of muscle activation and is strongly influenced by both motor unit recruitment and firing rates [347]. In this regard, it has been shown that MFCV increases as the firing rate of active motor units

increases and with the recruitment of type II motor units [344]. During electrically evoked activation of the tibialis anterior, MFCV was found to be strongly correlated with muscle twitch torque, half relaxation time and rise time, suggesting that MFCV may provide a non-invasive measure of fibre type recruitment [348]. This is supported by the findings of Sadoyama et al. [349] who observed a strong linear relationship ($r = 0.84$) between quadriceps MFCV during maximum voluntary contractions and the relative area of type II fibres observed after subsequent muscle biopsy. To date, two studies have explored changes in MFCV following trauma to the knee joint. Cruz-Martinez et al. [350] measured changes in quadriceps MFCV over time after the removal of a cast from the knee joint. The individuals involved had suffered joint damage due to a range of different conditions including inflammatory arthritis, menisectomy, tibial osteotomy, patella fracture and medial collateral ligament tear. Compared to the uninjured side, both MFCV and quadriceps strength were notably reduced on the injured side after cast removal, before gradually improving with a period of rehabilitation. Similarly, Mase et al. [351] compared quadriceps strength and MFCV in individuals with lower limb OA to that of matched healthy controls. Quadriceps strength was measured during maximum effort isometric contractions, while MFCV was estimated following electrically evoked contractions of the vastus medialis. Both quadriceps strength and MFCV were found to be significantly lower in individuals with joint disease and a significant positive correlation ($r = 0.63$) was observed between quadriceps strength and MFCV. Furthermore, in the months following various types of surgery, MFCV was reported to increase in

conjunction with quadriceps strength. Both Cruz-Martinez et al. [350] and Mase et al. [351] concluded that reduced MFCV likely reflects selective type II muscle fibre atrophy in individuals with joint pathology. Changes in quadriceps MFCV have not yet been explored after acute knee injury or experimental joint infusion.

While variables such as EMG amplitude and MFCV can provide valuable insights into muscle function after knee injury, surgery and pathology, there are a number of limitations and technical considerations that need to be taken into account when acquiring, analysing and interpreting the surface EMG signal. These are outlined below.

Methodological considerations

Noise and differential amplification

Unfortunately, the electrical signals recorded by surface EMG electrodes do not exclusively reflect the underlying muscle activity. In fact, several sources of “noise” can contaminate and in some cases swamp the EMG signal, which has a very low voltage. Noise can be defined as electrical signals that are not part of the wanted EMG recording and may arise from various sources, including surrounding electrical equipment, light fixtures, power lines and movement of the electrodes and/or recording cables [352, 353]. Thus,

when acquiring the EMG signal it is important to minimise noise as much as possible and, in doing so, maximise the signal to noise ratio.

One of the most important ways of maximising the signal to noise ratio is through the use of differential amplification. Differential amplification typically involves the use of a bipolar electrode configuration, with two electrodes placed in series along the muscle and third electrode placed distally over an electrically neutral (i.e. bony) site [353, 354]. As many forms of noise occur to a similar degree at all electrode sites, the recorded signals are relayed to an amplifier which subtracts the components of the EMG signal that are common to all sites and amplifies the difference [352]. In essence, any signal that originates far away from the muscle will appear as a common signal and be removed, whereas signals close to the recording electrodes will be amplified [352, 353]. This can greatly enhance the signal to noise ratio. However, additional noise from sources such as cable motion artefact and electrical power lines can creep into the EMG signal as it travels along the recording cables to the amplifier or at the input to the amplifier [353]. One solution to this problem has been the development of active electrodes, or preamplification, in which a primary differential amplification of the EMG signal takes place as close to the site of the recording electrode as possible, maximising the fidelity of the signal before any additional noise has a chance to contaminate it [353, 355]. The extent to which common signal components are removed during the process of differential amplification is

called the common mode rejection ratio [356]. While it is desirable to have the highest common mode rejection ratio possible, a preamplified differential electrode with a common mode rejection ratio of 32,000, or greater than 80 dB, is generally considered sufficient to achieve an appropriate signal to noise ratio [352, 357].

Volume conduction and skin preparation

The biological tissues between the active motor units and the surface electrodes act as a volume conductor with their properties strongly influencing the surface EMG signal [358]. In practice, the volume conductor typically refers to the resting part of the muscle, connective tissue, subcutaneous fat layers and the skin lying beneath the electrode. Before reaching the surface electrodes, the EMG signal has to travel through these tissues, which act as a low-pass filter [346, 358]. As such, the shape of the EMG signal is altered and it becomes lower in amplitude [340, 346, 358]. Part of this effect occurs at the interface between the skin and electrode [346, 353]. Importantly, if the skin impedance differs at the electrode recording sites, the signal will be distorted to differing degrees, which can interfere with the process of differential amplification [353]. For example, if the skin impedance alters the properties of power line noise dissimilarly at different recording sites, it will not be recognised as common and some of the power line noise will remain in the signal following differential amplification [356]. Furthermore, generally high skin impedance can increase the low pass

filtering effects of the volume conductor, reducing the amplitude of the EMG signal and altering the shape of the waveform [353]. Good skin preparation, both to reduce the overall skin impedance and to minimise any difference between the skin impedance at the recording electrode sites, is essential [353]. Various methods are used to reduce skin impedance, including shaving the area, abrading the skin and wiping it with alcohol [353, 355, 359, 360].

Motion artefact

Another potential source of noise in the EMG signal is motion artefact. Motion artefact can occur for a number of reasons, one of which is movement of the recording cables. This can be minimised by using preamplifiers close to the site of the recording electrodes [346, 352, 356]. However, motion artefact also occurs when there is relative movement between the recording electrode and the underlying skin. Having a layer of conductive gel or paste between the electrode surface and the skin (as with pregelled electrodes) can mitigate this problem as the intervening layer of gel or paste acts to damp any mechanical disturbances at the skin electrode interface, minimising any effect on the signal [353]. Finally, motion artefact can occur due to small changes in voltage across different layers of the skin as it is stretched or deformed [353]. This is not affected by the type of electrode used, but can be attenuated with thorough skin preparation [353].

Filtering and sampling

Motion artefact can also be reduced through the use of appropriate filters. As motion artefact typically occurs within a frequency range of 0-20 Hz, it has been argued that employing a high-pass filter with a frequency cut off of 10-20 Hz can effectively reduce this issue [352, 356]. Furthermore, as the frequency content of the surface EMG signal seldom exceeds 500 Hz [353, 361] a low pass filter of 500-1000 Hz is typically employed to remove unwanted components of the signal due to sources of electromagnetic interference [352, 353]. Following differential amplification and filtering, the analogue EMG signal (volts) is typically sampled using an analogue-to-digital (A/D) converter to allow storage and off-line processing on a computer. Both the sampling rate and the sampling resolution are important factors to consider in this process. The sampling theorem states that the sample rate should be at least twice the highest frequency component of the signal [353]. This is called the Nyquist limit [354, 362] and prevents the loss the vital signal information. Given that the frequency content of the surface EMG signal rarely exceeds 500 Hz, sampling frequencies of at least 1000 Hz are usually considered adequate [354, 362]. An exception to this is in the estimation of MFCV using the cross-correlation technique, which may require much higher sampling rates (10000 - 50000 Hz) to achieve adequate time resolution [346, 358, 363, 364]. In addition to sampling frequency, the sampling resolution is determined by the number of bits per sample, which defines the number of discrete levels into which the signal will be converted [353]. For most

purposes, using a 12 to 16-bit A/D converter is recommended to maximise the signal to noise ratio and avoid quantisation error [353].

The recording electrode

The type of electrodes used and their placement on the muscle can strongly influence the surface EMG signal. The material used for the recording electrodes is important for the stability of the recordings and to enhance the signal to noise ratio. In this regard, Ag–AgCl electrodes are considered highly electrically stable and are widely used in surface EMG [353, 360, 365].

Furthermore, with a bipolar or multipolar electrode configuration, the electrodes are aligned in series with a standardised interelectrode distance. This can be defined as the centre to centre distance between two adjacent electrodes [352]. There is some debate about the ideal interelectrode distance and the size of each electrode. Larger electrode sizes and a greater interelectrode distance maximises the “pick up” of active motor units within the target muscle [354], providing a more global indication of motor unit activity that is more reliable [366] and may better translate to overall muscle function. On the other hand, the larger the surface area of the electrodes and the greater the interelectrode distance the more likely it is that part of the EMG signal will be contaminated by electrical activity from adjacent muscles – a problem known as cross talk [352, 354]. In situations where cross talk is of concern, it is advisable to reduce both the size of the

electrode surface and the interelectrode distance [352, 354]. In general, electrodes with a maximum diameter of 10 mm and an interelectrode distance of 10-25 mm are recommended when using bipolar surface EMG techniques [352, 358, 360]. Another method that may help to reduce cross talk is double differentiation. This technique requires the collection of two bipolar EMG signals using three recording electrodes aligned in series along the muscle [352]. Double differentiation effectively eliminates signals that are common to each pair of electrodes and thus more likely to arise from distant motor units. Using this technique van Vugt and van Dijk [367] have reported that it may be possible to reduce cross talk by up to six fold when compared to standard single differentiation techniques.

Placement of the recording electrodes

When placing the recording electrodes on the skin, they should be orientated so that they are aligned in parallel with the direction of the muscle fibres [358, 362, 368]. This increases the likelihood that the electrodes capture the longitudinal propagation of action potentials along the same muscle fibres. As a result, alignment of the recording electrodes greatly influences both EMG amplitude and estimates of MFCV. For example, Andreassen and Rosenfalck [369] showed that aligning the recording electrode in the direction of the fibres increases the absolute number of muscle fibre action potentials contributing to the signal, leading to a greater EMG amplitude when compared to an electrode arrangement placed perpendicular to the

direction of the muscle fibres. Similarly, De Luca [352] reported that the amplitude of the surface EMG signal may be reduced by up to 50% if electrodes are placed perpendicular, rather than parallel, to the muscle fibres. More recently, Zuniga et al. [370] examined vastus lateralis EMG amplitude during cycling and compared an electrode orientation placed parallel to the muscle fibres to an orientation with the same centre point, but perpendicular to the muscle fibres. Significant differences in both absolute and normalised EMG amplitude were observed. Furthermore, it is known that the orientation of the electrodes in relation to the muscle fibres strongly affects the estimation of MFCV [346, 364, 368]. The estimation of MFCV involves the detection of action potentials as they are propagated from the neuromuscular junction, down the length of the muscle fibre to the tendon. For this reason, it is essential that the electrode is aligned as closely as possible with the direction of the muscle fibres. Misalignment of the electrodes has been shown to result in overestimation of MFCV and the greater the misalignment, the greater the degree of overestimation [349]. Furthermore, computer simulations have demonstrated that, in some cases, misalignment may also lead to the underestimation of MFCV [371].

Another important consideration with respect to electrode placement is their positioning in relation to both the neuromuscular and musculotendinous junctions. The area around a neuromuscular junction is known as the innervation zone. Electrode placement directly over the innervation zone can

greatly influence the surface EMG signal as the action potentials travel in opposite directions away from the motor point, towards both the origin and insertion of the muscle [372]. As a result, a number of recent investigations have shown that positioning a bipolar electrode configuration over the innervation zone results in significantly lower estimates of EMG amplitude compared to sites on the muscle that were proximal or distal to the innervation zone [372-376]. Similarly, MFCV is strongly affected by placement of the electrode over the innervation zone, with a number of authors demonstrating that this leads to a large overestimation of MFCV [364, 377]. The same effects on EMG amplitude and MFCV are observed if the electrodes are placed too close to the muscle tendon due to action potential extinction and the generation of non-propagating standing waves [344, 346, 354, 362, 364]. As a consequence, the best location for electrode placement is the area of the muscle between the innervation zone and tendon [352, 360, 364].

Quantifying EMG amplitude

The most commonly used estimators of EMG amplitude are the average rectified value and the root mean square (RMS) of the signal [346]. To determine the average rectified value the demeaned surface EMG signal is rectified and then integrated to calculate the area under the EMG signal within a certain time period. The RMS is determined by squaring all the values of the signal, averaging them and then taking the square root of the

average. Again, RMS is calculated over a certain epoch in the EMG signal, usually 0.5-1 second for maximum voluntary contractions [378, 379]. The RMS method is preferred to the averaged rectified value as it is argued that the RMS has a stronger mathematical basis as a measure of EMG amplitude, being directly related to the average power of the signal [352, 359].

Normalisation

It is not valid to compare the absolute EMG amplitude between different muscle groups or across individuals. This is because of individual and intermuscular differences in factors such as skin preparation, the properties of the volume conductor (e.g. relative thickness of the subcutaneous fat layer) and the distance of active motor units away from the electrode, all of which can strongly influence the EMG signal [346, 380, 381]. For these reasons, EMG amplitude is usually normalised by expressing it as a percentage of the maximum EMG amplitude recorded for the same muscle during a maximal voluntary contraction.

Reliability of EMG amplitude

Kollmitzer et al. [382] examined the intrasession and intersession reliability of quadriceps RMS amplitude during maximum effort isometric contractions. The within session Pearson correlation coefficients were 0.71 and 0.87 for the vastus medialis and vastus lateralis respectively. In contrast, the correlation

coefficients were 0.30 and 0.38 when RMS was measured 6 weeks apart, suggesting that intersession reliability is poor. Fauth et al. [383] investigated the reliability of quadriceps and hamstrings RMS amplitude during maximum effort isometric contractions at 90° of flexion. They observed excellent within session reliability with ICCs ranging from 0.94 – 0.97 for the medial hamstrings, lateral hamstrings, vastus medialis and vastus lateralis muscles. Gruet et al. [384] examined intersession reliability of quadriceps RMS amplitude during maximum voluntary contractions performed 6 weeks apart. The ICC values were 0.72 and 0.84 for the vastus medialis and vastus lateralis muscles. However, the standard error of the measurement was 36% and 22% respectively. Thus, while the within session reliability of quadriceps and hamstrings RMS is acceptable, the between session reliability is questionable.

Quantifying MFCV

In order to estimate MFCV, two variables must be quantified: the interelectrode distance and the time delay between the two detected signals [368]. MFCV can then be calculated as the interelectrode distance divided by the time delay (i.e. $\text{velocity} = \text{distance}/\text{time}$). With surface EMG the interelectrode distance is already known. The delay in the signal is commonly estimated by cross-correlating the recorded EMG signals from each electrode pair [346, 364, 368]. The peak in the cross-correlation function represents the displacement of the two signals relative to each other and can be used to estimate the time delay [347]. However, in order to

meaningfully calculate the delay in propagation between the signals, it is important that the signals are sufficiently similar [368]. In this regard, the cross-correlation coefficient provides a measure of the similarity in the two signals. MFCV estimations from signals with cross-correlation coefficients lower than 0.8 are not reliable and are typically discarded [346, 385].

Reliability of MFCV estimates

Only one study has examined the reliability of MFCV estimates in the quadriceps [379]. However, these authors examined the reliability of the slope of the change in MFCV during a fatiguing contraction, which is not relevant to this thesis. The reliability of MFCV estimates has been obtained from other muscles. Farina et al. [386] examined the within session and between session reliability of MFCV estimates from the biceps brachii during maximum effort isometric contractions. Using 2 single differentiated signals and a 10 mm interelectrode distance, they reported a within session coefficient of variation (CV) of ~20% and a between session CV of ~27%. In general, it was found that estimates using intermediate interelectrode distances (10-15 mm) and more signals (up to seven) were more reliable. Ollivier et al. [387] examined the between session reliability of MFCV estimates collected several days apart. Using a bipolar electrode configuration (3 single differentiated signals, 15 mm interelectrode distance) over the biceps brachii, these authors reported an ICC value of 0.80 and a coefficient of variance of < 15% for MFCV estimates during maximum isometric voluntary contraction.

Summary

Surface EMG is a valuable tool to quantify muscle function after knee injury, surgery and in patients with knee joint pathology. However, in order to properly interpret its physiological significance, there are a number of important methodological considerations that need to be taken into account. These include adequate skin preparation, the type, configuration and location of the electrodes and appropriate amplification, filtering, sampling and processing of the EMG signal.

METHODS

Participants

Sixteen participants (10 male and 6 female) volunteered to be involved in this study. The mean (\pm one standard deviation) age, height and mass of the participants are provided in Table 3.1. Volunteers were excluded from the study if they had a previous history of pathology in both knee joints; lower limb or spinal surgery; back pain in the last 6 months with associated neurological signs or symptoms; or any pathology that precluded their participation in maximum strength testing. In the event of a previous knee injury, the contralateral (uninjured) knee joint was used in testing. Participants provided written informed consent for all experimental procedures. Ethical approval for this study was granted by the Northern Regional X Ethics Committee.

Table 3.1. Participant characteristics.

	Cryotherapy group	Control group
Age in years, mean (SD)	34.3 (11.7)	36.5 (10.6)
Height in metres, mean (SD)	1.80 (0.04)	1.71 (0.11)
Mass in kilograms, mean (SD)	78.6 (12.2)	70.1 (10.2)

SD = One standard deviation. No significant between group differences were found for age, height or mass (all $p \geq 0.05$).

Knee extensor peak torque

Participants were positioned in an isokinetic dynamometer (Biodex 3, Biodex Medical Systems, Shirley, NY, USA) for the performance of maximum isometric quadriceps contractions (Figure 3.1). Straps were firmly secured over the distal third of the thigh, waist and chest, to limit extraneous movement. The lateral epicondyle of the femur was aligned with the dynamometer's axis of rotation. The dynamometer arm was attached to the distal tibia just above the malleoli. The leg and dynamometer arm were weighed by the Biodex for the purposes of gravity correction. With the knee positioned in 60° of flexion, three submaximal quadriceps contractions were performed as a warm up prior to data collection. Thereafter, a set of three (6 second) maximal isometric quadriceps contractions were performed at each measurement time point with participants receiving visual feedback of their performance

and a consistent level of verbal encouragement [320]. A 2 minute rest period was given between each contraction.

Electromyography

Surface EMG signals were collected from the vastus medialis during each maximal isometric contraction. This muscle was chosen as previous studies suggest it may be the most sensitive to swelling induced AMI [71]. Prior to the placement of electrodes, the skin was shaved, abraded (Green Prep., West Wawrick, USA) and cleaned with alcohol to reduce signal impedance. A custom designed double differentiated active electrode (Delsys Inc., Boston, USA) was placed over vastus medialis, superomedial to the patella. This electrode was made up of three 1 mm diameter rectangular Ag-AgCl bars arranged in parallel, with an interelectrode distance of 10 mm. The electrode was positioned perpendicular to a line running from the anterior superior iliac spine to the medial joint line of the knee, in accordance with the established surface electromyography for the non-invasive assessment of muscles guidelines [365]. A ground electrode (Red Dot, 3M, St Paul, USA) was positioned over the medial malleolus. All EMG signals were amplified (X1000), filtered (20-450 Hz) (Bagnoli 2, Delsys Inc., Boston, USA), and sampled at 10000 Hz. For each participant, the measurement of knee extensor torque, EMG amplitude and MFCV occurred at four intervals: baseline, preinfusion (10 minutes after baseline), postinfusion and postintervention (cryotherapy).



Figure 3.1. Participant positioning for quadriceps maximum voluntary isometric contractions.

Joint infusion

All participants received an experimental knee joint infusion (Figure 3.2). With the knee resting in slight flexion, a 23 gauge cannula was inserted into the superomedial aspect of the joint. All injections were performed without local anaesthesia, under sterile conditions. A pressure transducer (Medex Inc., Ohio, USA) and syringe were attached in parallel with the cannula via a three-way tap and pressure resistant tubing. Dextrose saline (4% dextrose and 0.19% NaCl) was injected into the joint space in increments of 15 ml or less. Intraarticular pressure was monitored for each participant and infusion stopped when intraarticular pressure reached 50 mmHg.



Figure 3.2. Experimental knee joint infusion to a standardised intraarticular pressure of 50 mmHg.

Cryotherapy

Participants were assigned by random number generation to either a cryotherapy (n=8) or control (n=8) group. Following postinfusion measurements, the cryotherapy group had three plastic bags of partially crushed ice cubes wrapped around their knee joint for a 20 minute period while remaining seated in the dynamometer. The bags were placed at least 2cm below the superior border of the patella. This was done to avoid cooling of the quadriceps muscle and prevent alterations in the EMG parameters due to cooling at the site of the electrode [388]. An infrared thermometer (Fluke, Eindhoven, Netherlands) was used to monitor surface temperature at

the joint line and the vastus medialis electrode site. The control group did not receive an intervention but remained seated in the dynamometer for an identical 20 minute period before performing the postintervention measurements.

Data analysis

Knee extensor peak torque, RMS of the EMG signals and MFCV were calculated by averaging data from the three trials at each measurement time. Peak torque was normalised to a percentage of body mass (kg) for each participant. RMS was calculated from a one second period of EMG activity corresponding to the time of maximum activation for each contraction. RMS at each measurement interval was normalised to a percentage of the baseline RMS calculated for each participant. MFCV estimates were obtained based on the time delay in the propagation of EMG signals between the 2 electrode pairs with a known interelectrode distance (10 mm) using the formula:

$$\text{Velocity} = \text{distance} / \text{time}$$

The time delay was estimated from the peak of a cross-correlation function (for further detail, refer to [364]). For each participant, MFCV data was discarded if cross-correlation coefficients were < 0.8.

Statistical analysis

Descriptive statistics were calculated and Shapiro-Wilk tests undertaken to check the normality of the respective distributions. Independent t-tests were used to analyse differences in baseline characteristics between the cryotherapy and control groups. As all participants underwent identical experimental procedures preintervention, a one way repeated measures analysis of variance was undertaken for each dependent variable up to and including the postinfusion measurement (baseline, preinfusion, postinfusion). Planned contrasts were used to assess whether the preinfusion and postinfusion measures differed from baseline. For each dependent variable, an analysis of covariance (ANCOVA) was utilised to analyse any between group differences in the change scores from the postinfusion to postintervention measurement intervals. The postinfusion (preintervention) values for each dependent variable were used as a covariate. Differences in surface temperature at the vastus medialis electrode site and medial joint line before and after the intervention period were analysed using paired t-tests. The alpha level for all statistical procedures was set to 0.05.

RESULTS

Fifteen participants completed the study. One participant from the control group had a vasovagal reaction during the joint infusion procedure that forced an abandonment of data collection. All other participants reported minimal discomfort during the experimental procedures. Feelings of “tightness” or “pressure” were commonly used to describe the sensation

within the knee joint and many expressed a general “lack of control” over their knee. All participants reported a full, pain-free range of motion within 48 hours of the experiment, with no visible swelling. There were no significant differences between groups for any of the participant characteristics provided in Table 3.1 (all $p > 0.05$).

Intraarticular pressure

Upon insertion of the catheter into the knee joint, intraarticular pressure was typically negative or slightly above atmospheric pressure. Intraarticular pressure increased with increasing volumes of infusion for all participants. The volume required to reach a standardised intraarticular pressure of 50 mmHg varied considerably between participants (median 75 ml; range 17 ml-110 ml).

Knee extensor peak torque

A summary of normalised peak torque values at each measurement interval is presented in Table 3.2. For the preintervention measures, there was a significant overall effect of time ($p < 0.001$). No significant difference in peak torque was seen between baseline and preinfusion measures ($p > 0.05$). In contrast, peak torque decreased significantly after experimental joint infusion ($p < 0.001$) (Figure 3.2). For the postintervention measures, cryotherapy led to

a significant increase in peak torque compared to the control group ($p < 0.05$) (Figure 3.3).

EMG amplitude

A summary of normalised RMS values at each measurement interval is presented in Table 3.2. There was a significant overall effect of time ($p < 0.01$). There was no significant change in EMG amplitude between baseline and preinfusion measures ($p > 0.05$). EMG amplitude decreased significantly following knee joint infusion ($p < 0.001$) (Figure 3.2). Despite EMG amplitude increasing after cryotherapy, this did not reach statistical significance when compared to the control group ($p > 0.05$) (Figure 3.3).

Muscle fibre conduction velocity

Acceptable MFCV data (cross-correlation coefficients > 0.8) were obtained from 10 participants (6 experimental, 4 control) and used in the subsequent analysis. As such, MFCV measures may be described as exploratory. A summary of MFCV values at each measurement interval is presented in Table 3.2. There was a significant overall effect of time ($p < 0.01$). No significant change in MFCV was observed between baseline and preinfusion measures ($p > 0.05$). MFCV decreased significantly after joint infusion ($p = 0.001$) (Figure 3.2). Cryotherapy led to a significant increase in MFCV compared to the control group ($p < 0.05$) (Figure 3.3).

Surface temperature

Surface temperatures at the joint line and vastus medialis electrode site are displayed in Table 3.3. These show that recording conditions at the electrode remained stable during the cryotherapy intervention ($p > 0.05$). In contrast, mean surface temperature over the joint line decreased from 29.7°C to 14.4°C following cryotherapy ($p < 0.001$).

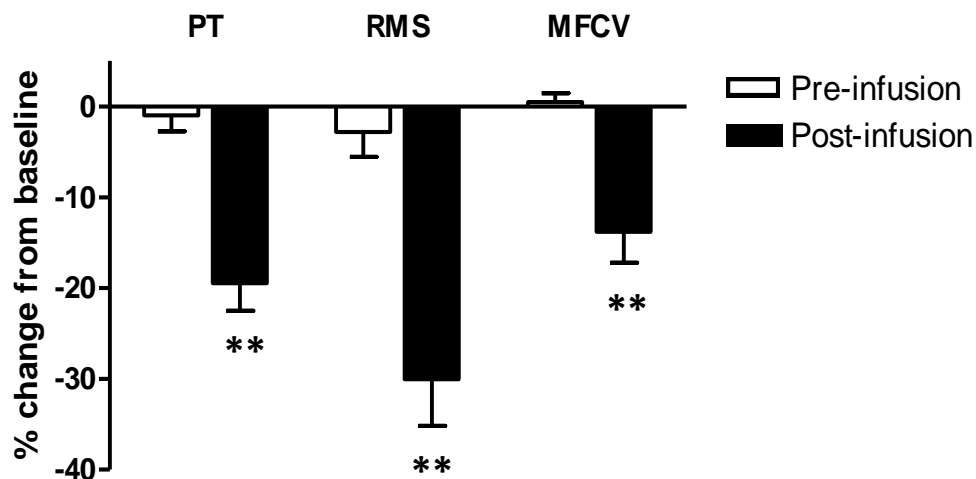


Figure 3.2. Percentage change in the dependent variables from baseline measures. PT = normalised knee extensor peak torque. RMS = root mean square of vastus medialis EMG signal. MFCV = estimated muscle fibre conduction velocity of vastus medialis. ** = significant difference from baseline ($p \leq 0.001$). Data are means and one standard error of the mean. Note that statistical analysis was not performed on percentage change values.

Table 3.2. Summary of dependent variables at each measurement interval for cryotherapy and control groups.

	Baseline ^a	Preinfusion ^a	Postinfusion ^a	Postintervention ^b	95% CI ^c	
<i>PT (Nm/kg*100)</i>						
Control	297 ± 46	292 ± 44	235 ± 44	251 ± 23	232	270
Cryotherapy	296 ± 61	295 ± 65	239 ± 50	277 ± 23	259	295
<i>MFCV (ms⁻¹)</i>						
Control	5.26 ± 1.14	5.27 ± 1.01	4.73 ± 0.57	4.52 ± 0.32	4.24	4.81
Cryotherapy	5.57 ± 0.79	5.64 ± 0.90	4.58 ± 0.74	4.97 ± 0.28	4.72	5.19
<i>RMS (% of baseline)</i>						
Control	100 ± 0	100.67 ± 11.80	73.82 ± 26.36	76.48 ± 16.26	63.09	89.88
Cryotherapy	100 ± 0	94.12 ± 8.97	66.58 ± 12.87	90.09 ± 16.24	77.58	102.60

a = mean ± SD. b = adjusted marginal mean ± SD. c = 95% confidence intervals for postintervention measures. PT = normalised knee extensor peak torque. MFCV = estimated muscle fibre conduction velocity of vastus medialis. RMS = normalised root mean square of vastus medialis EMG.

Table 3.3. Surface temperatures at the joint line and vastus medialis electrode site before and after the 20 minute intervention period.

Group	Electrode temperature (°C)		Joint line temperature (°C)	
	Pre	Post	Pre	Post
Control	31.49 ± 0.99	31.14 ± 0.81	30.34 ± 0.87	30.09 ± 0.97
Cryotherapy	31.25 ± 1.32	31.05 ± 1.25	29.68 ± 1.10	14.4 ± 1.12***

Data are means ± SD. *** = significant difference from preintervention ($p < 0.001$).

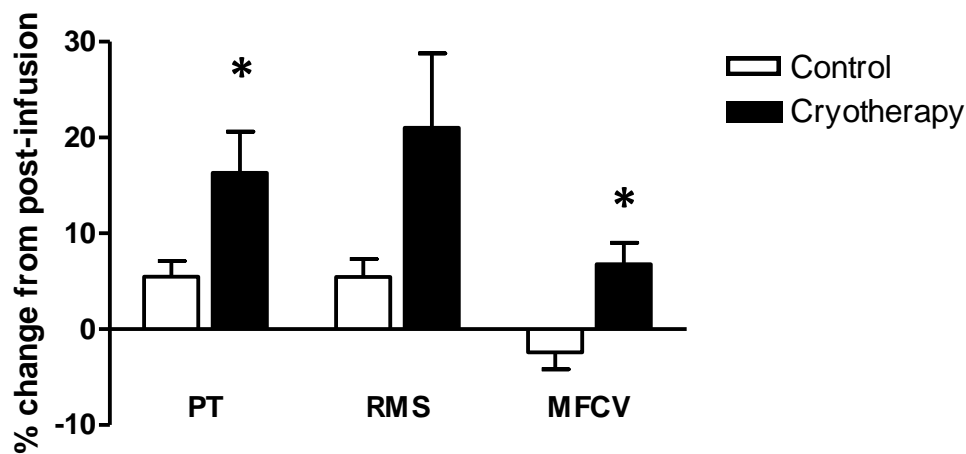


Figure 3.3. Percentage change in the dependent variables from postinfusion measures. PT = normalised knee extensor peak torque. RMS = root mean square of vastus medialis EMG signal. MFCV = estimated muscle fibre conduction velocity of vastus medialis. * = significant difference between groups ($p < 0.05$). Data are means and one standard error of the mean.

DISCUSSION

The results of this study confirm that icing the knee joint reduces the severity of experimentally induced quadriceps AML. These muscles are notably affected by knee joint pathology, with quadriceps strength an important predictor of functional ability in these patients [8, 27, 389, 390]. Normal activation of the quadriceps is essential for shock absorption, the regulation of stiffness at the knee and in maintaining dynamic stability of the joint [44, 51, 54, 113, 391]. The improvements in quadriceps activation observed after cryotherapy are notable in that strength measures returned to within ~6% of baseline values. This represents an effect size of 0.48, a moderate change based upon Cohen's classification of effect sizes [392]. These findings have important ramifications for the treatment of patients with arthritic joint disease and following knee injury and surgery. By temporarily reducing AML, cryotherapy may provide a therapeutic window during which more complete activation of the quadriceps musculature is permitted. Thus, if quadriceps strengthening is performed immediately after icing, earlier and more effective rehabilitation may be allowed, enhancing strength gains and minimising quadriceps muscle atrophy in patients with knee joint pathology.

The joint infusion technique used in the current study provides a controlled, experimental model of AML. The advantage of this model is that it permits

baseline measures of quadriceps activation, in the absence of inhibition. Had the intervention been applied to patients with knee damage and the dependent variables increased, cryotherapy could be said to have a positive therapeutic effect. However, without prior knowledge of the baseline level of inhibition, the degree to which cryotherapy reduces quadriceps activation deficits would be difficult to determine accurately. That is, cryotherapy may have eliminated a small portion of AMI or eradicated it completely. For this reason, the joint infusion model arguably allows a better appreciation of the magnitude and therefore clinical importance of cryotherapy's disinhibitory effect.

A limitation of the joint infusion model is that it does not precisely mimic the changes in articular afferent discharge that may occur when other factors associated with joint pathology (e.g. inflammation, pain and structural damage) are present. However, there are a number of reasons to suppose that cryotherapy may also be effective in reducing AMI in a patient population. These are outlined below.

Firstly, quadriceps inhibition of a similar magnitude occurs following the infusion of fluid into chronic arthritic knee joints [68, 98], suggesting that swelling is still an important cause of AMI when factors such as inflammation, pain and/or

structural damage are present. Secondly, it is widely accepted that swelling's inhibitory effect is caused by an increase in the discharge of articular mechanoreceptors innervated by large diameter, group II joint afferents [66, 107, 121, 125]. Similarly, there is strong evidence from animal studies that (as with swelling) inflammation is associated with an increase in joint afferent discharge due to the peripheral sensitisation of articular free nerve endings innervated by smaller diameter group III and IV afferents [115, 126]. An increase in joint afferent discharge may also occur due to structural damage (e.g. to ligaments) that increases joint laxity and subsequently the discharge of articular receptors involved in signalling the limits of joint motion [62, 155]. An increase in joint afferent discharge is thought to lead to AMI by enhancing the excitability of spinal interneurons [66, 70, 161] that in turn have inhibitory projections to the quadriceps α -motoneuron pool, preventing full activation of the muscle by descending pathways. Diminishing joint afferent discharge by aspirating or injecting a local anaesthetic into the knee joint may dramatically reduce AMI [16, 82], in some cases abolishing it almost entirely [71, 99, 125].

Cryotherapy may work via a similar mechanism, partially silencing the increased afferent traffic caused by swelling, inflammation and joint laxity. In this regard, icing the knee joint lowers intraarticular temperature [235, 237, 240] and a reduction in temperature has been shown to notably reduce the discharge rate

of articular sensory receptors [233]. Additionally, many of the nerve branches supplying the knee run superficially at the level of the joint [94]. Cooling peripheral nerves is known to reduce nerve conduction velocity in a near linear manner [243, 248], impeding the propagation of high frequency impulses [247, 248]. Thus, it may be that icing the knee joint reduces the discharge of sensory receptors and/or impairs articular nerve conduction, attenuating transmission of the aberrant joint afferent impulses responsible for AML. Cryotherapy may be as effective when factors such as inflammation, pain and joint laxity are present as the effects of moderate cooling are virtually identical on large and small diameter sensory fibres [243, 248] and it is well established that icing modulates the transmission of nociceptive impulses at the spinal level [254, 257, 393], seemingly via segmental and supraspinal mechanisms.

In addition, cryotherapy may have an excitatory effect on the quadriceps α -motoneuron pool, competing with AML and reducing the overall level of inhibition. In this regard, Hopkins et al. [205] found that not only did cryotherapy reverse the decline in quadriceps H-reflex amplitude caused by knee joint infusion, it facilitated the H-reflex amplitude more than 20% beyond preinfusion measures. While factors independent of α -motoneuron excitability may lead to an increase in H-reflex amplitude, prolonged icing of uninjured ankle joints has been shown to concurrently increase the amplitude of the soleus H-reflex: M-

wave ratio and plantar flexor peak torque (by ~15%) during maximum effort voluntary contractions [130]. Taken together, these findings suggest that cryotherapy may have an additional excitatory effect on the quadriceps α -motoneuron pool that competes with ongoing reflex inhibition, thus facilitating motor output.

An important aspect of this study is that it is the first to examine changes in MFCV using an acute model of joint injury. A key finding was that AMI may be reflected by a reduction in mean MFCV during maximal effort muscle contractions. MFCV is strongly correlated with motor unit size [348] and muscle fibre diameter [394] as type I muscle fibres making up smaller motor units typically display lower conduction velocities than group II fibres found within large, high threshold motor units. Thus, the ~15% drop in MFCV we observed after joint infusion provides novel information concerning the type of motor units affected by AMI, suggesting that swelling is likely to inhibit the quadriceps largely by limiting the firing of high threshold motor units with high MFCVs. A reduction in the firing rate of active motor units may also contribute to the observed effect [344]. Conversely, mean MFCV increased significantly after cryotherapy, indicating that icing enhances quadriceps force output by disinhibiting some of the high threshold motor units affected by AMI and/or enhancing motor unit firing frequency.

In this study we chose to standardise swelling to intraarticular pressure rather than infusing a set volume of fluid into the knee joint. Researchers [70, 98] have suggested that intraarticular volume provides a relatively poor estimate of capsular tension as both joint afferent discharge [121] and quadriceps inhibition [98] have a stronger correlation with pressure than volume. The relationship between intraarticular pressure and volume depends on factors that are likely to differ across participants such as joint size and capsular elastance [134]. This is clearly demonstrated in our study, as the volumes needed to reach a standardised intraarticular pressure of 50 mmHg ranged from 17 ml to 110 ml. Comparable findings have been reported in both normal [136] and arthritic [98] knee joints.

CONCLUSION

In conclusion, the findings of this study demonstrate that icing the knee joint for 20 minutes reduces the severity of quadriceps AML caused by intraarticular swelling. A reduction in AML may allow earlier and more effective quadriceps strengthening to take place in patients with arthritis or following knee injury or surgery. This has the potential to enhance rehabilitation and improve functional outcomes in patients with knee joint pathology.

Chapter Four: The effects of experimental joint swelling and cryotherapy on quadriceps corticomotor excitability, intracortical excitability and intermuscular coherence in the β -band

INTRODUCTION

To date, research investigating the neural mechanisms of AMI has focused almost exclusively on spinal reflex pathways. Potential cortical mechanisms for AMI have not been explored in detail, despite several lines of evidence that joint afferent impulses reach the level of the cortex and are consciously perceived. In this respect, joint afferents are known to have an exceptionally high transmission security to the somatosensory cortex [395]. Using the microstimulation technique, it has been shown that a single impulse in a single joint afferent is sufficient to reach conscious perception [396]. At the knee joint specifically, a number of animal studies have shown that both mechanoreceptive and nociceptive afferents project to many areas of the cortex, via multiple ascending pathways [397-401]. Furthermore, humans who experience experimental joint swelling invariably describe sensations such as pressure, tightness or heaviness in their knee joint [123, 402], while chemical activation of periarticular nociceptors produces strong sensations of pain in and around the knee joint [149, 403, 404]. Finally, in studies performed during knee joint surgery, mechanical and/or electrical stimulation of a number of

different joint structures produces identifiable sensory evoked potentials in the human primary somatosensory cortex [405-407].

Furthermore, there is evidence that a change in afferent output from a range of different sensory receptors can alter the excitability of the primary motor cortex [412-420]. Depending on the parameters used, experimental manipulation of afferent output can produce both inhibitory and facilitatory effects on corticomotor excitability and the excitability of local interneurons within the primary motor cortex. These observations have been made using diverse methods including local anaesthesia [408], muscle vibration [409], cutaneous application of capsaicin [410] and electrical stimulation of mixed nerves [411-415] and cutaneous receptive fields [271, 416].

Thus, it is apparent from the studies above that sensory output from the knee joint reaches the level of the cortex, is consciously perceived and has the potential to influence motor cortex excitability. Despite this, the effects of an acute change in articular sensory output (as with experimental swelling) on cortical excitability have not yet been explored. Such a study may have important implications in the understanding of AMI's underlying mechanisms. In this regard, it is possible that knee joint swelling alters the excitability of neurons within the primary motor cortex that in turn, decreases corticospinal

drive to the quadriceps and prevents the muscle from being fully activated, thus contributing to AMI (Figure 2.1).

In Chapter 3, we showed that 20 minutes of cryotherapy temporarily reverses AMI caused by experimental joint swelling. In order to determine which patients are most likely to benefit from cryotherapy, it is important to understand its mechanisms of action. There is evidence that icing an uninjured joint enhances maximal force production of the surrounding musculature [130] but it is unknown whether part of this effect may occur due to altered cortical excitability. Brain imaging studies have shown that application of noxious cold stimuli activates a large number of supraspinal structures including the insular cortex, anterior cingulate cortex, cerebellum, premotor cortex, primary and secondary sensory cortices and primary motor cortex [272, 417]. It is therefore possible that cryotherapy's excitatory effects may be partly explained by an increase in motor cortex excitability that enhances the corticospinal drive to the quadriceps α -motoneuron pool. This has not yet been investigated.

With these points in mind, the aims of the current study were to examine the effects of both knee joint swelling and knee joint icing on quadriceps corticomotor excitability, intracortical excitability, and intermuscular coherence in the β -band. Our main hypotheses were that 1) quadriceps

corticomotor excitability would be suppressed by experimental joint swelling and that 2) 20 minutes of knee joint icing would enhance quadriceps corticomotor excitability.

BACKGROUND AND METHODOLOGICAL CONSIDERATIONS

Before moving onto the methods section of this chapter, an overview is warranted concerning the background and key methodological considerations to be taken into account when measuring intermuscular coherence in the β -band and when using TMS to probe corticomotor and intracortical excitability.

Transcranial magnetic stimulation (TMS)

Barker and colleagues [418] were the first to use TMS to non-invasively probe the excitability of the human motor cortex. The method of TMS is based on Ampere's law, whereby passing an electric current through a wire coil produces a magnetic field that occurs at right angles to the direction of the electric current [419]. Unlike electrical stimulation, this magnetic field is able to painlessly pass through the skin and skull before inducing an electrical current in the underlying brain tissue that flows in the opposite direction to the current in the wire coil (Faraday's Law) [419]. When the coil is placed over the primary motor cortex, TMS activates corticospinal tract (CST) cells that project to α -motoneurons in the ventral horn and briefly cause the target muscle to

contract. As Barker et al. [418], describe in their seminal paper, TMS allows “movements of the opposite hand or leg [to be] easily obtained without causing stress or pain” (pg. 1107).

Single pulse TMS

The muscle twitch that arises from TMS can be measured via standard surface or intramuscular EMG techniques and is called a motor evoked potential (MEP). When measured using surface EMG, the MEP is a compound potential reflecting the corticospinal activation of multiple motor units in the target muscle by a single pulse of TMS. Evidence that TMS evokes descending volleys in the human CST has been provided using electrodes fixed in the epidural space following the implantation of a spinal cord stimulator [420-422]. These studies have shown that a single pulse of TMS applied to the human motor cortex produces a complex response made up of multiple descending volleys within the CST. In respect to time, the earliest volley to occur is termed a direct wave (D-wave) and results from direct depolarisation of the CST neurons in the motor cortex, while later waves occur at sequential intervals of 1.5 ms. These volleys are termed indirect waves (I-waves) due to their transynaptic nature, being elicited through the depolarisation of cortical interneurons which then synapse with and depolarise CST cells [419, 423-425]. More specifically, the first I-wave (I1) is thought to be generated by disynaptic activation of CST neurons while later I-waves (I2-I4) are thought to involve polysynaptic interneuronal circuits within

the primary motor cortex [426, 427]. Using standard TMS parameters, the threshold for I-wave generation is lower, with D-waves only occurring at stronger stimulation intensities [420, 426]. Thus, even at low stimulation intensities the descending volley generated by TMS is influenced not only by the excitability of CST cells (D-waves and I-waves) but also by the excitability of local interneurons that transynaptically activate CST cells (I-waves).

Some of the descending volleys evoked by TMS are transmitted monosynaptically to the target α -motoneuron pool, while the rest are transmitted polysynaptically via interneurons located in the intermediate zone of the spinal cord [428]. The proportion of CST fibres that have monosynaptic connections with the target α -motoneuron pool varies from muscle to muscle [429, 430]. In distal muscles of the upper limb, much of the corticospinal input to α -motoneurons is monosynaptic [429]. However, in lower limb muscles such as quadriceps, the monosynaptic input from CST fibres to motoneurons is much weaker [430]. In the quadriceps muscles, a significant portion of the corticospinal input to α -motoneurons is transmitted via lumbar group II interneurons [431], which are thought to form part of the lumbar propriospinal network [432, 433]. Thus, it is important to recognise that the excitability of both spinal interneurons and the α -motoneuron pool can influence quadriceps motor unit recruitment and firing patterns in response to the corticospinal volley.

Methodological factors that influence MEPs

A single muscle receives CST input from many different areas of the motor cortex [434]. This makes it impossible for non-invasive techniques such as TMS to focally and simultaneously recruit all of the corticospinal tract fibres from the primary motor cortex to a given muscle. However, at a gross level, corticospinal output appears to be organised in a reasonably somatotopic manner [435]. For instance, when using TMS, the upper limb and face muscles are most effectively activated by stimuli applied to the lateral aspect of the scalp, further away from the midline [434, 436, 437]. Conversely, the trunk and lower limb musculature is most easily activated by stimuli close to the midline [434, 436, 437]. When using TMS to probe corticomotor excitability, it is typical to search for the so called “hotspot” of the target muscle. This is done by methodically moving the coil on the scalp overlying the primary motor cortex until a position is found that consistently produces the largest MEP in response to suprathreshold stimulation [438]. The position of the coil is then marked and all subsequent stimulation takes place with the coil carefully held over this point on the scalp.

It is not only the position of the coil on the scalp that is important in generating a MEP. The orientation of the coil (relative to the motor cortex) can alter the recruitment of the underlying cortical neurons and thus influence the MEP. Most commonly, the coil is held so that the current propagates in a posterior to anterior direction, which preferentially activates

I-waves at lower stimulation intensities, with the I1 wave having the lowest threshold [425, 426]. In contrast, if an anterior to posterior direction is adopted, the I3 wave is preferentially activated [439]. Finally, D-waves can be generated at low stimulation intensities when the coil is held so that the current flow occurs in a lateral to medial direction [425, 426].

The stimulation intensity used for generating a MEP is usually expressed as a percentage of motor threshold. Resting motor threshold (RMT) is a measure of neuronal membrane excitability within the corticomotor pathway [440] and has been defined as the lowest stimulus intensity that evokes a MEP in 50% of consecutive trials, when the muscle is completely relaxed [435, 440]. With increasing stimulus intensity, the size of the MEP typically increases in the shape of a sigmoid, growing slowly around threshold, before rising steeply and then reaching a plateau at the highest stimulus intensities [441-443]. This plateau is thought to occur due to a parallel increase in the recruitment of inhibitory neurons within the nervous system (e.g. recurrent inhibition of large α -motoneurons) [442]. In turn, this inhibition may counteract the excitation produced by greater CST output with higher intensity stimulation, leading to a plateau in the size of the MEP [442].

When choosing a measure of corticomotor excitability, it is common to select a single “test” stimulus intensity. Provided the coil is held in the same position

and orientation and the stimulus intensity remains fixed, any alteration in the size of the resultant MEP is thought to reflect a change in the excitability of the underlying neural circuitry. Often, a test stimulus intensity of 120% or 130% of RMT is chosen, as this corresponds to the steeply rising portion of the input-output curve [442], where MEP variability is lowest [443]. The size of the MEP can be calculated using the peak to peak amplitude of the compound evoked potential, or by rectifying the EMG signal and calculating the area of the MEP response [444, 445]. For lower limb muscles such as quadriceps, the MEP is often polyphasic [445, 446]. Thus, analysing MEP area may allow a more accurate and robust appreciation of changes in corticomotor excitability.

Paired pulse TMS

While the size of the single pulse MEP reflects the excitability of the entire corticomotor pathway, paired pulse TMS is used to probe the excitability of local interneurons located within the primary motor cortex. In this paradigm, the normal suprathreshold TMS stimulus (test stimulus) is preceded by a (conditioning) stimulus in order to modify the size of the resultant MEP [426, 447]. Depending on the intensity and timing of the conditioning stimulus (relative to the test stimulus), the conditioned MEP is either increased or decreased in size when compared to a single pulse test MEP alone. This change in MEP amplitude with paired stimuli reflects the activation of different populations of local, intracortical interneurons by the conditioning

stimulus, which in turn, alters the recruitment of CST neurons to the later test stimulus [426].

Short interval intracortical inhibition (SICI)

Kujirai et al. [447] were the first to demonstrate inhibition of the test MEP by applying a subthreshold conditioning stimulus that preceded the test stimulus by 1-5 ms. Since the conditioning stimulus was itself below the threshold for evoking a MEP and did not alter H-reflex amplitude, Kujirai and colleagues argued that this inhibition likely occurred at a cortical level. This has since been confirmed by direct spinal cord recordings. Di Lazzaro and colleagues [448] showed that a conditioning stimulus of the same intensity used by Kujirai et al. did not evoke any descending spinal volleys, but instead suppressed the descending volleys created by the test stimulus. The later I-waves of the test volley (particularly I3) are greatly affected by the conditioning stimulus, with the I1 wave unchanged [448, 449]. These findings suggest that inhibition of the test MEP does not occur due to a direct change in the excitability of the CST neuron but instead occurs indirectly, via the activation of polysynaptic interneuronal pathways in the motor cortex [425]. This inhibitory response is termed short interval intracortical inhibition or SICI. SICI can be modified by drugs that are agonists of the GABA_A receptor [450]. GABA_A agonists have been found to increase the magnitude of SICI [451-454] and, more specifically, enhance the inhibition of later I-waves [455]. Thus, it

appears that SICI is mediated by low threshold, intracortical GABAergic interneurons that synapse with CST neurons via polysynaptic circuits.

Methodological parameters that influence the measurement of SICI

The strength of both the test and conditioning stimuli strongly influence the magnitude of SICI. Variation of the test stimulus intensity with a fixed conditioning stimulus typically results in a U shaped SICI input: output curve, with SICI magnitude greatest in the mid ranges of test stimulus intensity (~110-130% of RMT) [454, 456-458]. The drop in SICI at lower test stimulus intensities may reflect the fact that fewer late I-waves are recruited by the relatively weak test stimulus [421] and/or the non-linear input: output relationship of the α -motoneuron pool [95, 459]. On the other hand, higher test stimulus intensities are more likely to recruit D-waves [427], which will be unaffected by the conditioning stimulus. Thus, when measuring SICI, it is common to use a mid-range test stimulus intensity. Furthermore, when the size of the test MEP is altered by a change in corticomotor excitability (e.g. during muscle contraction or with a change in sensory input) estimates of SICI may be confounded by the different recruitment of I-waves and D-waves in the respective test MEPs, as well as changes in the population of motoneurons recruited by the descending CST volleys. For this reason, when measuring SICI many researchers have chosen to adjust the test stimulus intensity in order to match the size of the test MEP across time or conditions [460-463].

Varying the conditioning stimulus intensity while keeping the test stimulus fixed also results in a U shaped alteration of SICI [447, 454, 456, 464]. The decrease in SICI with lower conditioning stimulus intensity may reflect a reduction in the recruitment of the GABAergic inhibitory interneurons responsible for SICI. However, the decrease in SICI that is often observed with higher conditioning stimulus intensities is due to the simultaneous recruitment of a separate population of higher threshold interneurons that have a competing facilitatory effect on the CST neuron [426, 454, 457, 458]. These are termed short interval intracortical facilitatory (SICF) interneurons. Thus, at higher conditioning stimulus intensities, paired pulse paradigms with short (1-5 ms) interstimulus intervals (ISIs) may in fact reflect the net excitability of both SICI and SICF interneurons. To investigate this issue more closely, Pereula et al. [457] performed an in depth examination of the interaction between SICI and SICF. In agreement with previous work [458], they found distinct peaks and troughs in the magnitude of SICF depending on the ISI used. Specifically, SICF was found to peak at ISIs of ~1.5 ms, ~2.5 ms and ~4.5 ms. Conversely, troughs in SICF occurred at ISIs of ~2ms and ~3.5 ms. Importantly, the threshold intensity for the activation of SICF has been found to be ~80-90% of RMT and stimuli above this level are associated with a reduction in the magnitude of SICI [454, 457]. Thus, in order to measure SICI in isolation (i.e. uncontaminated by SICF) it appears necessary to use a conditioning pulse intensity that is below 80% of RMT and preferable to use an ISI of ~2ms, where SICF is at its weakest.

Intracortical Facilitation (ICF)

As well as demonstrating SICI for the first time, Kujirai et al. [447] showed that the test MEP could be facilitated if the ISI between the subthreshold conditioning and test stimuli was 10 ms or 15 ms, a phenomenon termed ICF. Despite its name, ICF has not yet conclusively been shown to be an intracortical phenomenon. To date, only one study has been completed utilising cervical epidural recordings of descending CST volleys evoked by the ICF paradigm [422]. Di Lazzaro and colleagues [422] found that although MEP amplitude was facilitated with ISIs of 10 ms and 15 ms, there was no clear evidence that the conditioning stimulus led to an increase in either the number or amplitude of descending volleys evoked by the test stimulus. This raises the possibility that ICF occurs due to changes in excitability at a spinal rather than a cortical level. However, earlier studies have demonstrated that the subthreshold conditioning stimuli typically used to evoke ICF do not produce a descending volley in the spinal cord [448] or alter H-reflex activity in the target muscle [447, 458]. Furthermore, Di Lazzaro et al. [422] showed that the same conditioning stimuli used to produce ICF failed to modify MEP amplitude produced by direct stimulation of the CST at the cervicomedullary junction (i.e. below the level of the motor cortex). Taken together, these findings provide strong, albeit indirect, evidence that the facilitatory effect of ICF occurs at a cortical rather than a spinal level. Di Lazzaro and colleagues [422] suggested that the conditioning stimulus activates intracortical neurons that alter the synchrony, rather than the number or amplitude, of the

descending volleys evoked by the test stimulus. This is likely to enhance motoneuron recruitment (and thus MEP amplitude) while escaping detection by epidural recordings.

The lowest conditioning intensity (threshold) used to elicit ICF is higher than the threshold for SICl [447, 456, 458]. Moreover, ICF can only be reliably elicited when the induced current runs in a posterior to anterior direction [458]. This differs from SICl, which can be elicited using current flowing in different directions [449, 458]. Together, these findings suggest that separate populations of interneurons are involved in mediating SICl and ICF. This is supported by the findings that NMDA receptor antagonists abolish or reverse ICF [450, 465], suggesting ICF occurs due to the activation of glutamatergic interneurons. However, ICF can also be reduced by the administration of a GABA_A receptor agonist [453] and abolished by ethanol [452], which potentiates GABA-mediated currents [466]. This is thought to be due to the persistent effects of SICl which can last for as long as 20 ms after the conditioning stimulus [449, 466]. Given that the threshold for SICl is lower than that for ICF and the ISI used to test ICF is usually 10-15 ms, this gives SICl ample opportunity to influence ICF [426]. Thus, ICF is likely a net facilitation, made up of glutamatergic facilitation that is curtailed by persisting GABAergic inhibition [426, 466].

Methodological parameters that influence the measurement of ICF

ICF can be elicited with ISIs ranging from 6-25 ms [426]. For the quadriceps, optimum facilitation occurs at an ISI of 15 ms [456]. The magnitude of ICF becomes larger with increasing conditioning stimulus intensity [447, 456], presumably due to increased recruitment of the interneurons mediating ICF. In contrast, ICF tends to decrease with increasing test stimulus intensity [467, 468]. This may occur due to the recruitment of CST neurons by the test stimulus which are more distant to the site of stimulation and thus less sensitive to ICF [468] and/or by the generation of D-waves at stronger test stimulus intensities.

Functional relevance of TMS parameters

The magnitude of SICI and ICF may help to determine the voluntary drive to a given muscle such as the quadriceps. Importantly, there is evidence that 1) the corticospinal fibres that are recruited by the "artificial" stimulation of TMS are the same corticospinal fibres recruited during a voluntary muscle contraction [469] and 2) activation of the intracortical interneurons responsible for SICI can modulate the corticospinal drive to a muscle during voluntary contraction [469-471]. In this regard, very low intensity TMS has been used to suppress ongoing EMG activity during gait [471] and isometric contractions [469, 470]. The intensity of this stimulation is well below the threshold for eliciting descending volleys in the spinal cord [448] and for

activating the interneurons involved in SICF [457] or ICF [456]. Furthermore, transcranial electrical stimulation (which preferentially recruits D-waves, thereby bypassing cortical interneurons) at a similar intensity fails to modulate the ongoing EMG in a comparable manner [471]. Together, these findings suggest that the inhibition observed with very low intensity TMS occurs due to the activation of interneurons in the primary motor cortex and does not involve changes in the excitability of local facilitatory interneurons. Rather, very low intensity TMS is thought to depolarise the GABAergic inhibitory interneurons involved in SICl, which in turn reduce the amplitude of the ongoing EMG by inhibiting corticospinal drive to the muscle [434]. Finally, single motor unit recordings have shown that the inhibition in the ongoing EMG occurs only 2-3 ms after the facilitation in EMG produced by suprathereshold TMS [469]. This suggests that it is activity in the same CST cells which drives voluntary activation of the motoneuron pool and the MEP produced by suprathereshold TMS [434, 469].

Summary

While not without its limitations, TMS permits the non-invasive study of corticomotor excitability in a given muscle. Coil position, orientation and stimulation intensity are all important determinants of the cortical neurons activated by TMS and need to be standardised across measurements. For the quadriceps muscle, the MEP represents the compound effects of multiple D and I-waves in the CST, some of which have direct actions on quadriceps a-

motoneurons and many of which are relayed to the α -motoneuron pool via spinal interneurons. This means that following single pulse TMS, the excitability of CST neurons, intracortical interneurons, spinal interneurons and α -motoneurons may all contribute to the size of the resultant MEP.

Paired pulse TMS allows the specific measurement of the excitability of local interneurons within the primary motor cortex. Importantly, these interneurons are involved in the modulation of corticospinal output to a muscle. With an ISI of 2 ms and a conditioning pulse intensity of < 80% of RMT, the excitability of GABAergic interneurons involved in SICI can be measured. These interneurons are inhibitory to CST cells and suppress the generation and size of late I-waves in particular. Conversely, at an ISI of 15 ms and conditioning pulse intensity of 80-90% of RMT, the excitability of a group of facilitatory interneurons can be measured (ICF). ICF involves a population of glutamatergic interneurons that excite CST cells via polysynaptic pathways. However, the stimulation parameters used in measuring ICF are also likely to recruit GABAergic interneurons involved in SICI. Thus, ICF can be thought of as the net measure of glutamatergic facilitation tempered by GABAergic inhibition of CST cells. SICI and ICF are strongly influenced by conditioning stimulus intensity, the size of the test stimulus and the ISI between the conditioning and test stimuli. These factors need to be carefully controlled in experimental settings.

Intermuscular coherence

Background

Another method of estimating the corticospinal output to a given muscle is to obtain a measure of intermuscular coherence. Intermuscular coherence explores the linear association between the frequency components of two EMG signals, usually recorded during isometric co-contraction of a pair of agonists [472-474]. Coherence is a bounded value, varying between 0 and 1, with 1 indicating a perfect linear association between the two signals and 0 indicating no association [475]. During weak to moderate intensity isometric contractions, intermuscular coherence typically occurs in two distinct bands, a lower frequency or α -band (4-12 Hz) and a higher frequency or β -band (15-35 Hz) [473, 474]. Physiologically, intermuscular coherence in both the α and β -bands is thought to reflect the rhythmic firing of common presynaptic inputs to the target motoneurons of the active muscles [473]. There are several lines of evidence to suggest that the β -band originates in the cortex, whereas the α -band is thought to be influenced by subcortical structures [473, 476].

Evidence that the β -band is of cortical origin arises from studies investigating the coherence between electroencephalography (EEG) or magnetoencephalography (MEG) signals over the cortex and simultaneous EMG recordings from a tonically active muscle (corticomuscular coherence). These studies have shown that coupling in the frequency components

between the cortex and muscle in both humans and primates occurs almost exclusively in the β -band [477-480]. Using a source mapping technique, Salenius et al. [480] further demonstrated that the strongest correlation occurred between signals recorded directly over the primary motor cortex and EMG signals. In addition, these authors found a spatial relationship between the muscle activated and the area of the primary motor cortex contributing most to β -band coherence, with activation of a hand muscle most strongly related to MEG activity in the lateral motor cortex and activation of foot muscles most closely related to MEG signals nearer to the midline [480]. More recent research has confirmed that β -band coherence is largely derived from activity in the primary motor cortex rather than other cortical areas [481].

Further evidence that intermuscular coherence in the β -band is cortical in origin comes from studies in people who have suffered a stroke [473, 482] or incomplete spinal cord injury [483, 484], where the amount of β -band coherence is significantly reduced compared to healthy controls. These findings are strengthened by observations in patients with complete spinal cord injuries, for whom intermuscular coherence in the β -band is missing [485, 486]. Furthermore, in a group of patients with incomplete spinal cord injury, Norton and Gorrasini [484] have shown that only those with a moderate ability to voluntarily activate their thigh muscles had appreciable β -band coherence during the early stance phase of gait. Following 3 to 5 months of

functional rehabilitation, β -band coherence increased only in those patients who improved their walking performance, with changes in coherence positively associated with increases in the size of the maximum MEP able to be elicited in these muscles using TMS [484]. Finally, Power et al. [474] used transcranial direct current stimulation (tDCS) to modify motor cortex excitability and examined subsequent changes in the intermuscular coherence between two upper limb muscles. tDCS is thought to alter the membrane excitability of local neurons within the motor cortex, including those involved in SICl and ICF (for reviews see [487, 488]). Anodal tDCS typically enhances motor cortex excitability, while cathodal stimulation depresses it [487]. In their study, Power et al. [474] showed that anodal stimulation enhanced MEP amplitude in the target muscles and increased intermuscular coherence in the β -band (15-35 Hz). No change was seen in α -band coherence. Conversely, when motor cortex excitability was depressed by cathodal stimulation, there was a parallel decrease in MEP amplitude and coherence in the β -band, with no change in the α -band. Across both stimulation conditions, there was a strong correlation ($r = 0.94$) between the change in MEP amplitude and the change in β -band coherence.

Methodological considerations

The calculation of coherence involves the transformation of each EMG signal from time series data into the frequency domain using the mathematical technique of Fast Fourier Transformation [473, 489]. This process involves

dividing the rectified EMG signal into equal, non-overlapping windows containing a defined number of data points, which are then transformed and averaged to estimate the power spectra for each muscle [490]. In choosing the length of each window (i.e. the number of data points), there is an inherent trade-off between the degree of variance in the spectra and the frequency resolution of the subsequent coherence estimate. Specifically, shorter windows reduce the variance in the spectra but come at a cost of lower frequency resolution [490]. In previous studies using the method of intermuscular coherence, frequency resolutions of ~ 1 Hz – 4 Hz have been used and are considered acceptable [473-475, 484-486, 491].

For a pair of muscles that have a close spatial relationship, coherence estimates can be confounded by cross talk between recording electrodes [483, 490, 492]. Such a problem will manifest itself in abnormally high coherence estimates across a large frequency range, with few peaks and troughs in the frequency bands where significant coherence is observed [483, 492]. Hansen et al. [483] have demonstrated that when using bipolar recording electrodes with a 2cm interelectrode distance, a ≥ 10 cm distance between the two sets of recording electrodes ensures that cross talk does not interfere with measures of intermuscular coherence.

Summary

In summary, β -band coherence provides an electrophysiological signature of common cortical drive to a pair of muscles. Taken together, the findings presented in the paragraphs above provide compelling evidence of a coupling between changes in primary motor cortex excitability, the resultant corticospinal output and the degree of intermuscular coherence in the β -band. Coherence can be influenced by factors such as the frequency resolution of the calculated power spectra and cross talk between pairs of recording electrodes. These factors need to be taken into account when performing coherence measures.

METHODS

Participants

Seventeen participants (11 male and 6 female) volunteered to take part in this study. Participants were screened and excluded based on contraindications to TMS including epilepsy, head injury, metal implants, or central nervous system altering medications. In addition, volunteers were excluded from the study if they had a previous history of pathology in both knee joints; a history of lower limb or spinal surgery; or a history of neurological disease. In the event of a previous knee injury, the contralateral (uninjured) knee joint was used in testing. Participants were asked to refrain from ingesting caffeine, alcohol or medication in the 4 hours prior to testing. All

participants provided written informed consent for the experimental procedures. Ethical approval for this study was granted by the Northern X Regional Ethics Committee, Auckland, New Zealand.

Experimental design

A repeated measures design was utilised for this study, with participants acting as their own control. All participants attended two sessions, at least a week apart. In each session, two baseline measures (B1, B2) of the dependent variables were collected before one of two interventions was applied, followed by a single postintervention measure (P1). In session one, the intervention was an experimental knee joint infusion, while in session two it was 20 minutes of knee joint cryotherapy. The order of sessions (joint infusion vs. cryotherapy) was randomly determined for each participant using a computer generated randomisation table.

Participant positioning

Participants were positioned in an isokinetic dynamometer (Biodex 3, Biodex Medical Systems, Shirley, NY, USA) for the duration of the experimental procedures. The lateral epicondyle of the femur was aligned with the dynamometer's axis of rotation and the knee fixed in 60° of flexion. Straps were firmly secured over the distal tibia, waist and chest to limit extraneous movement during the testing procedures.

Electromyography

Bipolar Ag-AgCl disc electrodes (Norotrode 20, Myotronics Inc., Kent, USA) with an interelectrode distance of 2.2 cm were placed on the skin overlying the vastus medialis and vastus lateralis muscle bellies in accordance with surface electromyography for the non-invasive assessment of muscles guidelines [365]. This placement ensured that the vastus medialis and vastus lateralis electrodes were always at least 10 cm away from each other, in order to minimise crosstalk that may confound measures of intermuscular coherence [483]. A ground electrode (Red Dot, 3M, St Paul, USA) was positioned slightly below the midpoint of the bony surface of the tibia. Prior to electrode placement the skin was shaved, abraded and cleaned with alcohol to reduce signal impedance. All EMG signals were amplified (X1000), filtered (10 Hz – 1000 Hz) (AMT-8, Bortec Biomedical, Alberta, Canada) and sampled at 2000 Hz (Micro 1401, Cambridge Electronic Design, Cambridge, UK) before being stored on a computer for further analysis.

Maximum voluntary contractions

Maximum effort quadriceps contractions were performed prior to the first baseline measurement of the dependent variables. This was done to obtain target levels of activation for the measurement of intermuscular coherence. With the knee positioned in 60° of flexion, four submaximal contractions (25%, 50%, 50% and 75% of perceived maximum effort) were performed as a warm

up. Thereafter, a set of three (6 second) maximal isometric quadriceps contractions were performed to assess maximal vastus lateralis EMG amplitude. Participants received visual feedback of their performance and a consistent level of verbal encouragement for each contraction [320]. A 2 minute rest period was given between each contraction. For each contraction, the root mean square (RMS) of the vastus lateralis EMG signal was calculated from a 1 second window corresponding to the highest amplitude EMG signal. The maximum RMS during any of the three contractions was taken as maximum vastus lateralis EMG activation.

Transcranial magnetic stimulation

In order to minimise the effect of strong voluntary contractions on corticomotor excitability [493], a 5 minute rest period was given between the performance of maximum voluntary contractions and the beginning of TMS procedures in each session. Magnetic stimuli were delivered using a BiStim 200² and a double cone coil (Magstim Company, Dyfed, UK). The coil was placed on the scalp over the contralateral primary motor cortex so that the induced current flow was in a posterior-anterior direction. First, the optimum site for stimulation (hot spot) was found by delivering a series of suprathreshold stimuli as the coil was systematically moved over the scalp until the largest MEP was elicited in vastus lateralis. This was typically found ~1-2cm lateral and anterior to the vertex over the contralateral motor cortex. The hot spot was marked on the scalp with a felt pen and all further testing

completed with the coil held directly over this position. RMT was then determined using a staircase method. RMT was defined as the lowest stimulation intensity (% of maximum stimulator output) evoking a clearly identifiable MEP in four out of eight consecutive stimuli in the relaxed vastus lateralis. The test stimulus was then set to 120% of RMT and the conditioning stimuli to 70% and 90% of RMT for SICl and ICF respectively. An interstimulus interval of 2 ms was chosen for SICl and 15 ms for ICF.

Stimuli were delivered at baseline (B1), again after a 10 minute rest period (B2) and after experimental knee joint infusion or knee joint cryotherapy (P1). At each measurement period, a combination of eight single pulse (test stimulus only) and sixteen paired pulse stimuli (8 SICl, 8 ICF) were delivered in a random order, once every 6 seconds. During stimulation, the location of the coil in relation to the head was repeatedly checked to ensure that the site and angle of stimulation remained constant. Following the joint infusion and cryotherapy interventions, eight single pulse stimuli were given first to assess the effect of each intervention on MEP area. If necessary, the test stimulus intensity was then adjusted for the subsequent measures of SICl and ICF to ensure that a test MEP of the same size ($\pm 10\%$) as the baseline test MEP was evoked. This was done to ensure that the level of SICl and ICF postintervention were not influenced by the size of the test MEP [456, 467, 468, 494, 495]. The conditioning stimulus intensity remained unchanged across all measurement periods.

Intermuscular coherence

Intermuscular coherence in the β -band (15-35 Hz) was measured immediately after the TMS parameters were collected, at each measurement interval. Coherence was calculated between vastus medialis and vastus lateralis during a 30 second isometric contraction of the knee extensors at an intensity of 10 ± 2.5 % of maximum vastus lateralis activation [474]. To achieve the target level of muscle activation, real time RMS of the vastus lateralis EMG signal was displayed on a computer screen positioned directly in front of the participants. Participants were presented with two horizontal target lines corresponding to 7.5% and 12.5% of maximum vastus lateralis EMG activation. They were asked to produce a steady, isometric contraction of their quadriceps muscles by extending their knee against the fixed dynamometer arm so that the EMG signal remained within these target lines for a period of 30 seconds.

Joint infusion

With the knee resting in slight flexion, a 23 gauge cannula was inserted into the superomedial or, on two occasions, the superolateral aspect of the joint. All injections were performed without local anaesthesia, under strictly sterile conditions. A pressure transducer (Medex Inc., Ohio, USA) and syringe were attached in parallel with the cannula via a three-way tap and pressure resistant tubing. A dextrose saline solution (4% dextrose and 0.19% NaCl) was

injected into the joint space in increments of 1.5 ml or less. Intraarticular pressure was monitored for each participant and infusion stopped when intraarticular pressure reached 50 mmHg. The sensation evoked by the joint infusion was then measured using a verbal numerical rating scale. This was collected after the withdrawal of the cannula from the joint and just prior to the postinfusion measurements of TMS parameters. Participants were asked to verbally rate the pain they felt in their knee on a scale from 0 (no pain) to 100 (worst pain imaginable).

Cryotherapy

Three plastic bags of partially crushed ice cubes were wrapped around the knee joint for a 20 minute period while the participants remained seated in the dynamometer. The bags were placed at least 2cm below the superior border of the patella to avoid cooling of the quadriceps muscle and prevent alterations in EMG parameters due to cooling at the site of the electrode [388]. An infrared thermometer (Fluke, Eindhoven, Netherlands) was used to monitor surface temperature at the medial joint line and the vastus medialis and vastus lateralis electrode sites.

Data processing and analysis

For each MEP collected, the EMG signal was rectified and the 50 ms preceding the stimulus artefact was visually checked for contamination by

voluntary muscle activity. Responses were removed from further analysis if silence in the EMG signal was not maintained (< 5% of recordings discarded). As MEPs in lower limb muscles often have a polyphasic shape, the area of the averaged, rectified MEP response was calculated from the first deflection of the MEP from baseline to the last return of the MEP to baseline [445]. For each participant, MEP areas were measured over the same time period at each measurement interval using an automated software algorithm. The MEP areas for the B2 and P1 measurement interval were normalised by dividing them by the MEP area for the B1 measurement interval. SICl and ICF were determined by expressing the averaged MEP area of the conditioned response relative to the averaged MEP area of the corresponding test response at each measurement period.

Intermuscular coherence between vastus lateralis and vastus medialis was calculated using automated MATLAB scripts developed by Halliday et al. [496]. The full wave rectified EMG files from vastus medialis and vastus lateralis underwent discrete Fourier transforms. Non-overlapping segments of 1026 data points were used giving a frequency resolution of ~1.95 Hz (2000 Hz/1026 samples). Cross- and auto-spectra were derived and coherence estimates generated. A 95% confidence limit was calculated for the coherence values using equations developed by Halliday et al. [496]. Coherence was only considered significant if it was above the calculated 95% confidence limit [473, 475]. To estimate the level of coherence in the β -

band, the coherence coefficients for each frequency bin that were above the 95% confidence limit and within the 15-35 Hz band were added together, giving a sum of significant β -band coherence at each measurement interval.

Statistical analysis

Data from the joint infusion and cryotherapy sessions were analysed separately. Descriptive statistics were calculated for each of the dependent variables and the normality of the respective distributions was checked using Shapiro-Wilk tests. As ICF and β -band coherence did not conform to a normal distribution, non-parametric tests were utilised to analyse these variables. One sample t-tests were used to assess whether normalised MEP area was significantly different from B1 at the B2 or P1 (joint infusion or cryotherapy) time points. Friedman's test was used to analyse differences in ICF over time for both sessions. As SICl followed a normal distribution, one sample repeated measures ANOVAs were utilised to analyse differences in SICl over time. Differences in the area of the test (non-conditioned) MEP used to calculate SICl and ICF at each measurement interval were assessed using Friedman's test.

Given that changes in the strength of quadriceps contraction may influence the corticospinal drive to the muscles and therefore β -band coherence, the RMS of both vastus medialis and vastus lateralis EMG signals during the 30

second isometric contraction period were checked using repeated measures ANOVAs to make sure that EMG amplitude remained stable across each measurement period. Friedman's test was used to analyse differences in β -band coherence over time for both sessions. In the cryotherapy session only, differences in surface temperature at the medial joint line and vastus lateralis electrode site before and after cryotherapy were analysed using paired t-tests. The α -level for all statistical procedures was set to 0.05.

RESULTS

Across both sessions, the average RMT was 61% of maximum stimulator output and ranged from 49 to 74%. Resting MEPs were unable to be obtained from vastus lateralis in 3 out of 17 participants. This left 14 participants who completed the study (9 males, 5 females). The mean (\pm one SD) age, height and mass of the participants were 32 ± 10 years, 1.78 ± 0.09 metres and 76 ± 14 kg respectively. Four participants had a history of knee joint pathology in the contralateral (untested) limb.

Part A: Experimental knee joint infusion.

Upon insertion of the catheter into the knee joint, intraarticular pressure was typically negative or slightly above atmospheric pressure. Intraarticular pressure increased with increasing volumes of infusion for all participants. The volume required to reach a standardised intraarticular pressure of 50 mmHg

varied considerably between participants (median 68ml; range 15 ml-121ml). Feelings of “tightness” or “pressure” were typically used to describe the sensation within the knee joint after experimental joint infusion. The average pain rating following the joint infusion procedure was 1.2 out of a possible score of 100, with a range of 0 to 5. Ten of fourteen participants judged themselves to be completely pain free, reporting a pain rating of 0 out of 100.

Motor evoked potential area

There was no significant difference in MEP area between the two baseline measurements ($p > 0.05$). Following joint infusion, vastus lateralis MEP area increased significantly compared to B1 ($p = 0.01$) (Figure 4.1).

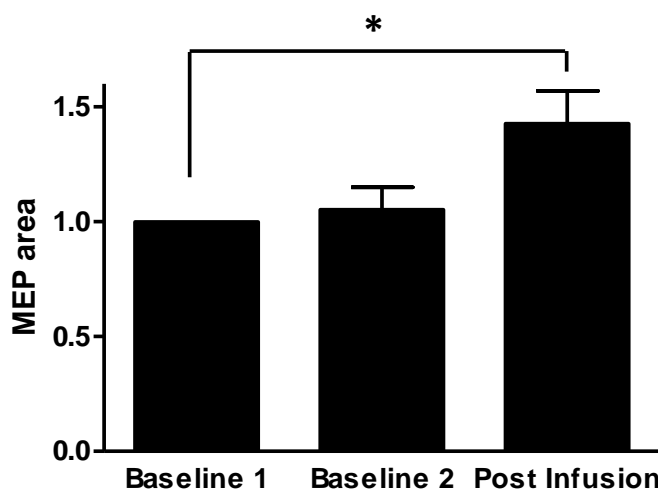


Figure 4.1. Motor evoked potential (MEP) area (normalised to Baseline 1 MEP area) in the vastus lateralis before and after experimental knee joint infusion.

* = significant difference from baseline 1 ($p = 0.01$). Data are means and one standard error of the mean.

Short interval intracortical inhibition

The area of the test MEP used to calculate SICI and ICF did not differ across measurement intervals ($p > 0.05$). Under the SICI paradigm, conditioned responses were typically inhibited by 60 to 70% compared to the test responses. However, there was no significant difference in the magnitude of SICI over time ($p > 0.05$) (Figure 4.2).

Intracortical facilitation

Under the ICF paradigm, there was strong facilitation of the conditioned MEP, which was an average of 3 to 4 times larger than the test MEP. Similar to SICI, there was no significant change in ICF over time ($p > 0.05$) (Figure 4.3).

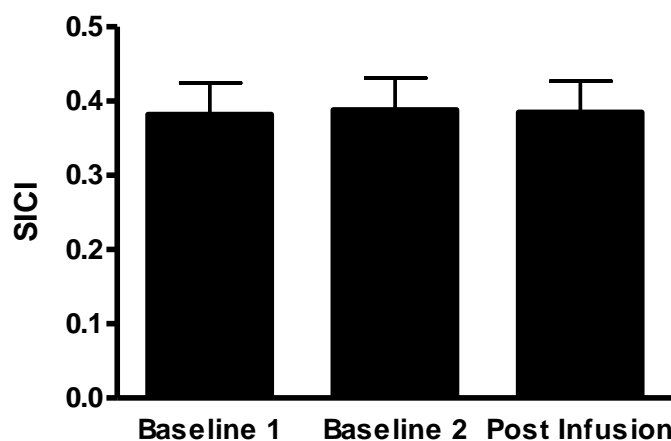


Figure 4.2. Short interval intracortical inhibition (SICI) [conditioned MEP area divided by test MEP area] in the vastus lateralis before and after experimental knee joint infusion. MEP = motor evoked potential. Data are means and one standard error of the mean.

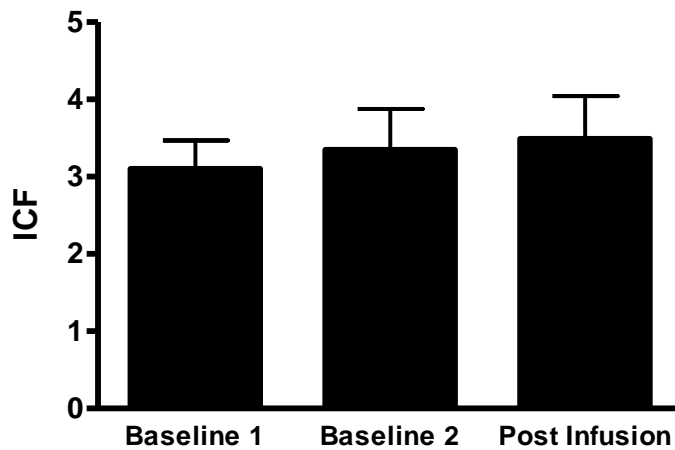


Figure 4.3. Intracortical facilitation (ICF) [conditioned MEP area divided by test MEP area] in the vastus lateralis before and after experimental knee joint infusion. MEP = motor evoked potential. Data are means and one standard error of the mean.

Intermuscular coherence in the β -band

There was no significant difference across measurement intervals in the RMS values of the vastus medialis ($p > 0.05$) or vastus lateralis ($p > 0.05$) EMG signal in the 30 second window used for estimating intermuscular coherence. Figure 4.4 demonstrates intermuscular coherence in a single participant. Significant coherence was commonly observed in the β -band, although not in every participant tested. Coherence estimates at individual frequency bins were typically low (< 0.05) and significant coherence never occurred across a large frequency range, suggesting that cross talk was unlikely to have contaminated the EMG recordings. As with SICI and ICF, there was no

significant change in the β -band coherence between vastus medialis and vastus lateralis across measurement intervals ($p > 0.05$) (Figure 4.5).

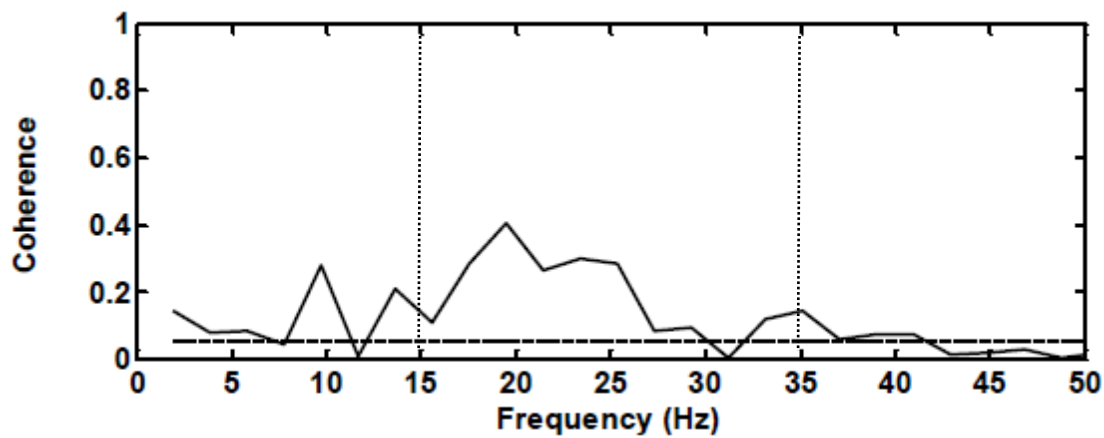


Figure 4.4. Example of intermuscular coherence between vastus medialis and vastus lateralis in a single participant. Hz = Hertz. Horizontal dotted line = 95% confidence interval for the coherence estimate. Note the peak of significant coherence in the β -band (15-35 Hz).

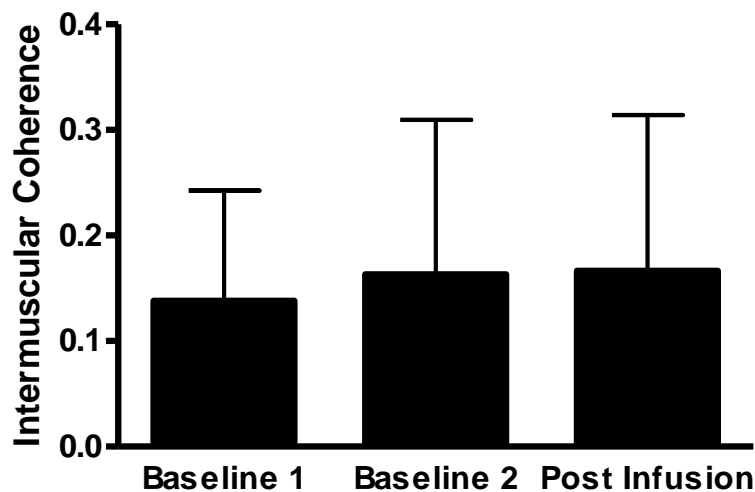


Figure 4.5. Sum of intermuscular coherence in the β -band (15-35 Hz) between vastus medialis and vastus lateralis before and after experimental joint infusion. Data are means and one standard error of the mean.

Part B: Cryotherapy

Surface temperatures at the medial joint line and the vastus medialis and vastus lateralis electrode site are displayed in Table 4.1. These show that recording conditions at the electrode sites remained stable following the cryotherapy intervention (both $p > 0.05$). In contrast, mean surface temperature over the joint line decreased significantly after cryotherapy ($p < 0.001$).

Table 4.1. Surface temperatures at the vastus medialis (VM) electrode site, vastus lateralis (VL) electrode site and medial joint line before and after 20 minutes of knee joint cryotherapy.

VM Electrode (°C)		VL Electrode (°C)		Medial joint line (°C)	
Pre	Post	Pre	Post	Pre	Post
31.13 ± 0.87	30.86 ± 0.84	31.40 ± 0.71	31.47 ± 1.07	30.09 ± 1.12	9.65 ± 4.60*

* = significant change in temperature from pre to post cryotherapy ($p < 0.001$). Data are means ± one standard deviation.

Motor evoked potential area

There was no significant difference in MEP area between the two baseline measurements ($p > 0.05$). Following cryotherapy, vastus lateralis MEP area increased significantly compared to B1 ($p < 0.01$) (Figure 4.6).

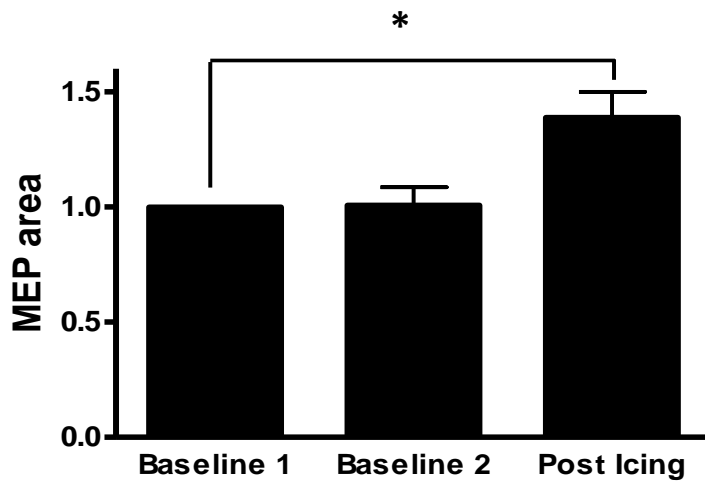


Figure 4.6. Motor evoked potential (MEP) area (normalised to Baseline 1 MEP area) in the vastus lateralis before and after 20 minutes of knee joint cryotherapy. * = significant difference between baseline 1 and post icing measures ($p < 0.01$). Data are means and one standard error of the mean.

Short interval intracortical inhibition

The area of the test MEP used to calculate SICI and ICF did not differ across measurement intervals ($p > 0.05$). The level of SICI observed was very similar to the joint infusion session, with a 60-70% reduction in the area of the conditioned MEP compared to the test MEP alone. There was no significant difference in the magnitude of SICI over time ($p > 0.05$) (Figure 4.7).

Intracortical facilitation

As in the joint infusion session, the conditioned response under the ICF paradigm was approximately 3 to 4 times larger than the test MEP alone. There was no significant change in ICF over time ($p > 0.05$) (Figure 4.8).

Intermuscular coherence in the β -band

There was no significant difference across measurement intervals in the RMS values of the vastus medialis ($p > 0.05$) or vastus lateralis (cryotherapy $p > 0.05$) EMG signals in the 30 second window used for estimating intermuscular coherence. Coherence in the β -band did not differ across measurement intervals ($p > 0.05$) (Figure 4.9).

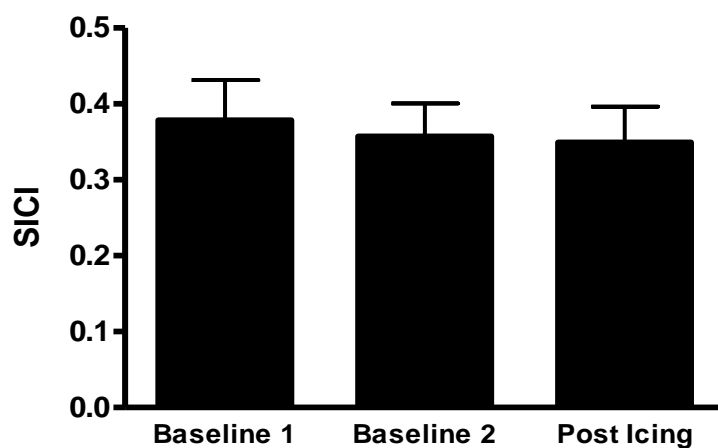


Figure 4.7. Short interval intracortical inhibition (SICI) [conditioned MEP area divided by test MEP area] in the vastus lateralis before and after 20 minutes of knee joint cryotherapy. Data are means and one standard error of the mean.

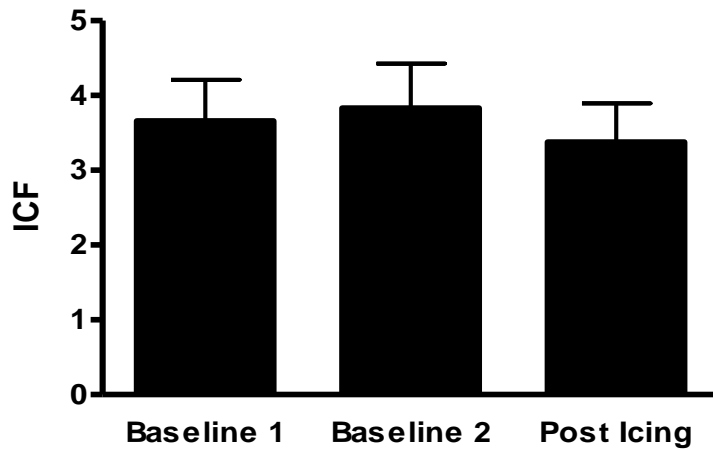


Figure 4.8. Intracortical facilitation (ICF) [conditioned MEP area divided by test MEP area] in the vastus lateralis before and after 20 minutes of knee joint cryotherapy. MEP = motor evoked potential. Data are means and one standard error of the mean.

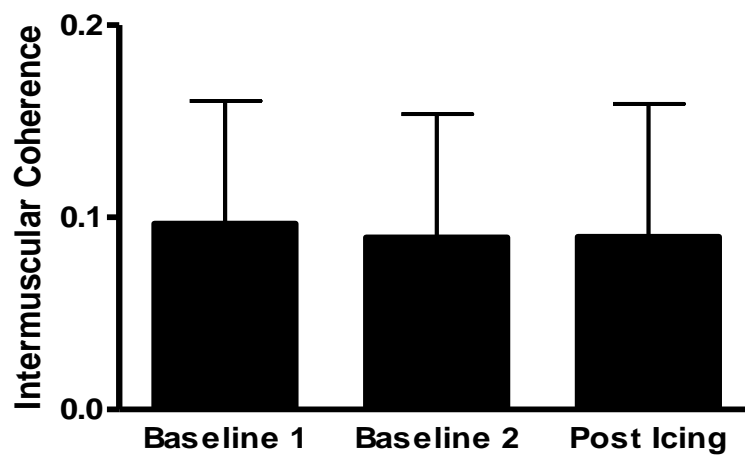


Figure 4.9. Sum of intermuscular coherence in the β -band (15-35 Hz) between vastus medialis and vastus lateralis before and after 20 minutes of knee joint cryotherapy. Data are means and one standard error of the mean.

DISCUSSION

In this study we found no evidence for a change in the excitability of intracortical interneurons or β -band coherence in the quadriceps muscle following experimental joint infusion or knee joint cryotherapy. However, the area of the vastus lateralis MEP in response to single pulse TMS increased from baseline following both knee joint infusion and cryotherapy. The main findings with respect to joint infusion and cryotherapy are discussed separately below.

Experimental knee joint infusion

Swelling in the knee joint has consistently been shown to suppress quadriceps muscle activity, which is at least partly due to a strong inhibitory effect at the spinal cord level [70, 71, 124, 125, 131, 402]. More specifically, experimental knee joint infusion is known to enhance Ib inhibition of the quadriceps α -motoneuron pool both at rest and during voluntary contraction [70], produce a marked suppression of quadriceps H-reflex amplitude [70, 94, 123, 131] and strongly suppress knee extensor torque production [98, 124, 125, 402], EMG amplitude [98, 402] and muscle fibre conduction velocity [402] during maximum effort voluntary contractions. Despite these consistent findings across many different studies, we observed a significant increase in vastus lateralis MEP area after experimental joint infusion, suggesting an increase, rather than a decrease, in quadriceps corticomotor excitability. While

somewhat paradoxical, this finding is supported by similar observations in patients with chronic patellofemoral pain [497] and chronic ACL injury [498], where quadriceps corticomotor excitability was found to be increased compared to healthy control participants or the uninjured limb. It was suggested that these observations may reflect a compensatory increase in cortical drive to the muscle, in order to counteract the ongoing inhibition at a spinal level [116].

In this regard, the current study is the first to examine changes in SICI and ICF to determine if such an increase in quadriceps MEP area could be explained by changes in the excitability of local interneurons within the primary motor cortex. We found no evidence that either SICI or ICF are altered by experimental joint infusion. Furthermore, intermuscular coherence in the β -band was not altered by joint infusion. These findings suggest that knee joint swelling may largely affect neural excitability at a subcortical level.

So if cortical excitability is seemingly unchanged and there is strong evidence from previous studies that α -motoneuron excitability and quadriceps activation is reduced with joint infusion, what could explain the *increase* in MEP area observed in the current study? In proximal lower limb muscles like the quadriceps, a significant portion of the corticospinal input to the motoneuron pool is relayed via lumbar group II interneurons [431], which are

thought to form part of the lumbar propriospinal system [432, 433]. Data from animals has shown that as well as receiving input from group I and II muscle afferents, these interneurons receive excitatory input from knee joint afferents [499]. Thus, while we cannot rule out undetected changes in cortical excitability, we hypothesise that the increase in MEP area seen in this study may be due to changes at a subcortical level, possibly due to a strong joint afferent mediated increase in the excitability of lumbar propriospinal-like neurons. In turn, this would greatly facilitate the portion of the MEP volley that is relayed via this pathway. This large facilitation at the premotoneuronal level may occur in concert with inhibition of the α -motoneuron pool via other pathways, as the effects of joint afferent discharge may have separate effects on two distinct populations of interneurons [188]. Importantly, if a greater *portion* of the TMS induced corticospinal volley is relayed via propriospinal interneurons when compared to the *overall* voluntary motor command (which will reflect the sum of input from multiple descending pathways, relayed via multiple neural systems) this could explain the paradoxical increase in quadriceps MEP area despite no evidence of a change in cortical excitability and the known inhibitory effects of swelling on quadriceps α -motoneuron excitability [71, 94, 123, 205] and quadriceps voluntary muscle activation [68, 82, 98, 124, 125, 132, 133]. Furthermore, at rest, TMS usually recruits less than 10% of the quadriceps motoneuron pool [456]. Thus, quadriceps MEP area may be particularly sensitive to the additional recruitment of a small portion of motoneurons by the enhanced propriospinal transmission of the corticospinal volley, despite ongoing

inhibition of the α -motoneuron pool by alternative pathways such as group I non-reciprocal (Ib) inhibition [70].

Cryotherapy

As there was no significant change in SICI, ICF or β -band coherence after cryotherapy, we found no evidence that icing the knee joint for 20 minutes alters the excitability of local interneurons within the primary motor cortex or enhances cortical drive to the quadriceps. However, following the cryotherapy intervention, we observed a significant increase the area of the vastus lateralis MEP evoked by single pulse TMS. Together, these findings suggest an increase in quadriceps corticomotor excitability that is most likely explained by a direct excitatory effect of cryotherapy at a subcortical level. In this regard, icing the ankle joint for 20 minutes has been associated with an increase in serum norepinephrine levels [262] and norepinephrine and other monoamines such as serotonin are known to have direct neuromodulatory effects on α -motoneurons, amplifying the effects of excitatory synaptic input from supraspinal and peripheral sources [269]. Thus, it is possible that the observed increase in vastus lateralis MEP area is explained by an excitatory effect of cryotherapy at the level of the ventral horn. This is supported by previous findings in uninjured controls that have shown an increase in H-reflex amplitude of the muscles surrounding an iced joint [130], as well as increased torque production [130] and central activation ratio [500] during maximum effort contractions. Furthermore, cryotherapy has been shown to reverse the

decline in quadriceps H-reflex excitability [131], peak torque production [Chapter 3] and muscle fibre conduction velocity [Chapter 3] following experimental knee joint infusion and enhance central activation ratio in patients with osteoarthritis of the knee [81]. Thus, it is clear that icing the knee joint can facilitate quadriceps muscle activation and it appears as if this facilitation is at least partly mediated by a direct excitatory effect on the central nervous system.

This has important clinical implications for the use of cryotherapy to enhance rehabilitation in patients with AMI. For example, if cryotherapy worked only by impairing the conduction of abnormal sensory output from the joint then it could be expected to reduce AMI in a number of knee joint pathologies where swelling and inflammation are present, as these factors are known to substantially increase joint sensory output [121, 161], leading to notable quadriceps inhibition [125, 142]. However, in those patients without detectable swelling or inflammation, where a reduction in articular sensory output (e.g. after ligament rupture) is thought to be the primary cause of AMI [157, 183, 501], icing may be ineffective. The findings from this and other studies that icing has a direct excitatory effect on the central nervous system suggests that cryotherapy is likely to be at least partially effective across a broad range of knee joint pathologies, as quadriceps muscle activation should be enhanced regardless of whether AMI is caused by an increase or a decrease in sensory output from the damaged knee joint.

Limitations

This study is not without its limitations. It should be emphasised that while experimental joint infusion provides a model of joint injury that induces potent quadriceps inhibition, it does not accurately mimic the afferent discharge from an acutely injured knee or from a joint affected by chronic pathology such as arthritis. Experimental swelling greatly increases the discharge of group II joint afferents [121] but in the absence of inflammation, is unlikely to stimulate a large portion of group III and IV afferents. This is demonstrated by the fact that joint infusion rarely evokes sensations of pain, as observed in the current and previous studies [68, 123, 402]. Thus, it remains to be seen whether an increase in nociceptive output from group III and IV knee joint afferents could alter motor cortex excitability and affect the corticospinal output to the quadriceps. This should be explored in future research.

Furthermore, we cannot rule out the possibility that either joint infusion or cryotherapy led to changes in cortical excitability that were undetectable using our experimental paradigm. In the current study, we measured SICl at 2 ms and ICF at 15 ms. There are various other intracortical inhibitory and excitatory influences on CST neurons that could also influence the size of the resultant MEP including SICF, long interval intracortical inhibition and interhemispheric inhibition [426, 454, 457]. Thus, it may be that joint infusion or cryotherapy enhanced the excitability of other cortical interneurons but we were unable to detect this in the current study using the TMS parameters we

employed. One possibility is that swelling increased the excitability of the interneurons mediating SICF. Importantly, ICF and SICF appear to be mediated by separate populations of interneurons [426]. In addition, while SICI and SICF may interact, SICF affects multiple I-waves generated by the test stimulus (including the I1 and I2 waves) whereas SICI's effects are largely confined to the I3 wave [449, 454, 457]. Furthermore, in the current study we deliberately chose an ISI of 2ms (corresponding to a trough in the strength of SICF) and a relatively low conditioning intensity of 70% RMT in order to measure SICI as selectively as possible (i.e. in the absence or near absence of contaminating effects from SICF) [457]. Thus, an increase in SICF could explain the increase in MEP area in response to single pulse TMS, despite no changes being observed in the paired pulse conditions. However, we consider this a less likely explanation for our findings given the lack of change observed in intermuscular coherence following both joint infusion and cryotherapy.

CONCLUSION

Despite finding no evidence for a change in intracortical excitability, we observed an increase in vastus lateralis MEP area after experimentally inducing swelling in the human knee joint. This is in agreement with studies of people with chronic knee joint pathologies, where a paradoxical increase in quadriceps corticomotor excitability has been reported. Given the wealth of evidence showing that knee joint swelling induces inhibition of the

quadriceps muscle during voluntary contraction and at the motoneuron level, it appears that our observations may either be explained by an undetected increase in motor cortex excitability or, perhaps more likely, a joint afferent mediated increase in the excitability of subcortical structures (e.g. lumbar propriospinal-like neurons) that transmit a major portion of the corticospinal volley to the quadriceps α -motoneuron pool. In addition, this study shows for the first time that icing the knee joint for 20 minutes increases quadriceps corticomotor excitability, probably through a direct excitatory effect at a spinal cord level. This has important clinical implications, suggesting that cryotherapy may be at least partially effective in reversing quadriceps AML across a wide range of knee joint pathologies.

Chapter Five: The effects of joint aspiration and intraarticular corticosteroid injection on flexion reflex excitability and knee extensor peak torque in patients with chronic knee joint arthritis

INTRODUCTION

The aspiration of fluid from a swollen knee joint has been shown to reduce quadriceps AML following experimental joint infusion [125, 132], acute knee injury [82], after knee surgery [99], and in patients with chronic knee joint pathology [98, 143]. However, joint swelling is not the only factor responsible for quadriceps AML. For instance, Fahrner et al. [143] showed that the infusion of local anaesthetic into OA knee joints led to further increases in quadriceps activation over and above that produced by aspiration. Similarly, in patients with RA, the combination of aspiration and intraarticular corticosteroid injection led to much larger improvements in quadriceps peak torque and EMG amplitude than aspiration alone [142]. In a recent study in healthy controls, Henriksen et al. [149] showed that experimentally induced knee pain reduces quadriceps peak torque production by up to 15%, with pain intensity positively correlated to the degree of strength reduction. As this model enhances nociceptive output from the knee in the absence of swelling, these findings provide strong evidence that nociceptive discharge alone is sufficient to induce quadriceps AML. Ongoing nociception is relevant to AML in arthritis, where the presence of inflammatory mediators substantially

increases nociceptive discharge due to ongoing peripheral sensitisation of articular free nerve endings, innervated by group III and IV afferents (for a review see [502]).

The underlying neurophysiological mechanisms by which abnormal joint afferent output leads to AML are only partially understood. One of the pathways that may be involved is the flexion reflex. Activation of the flexion reflex typically produces a pattern of flexor muscle facilitation and extensor muscle inhibition [167, 168]. As such, it has been suggested [66, 169] that enhanced flexion reflex excitability may be partially responsible for quadriceps AML. Importantly, the interneurons involved in the flexion reflex receive convergent input from a number of peripheral afferent sources, including knee joint sensory receptors [163, 172]. There is indirect evidence that flexion reflex excitability is enhanced in individuals with knee joint pathology. Compared to pain-free control subjects, flexion reflex threshold was significantly lower in individuals with anterior knee pain [79], knee joint OA [176] and anterior cruciate ligament deficiency [177]. Importantly, all of these studies were cross sectional in nature and, for practical reasons, had small sample sizes of between 6 and 20 participants per group. Even in healthy populations, the flexion reflex threshold is known to vary widely between individuals (4 mA – 40 mA) [178, 503]. Thus, the inclusion of only a small number of participants with flexion reflex thresholds at the extremes of this range may bias such between group comparisons.

An alternative approach is to modify afferent output from the knee and monitor changes in flexion reflex threshold over time. This has been successfully applied in animal models to show that acute inflammation of the knee joint dramatically increases flexion reflex excitability [161, 175, 504], and that intraarticular injection of local anaesthetic can return flexion reflex amplitude to baseline values [161]. For obvious ethical reasons, experimental arthritis cannot be induced in humans. However, therapeutic techniques such as joint aspiration and corticosteroid injection can be used to diminish joint swelling, inflammation and pain [505]. In individuals with RA, knee joint aspiration and corticosteroid injection has previously been shown to produce rapid increases in knee extensor peak torque and EMG amplitude [142], presumably due to a normalisation of joint afferent discharge from the arthritic joint. It remains unknown whether such a change in joint afferent discharge can alter flexion reflex excitability. Furthermore, measuring changes in flexion reflex excitability after aspiration alone may provide important new insights into the potential neural mechanisms of swelling-induced AML.

Thus, the primary aim of this study was to explore the effects of knee joint aspiration and corticosteroid injection on flexion reflex threshold in patients with chronic knee joint arthritis. A secondary aim was to examine changes in knee extensor peak torque after knee joint aspiration and corticosteroid injection. Our hypotheses were that: 1) Aspiration alone would increase

flexion reflex threshold and knee extensor peak torque and 2) corticosteroid injection would lead to further increases in flexion reflex threshold and knee extensor peak torque over and above aspiration alone.

BACKGROUND AND METHODOLOGICAL CONSIDERATIONS

Before moving onto the methods section of this chapter, an overview is warranted concerning the background and key methodological considerations that should be taken into account when using joint aspiration and intraarticular corticosteroid injection as an intervention and in the measurement of flexion reflex excitability.

The flexion reflex

Background

The flexion reflex is a polysynaptic spinal reflex response characterised by a co-ordinated multijoint withdrawal of the limb away from a noxious sensory input. In the lower limb, the flexion reflex is usually elicited via electrical stimulation of nociceptive cutaneous afferents supplying the foot [506]. The flexion reflex elicits a general pattern of flexor facilitation and extensor inhibition [507, 508] with the resultant muscle activation typically measured using standard surface EMG from flexor muscles such as biceps femoris or tibialis anterior.

Hugon [509] established the minimum latency of the biceps femoris flexion reflex as 85 ms and, as such, suggested that it was at least partly mediated by lightly myelinated group III (A- δ) afferents. This has since been confirmed using direct microneurographic recordings from single nerve fibres, where it has been shown that the recruitment of A- δ fibres is necessary in order for the flexion reflex to occur [510]. However, unmyelinated group IV afferents (C fibres) are also known to contribute to the flexion reflex response [511-514]. The maximum latency still considered to be a flexion reflex response in the biceps femoris is typically 150 ms as this is too short a time period after stimulation to be contaminated by the startle response or voluntary muscle contraction [260, 515].

The neurons responsible for mediating the flexion reflex appear to be located at a spinal cord level as studies in humans have demonstrated that flexion reflex responses can be elicited in patients who have suffered both incomplete and complete spinal cord injuries [516-518]. Evidence from studies involving animals suggests that wide dynamic range neurons play a major role in mediating the flexion reflex [170, 171, 519]. These interneurons are predominantly located in lamina V of the dorsal horn and receive convergent input from a number of peripheral afferent sources, including knee joint afferents [163, 172]. Due to this widespread pattern of convergence, spatial and temporal interactions between different sources of

afferent and descending input can strongly influence the flexion reflex response [163, 175, 520, 521].

Methodological considerations

Stimulus location

The flexion reflex is most commonly elicited via electrical stimulation of the sural nerve in its retromalleolar pathway [506, 522]. However, a number of studies have elicited flexion reflex responses following stimulation of the tibial nerve [523, 524], common peroneal nerve [525] or the skin on the plantar aspect of the foot [518, 526-529]. When stimulating the sole of the foot, the site of stimulation has been shown to strongly influence the resultant flexion reflex response. In this regard, both human and animal studies [526, 527, 530, 531] have shown that each muscle involved in the flexion reflex has a separate cutaneous receptive field corresponding to the skin area that will be withdrawn by contraction of that muscle. Furthermore, it has been shown that proximal muscles such as biceps femoris (which evoke large amplitude movements of the limb) have large receptive fields covering the entire plantar surface of the foot [526] while more distal muscles that produce smaller amplitude movements have smaller receptive fields covering only a part of the foot [526, 530].

Stimulus type

Previous studies [532, 533] have reported that use of a single electrical pulse to elicit the flexion reflex frequently results in a gradual decline in the amplitude of the response with repeated stimuli, a process known as habituation. In order to avoid habituation, more recent studies consistently use a train of impulses to elicit the flexion reflex (Figure 5.1). The composition of this train varies between studies but typically involves a series of 5-10 pulses, each with a pulse width of 0.2-1.0 ms and an interpulse interval of 1-5 ms [176, 259, 534-537]. Each train of stimuli is usually delivered with a delay of 4-20 seconds and it is common to randomise this interstimulus interval (e.g. a random interval between 8 and 12 ms) to further minimise habituation [532] and avoid stimulus predictability [178, 506]. Interstimulus intervals of less than 1 second need to be avoided as these typically lead to facilitation of the flexion reflex response via the process of temporal summation [538, 539].

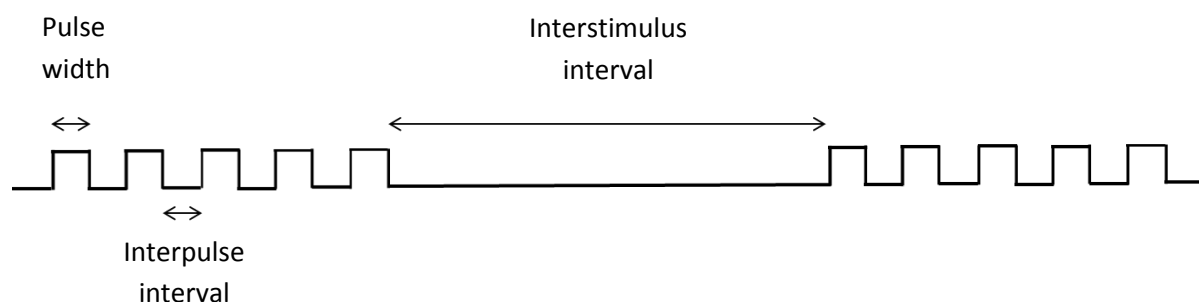


Figure 5.1. Schematic diagram illustrating the waveform characteristics of the electrical stimuli used to elicit the flexion reflex in humans.

Posture during stimulation

Participant posture during stimulation has been shown to influence the size of the resultant flexion reflex response. In this regard, Rossi and Decchi [540] stimulated the plantar surface of the foot and examined the flexion reflex response in tibialis anterior when in a seated position compared to standing positions with various degrees of weight bearing through the stimulated limb. Despite similar levels of background EMG, increasing the degree of weight bearing led to a progressive depression in the size of the flexion reflex response, a pattern interpreted by the authors as a functionally appropriate reflex modulation in order to maintain postural equilibrium [540]. Conversely, standing with the stimulated limb completely unloaded (i.e. 100% weight bearing through the contralateral limb) was found to greatly increase the magnitude of the flexion reflex response to an average 327% of the size of the response during standing with equal weight on both limbs. This was even larger than the flexion reflex response during sitting which was ~280% of the standing equal weight bearing response [540]. This finding has since been confirmed by Andersen et al. [541] who stimulated multiple sites on the plantar aspect of the foot and, in all lower limb muscles tested except iliopsoas, observed larger flexion reflex responses during standing compared to a seated position. Furthermore, plantar electrical stimulation was found to be significantly more comfortable in standing compared to sitting [541], a finding that has recently been confirmed in our laboratory [542].

Flexion reflex threshold

The excitability of the flexion reflex pathway can be quantified by steadily increasing the amplitude of the electrical stimulation to determine the flexion reflex threshold [506]. Flexion reflex threshold is defined as the lowest amplitude (mA) stimulation that consistently (variably described as 50-90% of trials) elicits a flexion reflex response in the target muscle [79, 537, 543, 544]. An alternative approach to determining flexion reflex threshold is to use a staircase method, whereby the stimulus is increased in large increments until a reflex is observed, then decreased in smaller increments until the reflex disappears and then increased and decreased again in progressively smaller increments (usually with a limit of 1 mA) until the reflex reappears and disappears several times [178, 522, 545, 546]. To establish the flexion reflex threshold, the average stimulation amplitude (mA) is calculated from the smallest increments (e.g. 1 mA) used [178, 522]. When measuring the flexion reflex threshold it is important to have reproducible criteria for establishing the definite "presence" or "absence" of a flexion reflex response in the EMG trace. A variety of criteria have been used, making comparisons across studies difficult. Recently, Rhudy and France [522], have provided a standardised approach to determining flexion reflex threshold, comparing the accuracy and reliability of a number of different measurement paradigms. The findings suggested that the flexion reflex interval peak z score may be the most appropriate method of determining the flexion reflex threshold. This is calculated by subtracting the mean prestimulus (resting)

biceps femoris EMG amplitude from the peak of biceps femoris EMG activity in the poststimulus reflex window and dividing the difference by the standard deviation of the prestimulus EMG amplitude. Using this equation, a z score of 10.32 was recommended as the most appropriate cut point when determining the presence or absence of a “true” flexion reflex response [522]. This cut point achieved a specificity of 0.91 and a sensitivity of 1.00 and was found to have good intrasession and intersession reliability when using it to establish the flexion reflex threshold.

Age

Several authors have examined the effects of participant age on flexion reflex threshold, with inconsistent findings. Sandrini et al. [547] recorded flexion reflex thresholds in a sample of 71 healthy subjects with an age range of 7–40 years, demonstrating a linear relationship between flexion reflex threshold and age. In contrast, Mylius, et al. [548] found no significant difference in flexion reflex threshold in a group of 39 young participants (aged 20 to 38) compared to a group of 52 older (aged 65 to 83) participants. Finally, in a large sample of 300 participants with an age range of 20–80 years, Neziri et al. [503] found that while flexion reflex threshold tended to increase with age, the effect was negligible with every 10 year increase in age increasing flexion reflex threshold by only 0.184 mA (~1%).

Gender

A number of studies have reported gender differences in flexion reflex threshold, with women reported to have lower flexion reflex thresholds and greater pain intensity ratings in response to electrical stimulation [549-551]. In contrast, other studies have found no difference in flexion reflex threshold between males and females [178, 552, 553]. In the largest sample tested to date, Neziri et al. [503] found no evidence for a gender effect on flexion reflex threshold in a group of 300 pain free participants.

Menstrual Cycle

Similarly, to date there are inconsistent findings with respect to the effects of hormonal status on flexion reflex threshold. In a sample of 14 women, Tassorelli et al. [554] recorded the flexion reflex threshold during the follicular and luteal phases of the menstrual cycle, as measured by basal body temperature. They observed a significant reduction in flexion reflex threshold during the luteal phase compared with the follicular phase. However, in a sample of 41 women, Rhudy et al. [555] found no difference in flexion reflex threshold between the luteal and follicular phases, as measured by basal body temperature, daily diaries and luteinizing hormone tests.

Time of day

Circadian changes in pain thresholds have been reported [556, 557]. To date, two studies have specifically examined the influence of circadian rhythms on flexion reflex threshold [537, 558]. These studies both showed strong effects for time of day in healthy pain free participants, with flexion reflex threshold consistently lower in the early morning and highest at midnight. Circadian effects on the flexion reflex were maintained in a group of 25 participants with episodic cluster headaches, but not in a group of 6 participants with chronic cluster headaches [537]. It has been postulated that circadian alterations of the flexion reflex can be explained by time related fluctuations in the descending serotonergic and opioidergic inhibition of wide dynamic range interneurons [537]. Regardless of the mechanism, it is apparent that repeated measures of flexion reflex threshold across days need to be performed at the same time of day in order to minimise the influence of circadian rhythms.

Leg dominance

In the only study to date that has assessed the effects of leg dominance on flexion reflex threshold, Neziri et al. [503] found a significant relationship between the limb tested and flexion reflex threshold, with flexion reflex threshold on the dominant leg approximately 6% lower than the non-dominant leg. The reasons for this are not clear. Neziri and colleagues [503]

speculate that this may relate to side to side differences in nerve conduction velocity and/or muscle strength that lowers the threshold for reflex muscle activation.

Physical activity

Guieu et al. [559] found that 20 minutes of cycling at an intensity of 200 W led to a 53% increase in flexion reflex threshold from baseline (before exercise) levels. However, sustained isometric muscle contractions (average 4.5 minutes) at 1%, 10% and 25% of maximum voluntary contraction using a grip strength dynamometer failed to modulate flexion reflex threshold in a group of 24 healthy control subjects. Thus, while it appears physical activity can modify flexion reflex excitability, whether this is confined to aerobic or lower limb exercise and the threshold for this effect remains unknown.

Ingested substances

Ingestion of a number of different substances has been shown to influence flexion reflex threshold. These substances include NSAIDs, opioids, monoamines, benzodiazepines and N-Methyl-D-aspartic acid receptor antagonists [544, 560-566]. Previous studies that have examined the flexion reflex threshold in chronic pain patients have either left drug use unchanged at the time of testing [534, 543, 567, 568] or asked patients to stop taking medications 6 hours – 2 weeks prior to each testing session [176, 259, 537,

569, 570]. In addition, commonly ingested substances such as nicotine, alcohol and caffeine are known to alter central nervous system excitability [571-573]. As a result, participants are frequently asked to avoid their consumption in the hours prior to flexion reflex testing [549, 551, 574].

Summary

The flexion reflex is a polysynaptic reflex response that produces a general pattern of flexor facilitation and extensor inhibition. It is known to be mediated in part by wide dynamic range neurons, which receive convergent input from a wide variety of non-noxious and noxious afferents, including those from the knee joint. The excitability of the flexion reflex pathway can be measured in humans by electrical stimulation of the sural nerve or the skin on the plantar aspect of the foot. The resultant reflex muscle response can be recorded from the biceps femoris muscle where it occurs at a latency of ~85-150 ms. It appears that the largest amplitude and most comfortable flexion reflex responses can be elicited in standing with the stimulated limb completely unloaded. It is common for a brief train of stimuli to be given every 4-20 seconds with the interstimulus interval randomised in order to avoid habituation and stimulus predictability. Flexion reflex threshold can be attained using a staircase method of testing and is reliably quantified using the flexion reflex peak interval z-score and a cut point of 10.32. The excitability of the flexion reflex can be influenced by a number of different factors including analgesic medications, the time of day of testing, the limb

tested (dominant/non-dominant) and strenuous physical activity. The effects of age, gender and hormonal status on flexion reflex excitability remain unclear.

Joint aspiration and intraarticular corticosteroid injection

Background

Joint aspiration and intraarticular corticosteroid injection are commonly used to relieve the signs and symptoms of synovitis, including swelling and pain [575]. A number of clinical guidelines recommend the use of intraarticular corticosteroid in the management of both inflammatory arthritis and OA of the knee [576-578], with level Ia evidence of its short term (1-3 weeks postinjection) efficacy compared to placebo injection [579-581]. It is beyond the scope of this thesis to provide a comprehensive review of joint aspiration and intraarticular corticosteroid injections. However, a brief overview is warranted, including information on their local and systemic effects, time course of action and methodological factors affecting their clinical efficacy.

Local effects

Joint aspiration and intraarticular corticosteroid injection strongly suppress aberrant sensory output from the arthritic joint. In turn, this is likely to alter the excitability of a number of neural pathways such as the flexion reflex that

may be involved in mediating AML. Aspiration of the distended knee reduces intraarticular pressure [582-584], attenuating the discharge of stretch and pressure sensitive mechanoreceptors innervated by group II joint afferents. Furthermore, in the presence of inflammation, the mechanical sensitivity of articular free nerve endings is greatly increased due to ongoing peripheral sensitisation [116, 126, 145]. Thus, aspiration is likely to reduce the mechanical activation of a portion of free nerve endings innervated by group III and IV joint afferents. Finally, aspiration is thought to reduce the concentration of pro-inflammatory chemicals within the synovial cavity [582, 584, 585]. This is likely to attenuate the direct activation of chemosensitive free nerve endings as well as indirectly reduce joint afferent output by minimising ongoing peripheral sensitisation and intraarticular swelling [586]. The effects of aspiration on joint afferent discharge are strongly augmented by intraarticular corticosteroid injection. Corticosteroid injection is known to directly inhibit C-fibre membrane excitability [114] as well as powerfully suppress local joint inflammation [587], further reducing joint swelling, peripheral sensitisation and the activation of chemosensitive articular free nerve endings. The anti-inflammatory mechanisms of intraarticular corticosteroid injection have been examined using pre and postintervention arthroscopy and biopsy of synovial tissue from the injected joint. These studies have shown that intraarticular corticosteroid injection exerts its anti-inflammatory effects by a number of synergistic mechanisms including reducing mast cell [588, 589] and macrophage [589, 590] numbers,

suppressing T-cell production [591, 592] and reducing both pro-inflammatory cytokine [591] and leukotriene [593] expression in the synovium.

Systemic effects

While the anti-inflammatory effects of corticosteroid injections are maximal in the injected joint, some diffusion into the blood stream is inevitable. In this regard, Armstrong et al. [594] showed that increased levels of serum corticosteroid began 30 minutes after intraarticular corticosteroid injection of the knee, with peak levels observed after 2–12 hours and complete clearance from the blood taking approximately 5 days. Increased levels of serum corticosteroid following intraarticular injection may affect the hypothalamic-pituitary-adrenal axis, altering the endogenous production of cortisol. In patients with inflammatory arthritis, a significant decrease in serum cortisol levels has frequently been observed after a single corticosteroid injection into the knee joint [595]. The earliest decrease in cortisol levels has been seen within 4 hours [596]. Maximal suppression is usually seen after 24–48 hours with serum cortisol recovering to normal levels within 1–4 weeks [595]. Furthermore, intraarticular corticosteroid has been shown to reduce plasma levels of pro-inflammatory cytokines including tumor necrosis factor- α , interleukin-2, interleukin-4, interleukin-6, interleukin-7 and interleukin-17 [597, 598]. Similarly, serum biomarkers of whole body inflammation are suppressed following intraarticular corticosteroid injection. In patients with RA, a significant decrease in plasma C-reactive protein and erythrocyte

sedimentation rate was observed 1 week after corticosteroid injection at one or both knees [599]. Both C-reactive protein and erythrocyte sedimentation rate dropped by an average of 46%, with the effect lasting up to 6 months in some patients. In another study in RA patients, there was a 16% decline in erythrocyte sedimentation rate 3 days and a 24% decline in erythrocyte sedimentation rate 28 days after intraarticular corticosteroid injection at the knee joint [600].

The systemic effects of corticosteroid injection may lead to clinical improvements in distant, uninjected joints. For example, in patients with inflammatory arthritis, intraarticular corticosteroid injection has been shown to decrease the temperature of both the injected knee and the contralateral, uninjected knee joint, suggesting a bilateral anti-inflammatory effect [601, 602]. Furthermore, after a single intraarticular corticosteroid injection at the knee, improvements in clinical measures such as articular index, swollen joint count and joint effusion were frequently noted in patients with polyarticular inflammatory arthritis [603]. In seven out of 18 patients in the same study, hand grip strength was also improved after corticosteroid injection in the knee [603]. Thus, while it is apparent that the strongest anti-inflammatory effect of corticosteroid injection occurs locally at the site of the injected joint, some diffusion of the corticosteroid into the blood stream does occur. This may have systemic effects, affecting cortisol production, reducing the levels

of inflammatory mediators in the blood and having therapeutic effects at distant, uninjected joints.

Time course

Anti-inflammatory effects may occur within hours of aspiration and corticosteroid injection [604], although peak effects take longer to manifest. To explore this time course in more detail, Ostergaard et al. [505] examined the effects of aspiration and corticosteroid injection (80 mg methylprednisolone acetate) on synovial membrane volume (a biomarker of joint inflammation) and joint swelling using serial MRI measurements in patients with various forms of knee joint arthritis. In respect to swelling, intraarticular fluid volumes ranged from 17-77 ml at baseline, with a median of 35 ml. One day after aspiration and corticosteroid injection, swelling had reduced to a median of 15 ml (range 8-68 ml). Further reductions in joint swelling were noted 7 days postintervention, with the percentage reduction from baseline ranging from 12-97%. It is notable that at day 7, despite swelling reducing in all treated joints, residual effusion was common, with observed volumes of between 1 and 52 ml (median 13 ml). Similarly, synovial membrane volumes decreased in all patients following knee joint aspiration and corticosteroid injection. While reductions in inflammation were apparent as early as 1 day postintervention, greater improvements were observed after 7 days with synovial membrane volume decreasing by a median of 49% (range 15-73%) [505]. In another study [591] in patients with RA, clinical signs

of inflammation were markedly suppressed after aspiration and corticosteroid injection yet persistent signs of synovitis, such as villus formation and increased vascularity, were reported during arthroscopy 9-15 days postintervention. Similarly, cytokine expression and vascular growth factors were suppressed after corticosteroid injection, but remained elevated compared to healthy controls [591]. Taken together, these findings suggest that following knee joint aspiration and intraarticular corticosteroid injection: 1) it takes more than 1 day for the peak reduction in swelling and inflammation to occur and 2) swelling and inflammation are attenuated but rarely, if ever, abolished entirely.

Methodological considerations

Injection technique

Clinically, there is no consensus on the best technique for knee joint aspiration and corticosteroid injection, with medial, superolateral and anterior approaches commonly used [582, 583, 605]. It has been suggested that the anterior approach is less desirable due to the presence of the infrapatellar fat pad and a greater likelihood of injuring the joint surface [582]. Aspiration of synovial fluid appears to greatly improve the accuracy of subsequent corticosteroid injection. In this respect, Jones et al. [606] showed in a contrast radiography study that up to one third of corticosteroid injections at the knee joint may be extraarticular but that the prior aspiration of synovial fluid

significantly improved injection accuracy. In a recent systematic review of 9 studies examining different needle placements, Hermans et al. [607] reported that the superolateral approach had the greatest pooled accuracy with 91% (95% CI 85-99%) of injections deemed to be intraarticular. Moreover, Hirsch et al. [608] performed a high resolution ultrasound study of the location of knee effusion in patients with a variety of arthritic conditions. These authors showed that the greatest volume of synovial fluid was typically found in the superolateral compartment of the knee joint; and for those patients with small effusions, the lateral compartment was most likely to be affected. In the case of "dry taps" or where substantially less synovial fluid than expected is withdrawn, it has been suggested that both medial and lateral approaches are trialled and that the opposite side of the knee joint is manually compressed in order to force the effusion into the articular compartment in which the needle is located [609]. Recently, Zhang et al. [610] compared the success of knee joint aspiration in two different patient positions, sitting with the knee flexed and supine with the knee extended. Forty arthritic knee joints were randomly aspirated in one of these positions and then ultrasound imaged in order to determine the degree of residual effusion. Three quarters of knees aspirated in the sitting position required a second arthrocentesis in order to fully drain the joint while only 30% of those in a supine position required reaspiration.

Type of corticosteroid and dosage

The corticosteroids most commonly used for intraarticular injection include methylprednisolone acetate, triamcinolone hexacetonide and triamcinolone acetonide [582, 585]. In a survey of American rheumatologists, methylprednisolone acetate was the most commonly used preparation, followed by triamcinolone hexacetonide and triamcinolone acetonide [611]. The efficacy of different dose rates for intraarticular corticosteroid injection has not been investigated in any detail. However, recommended dosages for methylprednisolone acetate range from 20-80 mg for large joints such as the knee [582, 585, 605, 612].

Activity in the first 72 hours postinjection

A period of relative rest and reduction of weight bearing for 24-72 hours after intraarticular corticosteroid injection is commonly advised [582, 583, 605]. This is thought to reduce the rate of absorption of the corticosteroid from the synovial cavity and maximise its therapeutic efficacy [582, 583, 605]. In support of this conjecture, Chakravarty et al. [613] demonstrated a superior outcome at 6 months for those patients who underwent a 24-hour period of complete bed rest after corticosteroid injection. In contrast, another study found no benefit of resting the joint after corticosteroid injection [614]. Thus, it remains unclear whether relative rest of the joint is important in the first few days after injection.

Summary

Joint aspiration and corticosteroid injection is commonly used to suppress inflammation in arthritic joints, with level 1a evidence of its short term efficacy compared to placebo [579-581]. There is strong evidence of a reduction in local inflammation within the damaged joint, with changes in the expression of numerous inflammatory and/or immune cells in the synovium. Systemic effects may also occur, with changes in serum cytokines and cortisol levels and reports of anti-inflammatory effects at distant, untreated joints. The time course of therapeutic action is relatively quick, with symptom relief often occurring within hours after aspiration and corticosteroid injection. However, the maximal anti-inflammatory effects of corticosteroid injection may take several days to manifest and it is apparent that residual levels of swelling and inflammation often remain. The optimum type and dosage of corticosteroid have not been explored in any detail. Joint aspiration improves the accuracy of needle placement while the evidence suggests that a superolateral approach with the patient in a supine position and the knee extended will maximise both the accuracy of intraarticular needle placement and the amount of synovial fluid aspirated from the joint. When aspiration retrieves notably less synovial fluid than expected, there may be value in adopting both a lateral and medial approach and compressing the opposite side of the joint in order to push or “milk” the fluid towards the needle. While findings to date are equivocal, a 24-72 hour period of relative rest has been suggested after intraarticular corticosteroid injection.

METHODS

Participants

Sixteen participants with chronic knee joint arthritis (10 RA, 5 OA, 1 psoriatic arthritis) who were attending outpatient Rheumatology or Physiotherapy clinics volunteered to take part in this study (Table 5.1). Participants were included if they had a clinically detectable knee joint effusion as well as other signs and symptoms of knee joint synovitis including pain on motion, heat and tenderness on palpation. They were excluded if they had received an intraarticular injection in the last 4 months, a cardiovascular condition precluding the performance of maximum effort strength tests, a documented loss of normal sensory function or a history of low back pain with associated neurological symptoms and signs. Participants were asked to refrain from caffeine, alcohol, nicotine, and strenuous exercise for 4 hours prior to each testing session [615-617]. In addition, they were asked not to take any analgesic medication in the 12 hours prior to each testing session but were otherwise free to take their usual medication. Finally, participants were asked not to start any new medication or undertake any unaccustomed exercise during the testing period. Ethical approval for this study was given by the Northern Regional X Ethics Committee of New Zealand in accordance with the principles of the Declaration of Helsinki.

Table 5.1. Participant characteristics.

Age in years, mean (SD)	61.0 (13.7)
Height in metres, mean (SD)	1.65 (0.09)
Mass in kilograms, mean (SD)	81.9 (22.6)
Female, number (%)	11 (68.8)
Duration of pathology in months, mean (SD)	98.8 (78.3)
Type of pathology, number (%)	
Rheumatoid arthritis	10 (62.50)
Osteoarthritis	5 (31.25)
Psoriatic arthritis	1 (6.25)
Number of painful joints, mean (SD)	3 (3.25)

SD = standard deviation.

Experimental protocol

Participants attended three testing sessions: baseline, 5 ± 2 days postintervention and 15 ± 2 days postintervention. For each participant, testing was conducted at the same time of day in each session to minimise circadian variations in flexion reflex threshold [618] and muscle strength [324]. At the baseline session, measures of knee pain, flexion reflex threshold and knee extensor peak torque were performed. A second baseline measure of flexion reflex threshold was undertaken 15 minutes later to ensure the stability of this measure following maximum effort quadriceps contractions. Following this, participants had their knee joint aspirated. Immediately following joint

aspiration, flexion reflex threshold and then knee extensor peak torque were measured again. At the end of the baseline session, participants received an intraarticular corticosteroid injection. Patients were advised to rest the injected knee as much as possible in the first 72 hours after corticosteroid injection [582, 605, 613]. Five \pm 2 days and 15 \pm 2 days postintervention, knee pain, flexion reflex threshold and knee extensor peak torque were retested.

Knee pain

Knee pain ratings were obtained using the P4 instrument [619] with each participant asked to mark the scale while thinking specifically of the pain in the knee joint undergoing aspiration and corticosteroid injection. The P4 was administered at baseline, 5 \pm 2 days postintervention and 15 \pm 2 days postintervention.

Flexion reflex threshold

A Nicolet bar electrode (stimulating electrode) with 9 mm gold cups and 30 mm interelectrode distance (D.O. Weaver & Co., CO, USA) was secured under the medial arch of the foot and covered with an elastic bandage. The stimulating electrode was placed in a standardised position 2cm proximal to the first metatarsophalangeal joint and directly underneath the first metatarsal on the plantar aspect of the foot, with the anode positioned distally. For recording purposes, bipolar Ag-AgCl disc electrodes with an

interelectrode distance of 2.2cm were placed over the biceps femoris muscle of the ipsilateral leg, with the inferior edge positioned 10 cm above the popliteal crease [522]. A ground electrode was placed on the anterior surface of the ipsilateral tibia. Prior to electrode placement, the electrode sites were shaved, abraded and cleansed with an alcohol wipe to reduce skin impedance. EMG signals were amplified (1000x), filtered (10-1000 Hz; AMT-8 amplifier, Bortec Biomedical, Alberta, Canada), and sampled at 2000 Hz (Micro 1401, Cambridge Electronic Design, Cambridge, UK).

Once the stimulating and recording electrodes were in place, participants stood on a carpeted wooden block (150 x 40 x 26 cm) and were asked to support their weight through their contralateral leg and arms while holding onto the back of a chair for balance. The stimulated leg hung in a relaxed position without touching the ground. Participants were asked to look straight ahead during testing and to keep the muscles in their stimulated leg completely relaxed. Muscle relaxation was ensured by continuous monitoring of biceps femoris surface EMG in real time on an oscilloscope (Textronix TDS2014B, Beaverton, Oregon, USA). Electrocutaneous stimuli were then delivered to the medial arch of the foot using a DS7A constant current stimulator (Digitimer Ltd, Hertfordshire, UK) controlled by a specialised software programme (Signal 3, Cambridge Electronic Design, Cambridge, UK). Each stimulus train consisted of 5 rectangular pulses of 1 ms pulse width with a 3 ms interpulse interval (17 ms total duration) [178] and each train was

separated by a random interstimulus interval of 8-12 seconds [616, 617]. Prior to threshold testing participants were first familiarised with the stimulation procedures by receiving a series of 15 stimuli trains of varying intensity. The flexion reflex threshold was then determined using an up-down staircase method [617]. Specifically, the stimulation intensity was increased from 0 mA in 4 mA increments until a flexion reflex was observed. Once a flexion reflex response was observed, the intensity was decreased in 2 mA steps until a flexion reflex was no longer evident. The stimulation intensity was then increased and decreased four more times in 1 mA increments until a flexion reflex appeared twice and disappeared twice. These final four stimulation intensities were recorded and averaged to calculate flexion reflex threshold. The presence of a flexion reflex response was defined using a flexion reflex interval peak z-score of biceps femoris EMG activity, derived using the formula [617]:

$$\text{z-score} = (\text{flexion reflex interval peak} - \text{baseline mean}) / \text{baseline standard deviation}$$

The baseline mean and standard deviation of biceps femoris EMG activity was recorded from a 65 ms period (-70 to -5 ms) prior to stimulation. The flexion reflex interval peak refers to the peak EMG activity of the biceps femoris muscle during the poststimulation window of 85-150 ms. This window was chosen to avoid signal contamination via non-nociceptive reflexes and startle responses [515]. A z-score of 10.32 or greater was considered a true flexion reflex response [617].

Knee extensor peak torque

Participants were seated in a custom designed chair with the hips and knees flexed to 90°. Straps were firmly secured over the distal third of the thigh and across the chest to limit extraneous movement. A rigid strap was secured around the ankle, slightly superior to the malleoli. This was coupled to a metal attachment that was connected in series to a uniaxial load cell (Precision Transducers, Auckland, New Zealand), aligned horizontally with the ankle joint. All participants were asked to perform isometric maximum voluntary contractions of their knee extensors by pushing as hard as possible against the ankle strap. Prior to maximum effort contractions, a series of 4 submaximal knee extensor contractions (25%, 50%, 50% and 75% of perceived maximum effort) were performed, with a 1 minute rest given between each contraction. Thereafter, a 2 minute rest was given before a set of three (6 second) maximum voluntary contractions were performed. Participants received visual feedback of their force trace and a consistent level of strong verbal encouragement [320] during each contraction. A 2 minute rest period was given between each maximum effort contraction. In the event that the peak force (N) produced during the maximum voluntary contractions continued to increase with each subsequent trial, a 4th, and in some cases a 5th, contraction was performed until force plateaued or decreased. This was done in an effort to elicit a true maximum effort from each individual [339]. Force (N) signals were recorded from the load cell during each contraction, where they were amplified (x100), sampled (1000 Hz) and stored on a

computer for later analysis. At each measurement interval, peak isometric knee extensor strength was calculated as the highest force (N) produced during any of the 3-5 maximum voluntary contractions. The length of the lever arm was measured from the lateral epicondyle of the femur to the centre of the ankle strap, which was parallel to the load cell. Lever arm length (m) was then multiplied by peak isometric force (N) to calculate peak torque (Nm).

Joint aspiration and corticosteroid injection

All participants had their knee joint aspirated while lying in a supine position with the knee resting in or near full extension. A 21 gauge needle was inserted into the superolateral aspect of the joint and as much synovial fluid as possible withdrawn from the knee. Where aspiration obtained notably less synovial fluid than expected from clinical examination (n=5), a second aspiration was attempted using a medial approach and, where relevant, “milking” of the joint performed to maximise the amount of fluid recovered. Following the postaspiration measures of the dependent variables, 40 mg of methylprednisolone acetate (Depomedrol ®) was injected into the knee joint. All joint injections were performed without local anaesthesia, under sterile conditions.

Statistical analysis

To assess whether the dependent variables conformed to a normal distribution Shapiro-Wilk tests were completed. As the P4 pain scores were not normally distributed, a Friedman's test was used to examine changes in knee pain over time. Thereafter, Wilcoxon signed rank tests were utilised to compared baseline P4 values to P4 values on day 5 and day 15. One way repeated measures ANOVAs were undertaken to analyse differences in flexion reflex threshold and knee extensor peak torque over time (baseline, day 5, day 15). Where the assumption of sphericity was violated, Greenhouse-Geisser corrections were utilised. As our a priori hypotheses were that flexion reflex threshold and knee extensor torque would increase following aspiration and corticosteroid injection, planned contrasts were used to assess the differences between the first (baseline 1) and each subsequent measure. The alpha level for all statistical procedures was set to 0.05.

RESULTS

Baseline characteristics of the participants are provided in Table 5.1. The mean volume of fluid aspirated from the knee joint was 24 ± 27 ml. P4 pain scores and knee extensor peak torque were collected from all 16 participants. Complete data sets for measures of flexion reflex threshold could only be obtained from 11 of the 16 participants. One participant with RA was unable to support their body weight on the contralateral leg due to

painful synovitis in the 1st metatarsophalangeal joint. Equipment failure prevented complete data sets from being collected in the other four participants. All participants acted appropriately in respect to the instructions regarding exercise changes and medication use during the testing period.

Knee Pain

A summary of P4 pain ratings at each measurement interval is presented in Figure 5.2. There was a significant change in knee pain over time ($p < 0.001$). Compared to baseline, there was a significant reduction in knee pain at 5 ± 2 days ($p = 0.001$) and 15 ± 2 days ($p < 0.01$) after aspiration and corticosteroid injection.

Flexion reflex threshold

A summary of flexion reflex thresholds at each measurement interval is presented in Figure 5.3. A significant change in flexion reflex threshold was observed over time ($p < 0.01$). Compared to baseline 1, flexion reflex threshold increased immediately postaspiration ($p < 0.01$) and at 5 ± 2 days ($p < 0.05$) and 15 ± 2 days ($p < 0.01$) after aspiration and corticosteroid injection. No significant difference was found between the baseline 1 and baseline 2 measures of flexion reflex threshold ($p > 0.05$).

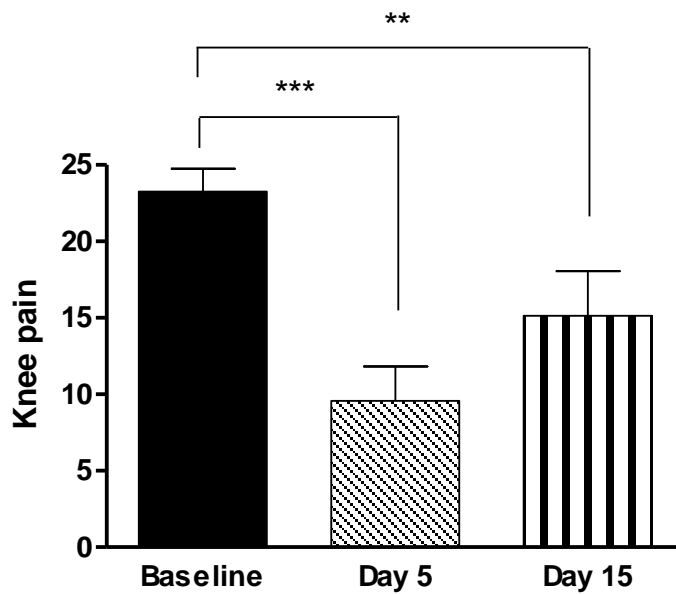


Figure 5.2. Knee pain as measured on the P4 instrument before and after knee joint aspiration and corticosteroid injection. Baseline = before intervention. Day 5 = Five \pm two days after knee joint aspiration and corticosteroid injection. Day 15 = Fifteen \pm two days after knee joint aspiration and corticosteroid injection. ** = significant change from baseline ($p < 0.01$). *** = significant change from baseline ($p = 0.001$). Data are means and one standard error of the mean.

Knee extensor peak torque

A summary of knee extensor peak torque values at each measurement interval is presented in Figure 5.4. There was a significant change in knee extensor peak torque over time ($p < 0.001$). Compared to baseline values, knee extensor peak torque increased immediately after aspiration ($p < 0.01$)

and at 5 ± 2 days ($p = 0.001$) and 15 ± 2 days ($p < 0.001$) after aspiration and corticosteroid injection.

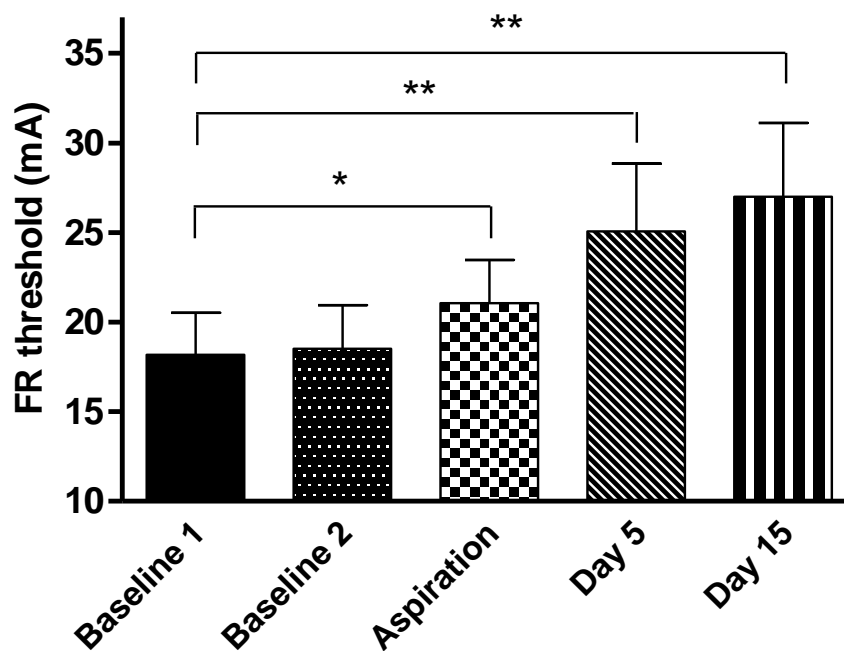


Figure 5.3. Flexion reflex (FR) threshold before (baseline 1 and baseline 2) and after knee joint aspiration and corticosteroid injection. mA = Milliamps.

Aspiration = Following joint aspiration only. Day 5 = Five \pm two days after knee joint aspiration and corticosteroid injection. Day 15 = Fifteen \pm two days after knee joint aspiration and corticosteroid injection. * = significant change from baseline 1 ($p < 0.05$). ** = significant change from baseline 1 ($p < 0.01$). Data are means and one standard error of the mean.

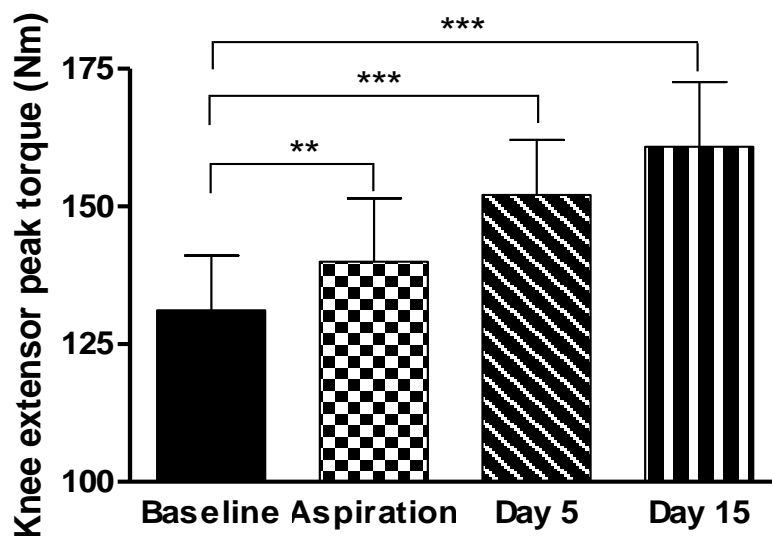


Figure 5.4. Knee extensor peak torque at baseline and following knee joint aspiration and corticosteroid injection. Nm = Newton metres. Aspiration = Following joint aspiration only. Day 5 = Five \pm two days after knee joint aspiration and corticosteroid injection. Day 15 = Fifteen \pm two days after knee joint aspiration and corticosteroid injection. ** = significant change from baseline ($p < 0.01$). *** = significant change from baseline ($p \leq 0.001$). Data are means and one standard error of the mean.

DISCUSSION

The findings of this study provide evidence that altered knee joint afferent discharge modifies flexion reflex excitability in humans, as has previously been demonstrated in animals. We observed an increase in flexion reflex threshold following knee joint aspiration alone followed by larger increases in flexion

reflex threshold 5 and 15 days after subsequent corticosteroid injection. The change in flexion reflex excitability following aspiration alone is a novel finding, suggesting that intraarticular swelling may enhance flexion reflex excitability as well as increasing the excitability of other spinal reflex pathways such as group I non-reciprocal (Ib) inhibition [70]. In animal studies, it is well established that swelling enhances the discharge of articular mechanoreceptors innervated by group II afferents [121]. Group II knee joint afferents provide input to wide dynamic range neurons, but these neurons are more strongly activated by group III and IV joint afferents [167, 620]. In humans, experimental joint infusion rarely evokes sensations of pain [68, 71, 124, 205], suggesting that that swelling alone is unlikely to stimulate a significant number of group III and IV afferents. Furthermore, in animal studies, the infusion of saline into undamaged knee joints failed to facilitate the flexion reflex response [161, 175]. However, the afferent response to swelling may differ markedly in arthritic joints, where the presence of inflammatory mediators can be expected to directly activate a portion of group III and IV joint afferents while at the same time greatly reducing their mechanical threshold via the process of peripheral sensitisation [147, 502]. Thus, aspirating synovial fluid from the arthritic joint may diminish the direct activation of chemosensitive joint afferents while at the same time reducing capsular distension and diminishing the mechanical stimulation of group II and sensitised group III and IV afferents. Furthermore, central sensitisation of wide dynamic range neurons is likely to greatly enhance the synaptic efficacy of group II input to the flexion reflex pathway [127]. As such, in arthritic joints it is

likely that aspiration reduces the discharge of group II, III and IV knee joint afferents, all of which have the potential to influence flexion reflex excitability.

We observed a significant increase in flexion reflex threshold 5 and 15 days after aspiration and corticosteroid injection. Such a change is likely to be explained, in part, by the anti-inflammatory and/or analgesic effects of corticosteroid injection. Locally administered corticosteroid has been shown to have a direct inhibitory effect on the ability of group IV afferents to generate action potentials [114]. Furthermore, corticosteroid injections are known to strongly suppress local joint inflammation [505, 587] and thus can be expected to reduce the activation of chemosensitive free nerve endings, raise the threshold of group III and IV joint afferents affected by peripheral sensitisation and attenuate any residual intraarticular swelling. The net result of these changes would be a reduction in the peripheral afferent barrage from the injected knee joint. Furthermore, as some diffusion of the corticosteroid into the bloodstream is likely [595], it is possible that nociceptive output from other joints was attenuated too [602]. The overall reduction in articular sensory output may partially reverse neuroplastic changes in wide dynamic range neurons, diminishing the excitability of the flexion reflex. Such a reduction in central nervous system excitability following the blockade of peripheral afferent input has been observed previously [621-628].

Alternatively, the increase in flexion reflex threshold may reflect a reduction in spatial facilitation between nociceptive cutaneous afferents in the foot and

articular afferents, without direct changes in the membrane excitability of neurons in the dorsal horn [167, 521, 629, 630]. Finally, it is possible that aspiration and corticosteroid injection led to a change in descending inhibition and/or facilitation of wide dynamic range neurons from supraspinal structures. In this regard, Herrero et al. [504] have shown that supraspinal pathways are at least partly responsible for the increase in flexion reflex excitability following the induction of experimental arthritis in the cat's knee joint. Furthermore, a reversal in the dysfunction of conditioned pain modulation pathways has been shown after both knee [629] and hip [212] arthroplasty. As conditioned pain modulation is at least partly determined by brainstem mediated inhibition of wide dynamic range neurons [631], this suggests that reducing the nociceptive barrage from the damaged joint may help to restore the normal balance between descending inhibition and facilitation of wide dynamic range neurons [212, 629].

Another important finding from the current study is that aspiration and corticosteroid injection led to a notable increase in knee extensor peak torque in individuals with chronic knee joint arthritis. These findings support the earlier work of Geborek et al. [142] in patients with RA, who reported a small (9 Nm) but significant increase in knee extensor torque immediately after knee joint aspiration and a larger (26 Nm) increase 14 days after subsequent corticosteroid injection. Similarly, we observed a 9 ± 10 Nm increase in torque immediately following aspiration and a 31 ± 19 Nm increase by day 15, an

average 25% increase in knee extensor peak torque from baseline levels. Such a rapid change in peak torque likely reflects enhanced neural activation of the quadriceps muscle. Muscle force production is determined by the morphological characteristics of the muscle and its neural activation. Even during heavy resistance training, changes in quadriceps morphology take longer than 15 days to occur [632-634]. In the current study, participants did not perform any resistance training and were asked to avoid any unaccustomed exercise in the 15 day follow up period. Furthermore, corticosteroid injection may even reduce the rate of muscle protein synthesis [635]. In this context, it is reasonable to suggest that the observed increase in knee extensor peak torque is largely explained by an increase in neural activation of the muscle. Neural activation deficits commonly contribute to quadriceps weakness in patients with RA [8, 76, 636] and OA [22]. This is supported by our findings of an immediate increase in knee extensor peak torque following knee joint aspiration. Furthermore, it has previously been shown that quadriceps EMG amplitude is strongly depressed by knee joint swelling ([98] and Chapter 3 of this thesis) while in patients with knee joint arthritis, aspiration [143] and intraarticular corticosteroid injection [142] have been shown to increase quadriceps EMG amplitude during subsequent maximum voluntary contractions.

As well as being a direct cause of quadriceps weakness [75], AMI may contribute to muscle atrophy [66] and can hinder effective quadriceps

strengthening [20, 28, 637]. Interestingly, the ~25% increase in knee extensor peak torque we observed 15 days after aspiration and corticosteroid injection is of a similar magnitude to the reported change in knee extensor strength produced by 5-6 months of high intensity resistance training in patients with chronic arthritis [638, 639]. While AMI does not always preclude strength gains [8, 11, 23], even mild AMI can restrict their magnitude as a portion of the muscle cannot be activated [24, 25]. Recent work in patients with OA suggests that combining TENS (an intervention known to reduce AMI [81]) with resistance training produces greater improvements in quadriceps activation and torque than resistance training alone [25]. Furthermore, in patients with OA, the combination of either glucosamine and resistance training or NSAIDs and resistance training has recently been shown to lead to greater increases in quadriceps muscle strength compared to resistance training and placebo tablets [24]. Given the 25% increase in knee extensor peak torque we observed in the current study, it may be that aspiration and corticosteroid injection followed by a period of resistance training will lead to greater long term quadriceps strength gains than resistance training alone. This should be investigated in future studies.

Although statistically significant, the magnitude of improvement in knee extensor peak torque following aspiration alone was relatively small (mean increase of 6%). This is comparable to previous findings exploring aspiration in patients with chronic knee joint pathology, in which either no significant

change or moderate increases (~14-18%) in quadriceps activation were observed [76, 143]. In contrast, aspiration (36-85 ml) of acutely swollen knee joints is reported to consistently reduce, and in some cases abolish AMI [99]. Furthermore, a recent case study [82] has reported absolute increases in knee extensor peak torque of approximately 400% one to two hours after aspirating 150 ml of synovial fluid from an acutely injured knee joint. The reasons for this discrepancy remain unclear. It may be that chronic joint pathology leads to changes in capsular compliance [119, 139] and/or damage to articular receptors that reduces the afferent response to swelling, thereby reducing its inhibitory effect. Against this argument, experimental joint swelling is reported to produce quadriceps AMI of a similar magnitude when the fluid is infused into chronic arthritic knee joints [68, 98, 640] and a significant correlation has been found between clinical measures of joint swelling and the reduction in knee extensor torque in the early stages after TKA [641]. An alternative explanation for the reduced efficacy of aspiration in chronic joint pathology may relate to the volume of fluid aspirated, which is typically less than that withdrawn following acute joint damage. In the chronic arthritic knee, capsular thickening and increased viscosity of synovial fluid can complicate aspiration, with imaging studies showing that it is often difficult to completely drain the joint [609, 642, 643]. Thus, it may be that despite our and others' efforts to aspirate the joint completely, some residual joint swelling remains. Previous studies [68, 125] have shown that even small, clinically undetectable effusions can induce quadriceps AMI. Thus, a failure to completely drain the swollen joint may partially explain the smaller effects

of aspiration on knee extensor peak torque in individuals with chronic joint pathology. Finally, it could be that the afferent discharge produced by swelling and inflammation have convergent inhibitory effects on the same quadriceps α -motoneurons. Even with a reduction in joint swelling, if the inhibitory effects of ongoing nociception (e.g. due to peripheral sensitisation) are sufficient to continue to prevent α -motoneuron depolarisation then it is conceivable that aspiration alone may have a limited effect on quadriceps activation.

A limitation of the current study is the lack of a control group who received a placebo/sham injection. Thus, while it is likely that a reduction in peripheral afferent output from the treated joint explains our findings, we cannot rule out non-specific treatment effects such as placebo partially explaining the observed changes in pain, flexion reflex threshold and knee extensor peak torque. However, corticosteroid injection is known to have potent anti-inflammatory effects at the injected joint; reducing mast cell [588, 589] and macrophage [589, 590] numbers; suppressing T-cell production [591, 592] and reducing both pro-inflammatory cytokine [591] and leukotriene [593] expression in the synovium. Furthermore, placebo injection has previously been shown to have no effect on quadriceps strength [215] and there is level Ia evidence that corticosteroid injection has a significant physiological effect over and above placebo injection [579-581]. Another possible limitation of the current study is that we did not take into account the potential effects of

the hormonal status on flexion reflex excitability. Tassorelli et al. [554] has previously shown that flexion reflex threshold varies during different phases of the menstrual cycle, although these findings are not unequivocal [555]. As our subjects all presented with painful synovitis, it was considered unethical to make them wait until a defined phase of their menstrual cycle to receive an intervention that is known to provide good symptomatic relief. As such, hormonal fluctuations could have influenced the measurement of the flexion reflex threshold over time. However, this is unlikely to have had a systematic effect on flexion reflex thresholds, accounting for the large and consistent changes we observed in flexion reflex threshold following aspiration and corticosteroid injection. Rather, it is likely that this represents a true physiological change in spinal reflex excitability, due to a reduction in the barrage of aberrant afferent output from the arthritic joint. Finally, we used a peak interval z-score of 10.32 as the cut-off for establishing the presence or absence of a true flexion reflex response [522]. It has since been shown that while the peak interval z-score is a reliable way of quantifying the flexion reflex response, the average interval z-score may be more stable [545]. In future research, it may be more appropriate to use the average interval z-score to determine the presence or absence of a true flexion reflex response.

CONCLUSION

In this study, aspiration and corticosteroid injection of chronic arthritic knee joints led to a significant increase in flexion reflex threshold, suggesting that

aberrant joint afferent output due to swelling and inflammation enhances flexion reflex excitability in humans as has previously been observed in animals. Furthermore, aspiration and corticosteroid injection led to a 25% increase in knee extensor peak torque after 15 days, suggesting a clinically important reduction in quadriceps AML. Future research in patients with chronic arthritis should explore whether quadriceps resistance training can be enhanced by the prior aspiration and injection of corticosteroid into the knee joint.

Chapter Six: The effects of tendon vibration on knee extensor peak torque and quadriceps EMG amplitude in healthy controls, individuals with knee joint osteoarthritis and individuals who have recently undergone anterior cruciate ligament reconstruction

INTRODUCTION

It has been reported that AMI may continue to be present in the absence of clinically detectable knee joint swelling, inflammation and pain [11, 62, 80]. This suggests that mechanisms unrelated to swelling and inflammation may also contribute to AMI [62]. One possible mechanism may be a dysfunction in the quadriceps γ -loop. Hagbarth and colleagues [276] were the first to demonstrate that excitatory input from the γ -loop is necessary to achieve full muscle activation. These authors showed that preferential anaesthetic block of γ -efferents reduced muscle force production and the firing rate of tibialis anterior motor units during subsequent maximum voluntary contractions [276]. Further investigations into the importance of the γ -loop have relied on prolonged vibration to experimentally attenuate Ia afferent input to the quadriceps α -motoneuron pool. In healthy participants, prolonged vibration (20-30 minutes) causes a reduction in muscle force output [157, 180-182], EMG amplitude [157, 180, 181] and motor unit firing rates [180] during subsequent maximum voluntary contractions. However, in individuals who have ruptured their ACL [157] or recently undergone ACL reconstruction [77,

83, 183], prolonged vibration has no effect on quadriceps muscle activation. These findings suggest that ACL injury leads to dysfunction in the γ -loop pathway that limits quadriceps α -motoneuron depolarisation, thus contributing to AMI [157]. However, it remains unknown whether γ -loop dysfunction contributes to AMI in other knee joint pathologies such as OA. Thus, the aim of Part A of the current study was to explore the effects of prolonged vibration on knee extensor peak torque and quadriceps EMG amplitude in individuals with OA, individuals who had recently undergone an ACL reconstruction and healthy controls. Our hypothesis was that prolonged vibration would lead to a significant decrease in knee extensor peak torque and quadriceps EMG amplitude in healthy controls but not in individuals with OA or in individuals who had recently undergone an ACL reconstruction.

The neural mechanisms explaining γ -loop dysfunction remain poorly understood. There is evidence that this problem may be caused by a loss of excitation from knee joint mechanoreceptors to the quadriceps γ -motoneuron pool (i.e. a disfacilitation) [78, 157, 160]. This may occur directly, via damage to the afferents themselves [155, 156, 644-646] or indirectly, via enhanced group IV joint afferent output that blocks the normal excitatory effects of joint afferent discharge on extensor γ -motoneurons [184]. The loss of excitatory input from joint afferents to γ -motoneurons is thought to impair γ -efferent drive to the quadriceps muscle spindles, in turn diminishing excitatory input from Ia afferents to the α -motoneuron pool and preventing full muscle

activation [157, 647]. This hypothesis is supported by earlier findings in animals which showed that knee joint mechanoreceptors have excitatory inputs to γ -motoneurons of muscles surrounding the knee [184, 648, 649] and that anaesthetising knee joint afferents leads to a block in extensor γ -motoneuron facilitation [648]. Furthermore, the injection of local anaesthetic into uninjured human knee joints has been shown to lead to a decrease in knee extensor peak torque and quadriceps EMG amplitude during subsequent maximum voluntary contractions [157]. Finally, prolonged vibration failed to impair quadriceps muscle activation in individuals with uninjured, but anaesthetised, knee joints [157].

Importantly, if a reduction in γ -motoneuron excitability is sufficient to explain γ -loop dysfunction, then the afferent portion of the γ -loop (the muscle spindles and Ia afferent fibres themselves) may be unaffected by joint pathology and could be artificially stimulated in order to restore Ia afferent drive to the quadriceps α -motoneuron pool. For instance, despite an anaesthetic block of γ -efferent fibres, Hagbarth et al. [276] have shown that short duration vibration was able to enhance motor unit recruitment and firing rates during maximum effort voluntary contractions. Given these findings, it may be possible to apply short duration tendon vibration during quadriceps muscle contractions to temporarily reverse γ -loop dysfunction in individuals with knee joint pathology. This may have important implications for rehabilitation; as such an intervention would enhance quadriceps neural

activation, allowing more effective muscle strengthening to take place. Thus, the aim of Part B of the current study was to examine the effects of short duration tendon vibration (~5 seconds) on knee extensor peak torque and quadriceps EMG amplitude in healthy controls, individuals with knee joint OA and individuals who had recently undergone an ACL reconstruction. It was hypothesised that short duration vibration would increase knee extensor peak torque and quadriceps EMG amplitude in knee injured populations (with an established γ -loop dysfunction) but have no effect on these variables in healthy controls.

BACKGROUND AND METHODOLOGICAL CONSIDERATIONS

Before moving onto the methods section of this chapter, an overview is warranted concerning the background and key methodological considerations related to vibration generally and specifically, the use of prolonged vibration to experimentally induce γ -loop dysfunction.

Neurophysiological effects of local vibration

Background

Vibration can be applied locally, to a muscle or its tendon, to experimentally manipulate the sensory output from intramuscular sensory receptors. The relative recruitment of different types of muscle afferents is affected by the

activation state of the muscle [650] (relaxed vs. contracting) and the mechanical properties of the vibration stimulus [651]. These properties include the shape of the vibration wave-form [651-653], its frequency [650-655], peak-to-peak amplitude [651, 652] and the force [651] with which the vibration probe is applied to the muscle or its tendon. The technique of microneurography has been used to carefully examine how each of these parameters influences the firing patterns of different muscle afferents. Microneurography involves the insertion of a fine needle electrode directly into the trunk of a peripheral nerve and allows the recording of sensory impulses from single afferent fibres. The findings from these studies are summarised below.

Primary muscle spindles are exquisitely sensitive to the minute changes in muscle length that occur with tendon vibration. As a result, tendon vibration strongly activates Ia afferent nerve fibres. Microneurography studies have consistently found that 100% of Ia afferents tested are vibration sensitive, with many increasing their firing rate in a 1:1 manner with the frequency of the vibration stimulus, in some cases as high as 180-220 Hz [650, 655]. This is far greater than their natural response to muscle lengthening (20-30 Hz) [656] and higher even than their maximum response during voluntary isometric contraction (~140 Hz) [657]. While tendon vibration is a potent stimulus for Ia afferent fibres, it does not activate these afferents selectively. Rather, studies have shown that even in a relaxed muscle, vibration can activate a portion

of group II and group Ib muscle afferents. It should be noted that (in comparison to Ia afferents) group II and Ib afferents are remarkably less sensitive to vibration, provided the muscle is completely relaxed. In this regard, Roll and colleagues [653, 655] observed that 30-40% of group II and ~30% of group Ib afferents were completely insensitive to tendon vibration. More recently, Cordo et al. [651] reported that none of the Ib afferents they tested were sensitive to vibration across a wide range of vibration frequencies, amplitudes and force levels. Finally, Fallon and Macefield [652] reported that 5 out of 6 (83%) Ib afferents were completely insensitive to vibration. In this study, a single Ib afferent responded when the tip of the vibrator was held directly over the previously palpated location of a Golgi tendon organ. However, any movement of the vibrator tip away from this position resulted in the Ib afferent falling silent [652].

Methodological considerations

Vibration frequency

The firing rate of muscle afferents has been found to increase in a 1:1 manner with vibration frequency (i.e. their discharge is phase-locked to each vibration cycle) until they reach what has been termed a frequency limit [656]. Once this limit is breached, afferents commonly fire at subharmonics of the vibration frequency [650, 653, 655]. That is, rather than firing in a 1:1 manner with each vibration cycle, the discharge rate of the afferent reduces

to fire only 1 in every 2 or 1 in every 3 vibration cycles. The frequency limit at which this drop off occurs is generally much higher for Ia afferent fibres when compared to vibration sensitive group II and Ib afferents. However, even amongst Ia afferents this limit can vary markedly from ~30 Hz for the least responsive afferents up to 220 Hz for the most sensitive Ia afferents recorded [650, 653, 655]. The most common frequency limit for Ia afferents occurs between 80 Hz and 100 Hz [655]. Consequently, further increases in vibration frequency beyond 100 Hz will not necessarily lead to an increase in the population response of Ia afferent fibres. Rather, it appears as if 80-100 Hz may be the optimum frequency at which a large portion of Ia afferents continue to fire in a 1:1 manner. Once vibration frequency is increased beyond this point, overall Ia afferent discharge will in fact decrease, as many Ia afferents drop off to fire at subharmonics of the vibration frequency [655].

In contrast to Ia afferents, Burke et al. [650] reported that ~40% of vibration sensitive group II muscle afferents had a frequency limit of 50 Hz, being unable to follow the vibration frequency higher than this even when the muscle was placed on stretch (thus maximally enhancing spindle sensitivity). Similarly, using vibration frequencies up to 180 Hz, Roll and colleagues [653, 655] found that most group II afferents failed to fire at frequencies higher than 10-20 Hz, with a single group II afferent managing a frequency limit of 60 Hz. These findings are similar to those of Cordo et al. [651] who reported that even the most sensitive group II afferents had a frequency limit of 20-40 Hz.

Burke et al. [650] reported that Ib afferents were notably less sensitive to vibration than Ia afferents, typically only firing 1 in every 2 or 1 in every 3 vibration cycles. Similarly, Roll et al. [655] found that of the ~70% of Ib afferents that responded to vibration, most had a frequency limit of ≤ 10 Hz, even when vibration frequency was increased up to 180 Hz. Approximately 25% of Ib afferents tested could fire in a 1:1 manner up to (but never beyond) 50 Hz [655]. In contrast, more recent studies have found Ib afferents to be markedly less sensitive to vibration. Independent of vibration frequency [651] and amplitude [652], Ib afferents were found to be almost completely insensitive to tendon vibration.

Vibration amplitude

The peak to peak amplitude of the vibration stimulus is an important determinant of the muscle afferent response. Using sinusoidal tendon vibration, Fallon and Macefield [652] reported that the average amplitude at which Ia afferents began to respond was ~0.225 mm, although some units were responsive to vibration at amplitudes as low as 0.05 mm. As the vibration amplitude was increased up to a maximum of 1 mm the recruitment of both Ia afferents and group II afferents was progressively enhanced [652]. However, at these amplitudes (≤ 1 mm), fewer than 15% of Ia afferents were driven in a 1:1 manner with vibration frequency, even at the lowest frequency used (20 Hz). In contrast, Hagbarth and colleagues [656] were able to drive 96% of Ia afferents in a 1:1 manner at frequencies ≥ 50 Hz when

using a vibration amplitude of ~1.5 mm. Interestingly, at amplitudes < 1 mm five out of six Ib afferents were completely unresponsive to vibration [652]. However, with an amplitude of ~1.5 mm Burke et al. [650] found that three out of three Ib afferents tested were responsive to vibration, albeit with much lower firing frequencies when compared to Ia afferents. This finding is difficult to reconcile with the observations of Cordo et al. [651], who found that all Ib afferents were completely insensitive to vibration, even at an amplitude of 2 mm.

In summary, it appears that vibration amplitudes > 1 mm may be necessary to drive Ia afferents optimally when using a sinusoidal waveform. However, greater amplitudes may also reduce the selectivity of the Ia afferent response, potentially enhancing both group II and Ib afferent discharge. While some studies have shown that Ib afferents may be more responsive to vibration at amplitudes >1 mm, others have found Ib afferents to be remarkably insensitive to vibration, even at amplitudes of up to 2 mm.

Shape of the vibration waveform

The shape of the vibration wave form influences the speed at which the vibration probe deforms the tendon [651, 652]. It is thought that rectangular pulses lead to a greater rate of tendon deformation than sinusoidal pulses [652], which can in turn influence the relative activation of muscle afferents.

While both Ia and group II afferents are sensitive to changes in muscle length, Ia afferents have greater velocity sensitivity, suggesting that rectangular pulses may bias the afferent response in favour of Ia afferent recruitment [651, 652]. This assertion may help to explain the seemingly contradictory findings of Fallon et al. [652] and Roll et al. [653, 655] who used microneurography to measure afferent responses to tendon vibration but employed sinusoidal and rectangular wave forms respectively. Despite Fallon and Macefield employing a larger vibration amplitude (up to 1 mm) compared to Roll's group (0.2-0.5 mm), they were only able to engage ~14% of Ia afferents to fire in a 1:1 manner with the vibration frequency. In contrast, using a rectangular waveform, Roll et al. [653, 655] reported that 100% of Ia afferents fired in a 1:1 manner with vibration frequency, with most able to follow vibration frequency up to at least 80 Hz. These findings suggest that the use of a rectangular waveform greatly enhances the population response of Ia afferents to tendon vibration, even when the amplitude of vibration is low (≤ 0.5 mm).

Force of vibration application

The force with which the vibration probe is applied to the tendon may also influence the recruitment of muscle afferents. At force levels < 10 N, a 1 N increase in force has been shown to as much as triple Ia afferent discharge when all other stimulation parameters remain unchanged [651]. For a given amplitude and frequency of vibration, Cordo et al. [651] observed a set level

of force at which maximum Ia afferent firing began to plateau. Using a sinusoidal wave form with a frequency of 110 Hz and amplitude of 1.5 mm the force level at which maximum Ia afferent firing first occurred was found to be < 8 N, with no appreciable increase in Ia afferent firing at greater force levels. However, when a lesser amplitude (0.25 mm) was employed, Ia afferent firing continued to increase in a linear manner up to at least 12 N of force. Thus, in order to recruit Ia afferents maximally it appears necessary to apply a force of at least 8 N and when lower vibration amplitudes are used (< 1.5 mm) greater force may need to be applied.

The effects of voluntary muscle contraction

Voluntary activation of the target muscle has a marked effect on the vibration sensitivity of all muscle afferents. Burke et al. [654] found that weak isometric muscle contractions greatly increase the response of both Ia afferents and group II afferents to vibration, provided they were not already firing at their frequency limit. This has been attributed to coactivation of α and γ motoneurons during a voluntary contraction, with the resultant γ -efferent drive greatly enhancing the vibration sensitivity of both primary and secondary spindle endings [654, 657, 658]. In addition to its effects on muscle spindles, voluntary muscle activation has been shown to greatly enhance the vibration sensitivity of Ib afferents. For instance, during isometric contractions, Burke et al. [654] found that Ib afferents were able to fire in a 1:1 manner at vibration frequencies as high as 70-110 Hz. Similarly, Roll et al. [655] observed

that even weak voluntary contractions of the receptor bearing muscle can lead to a single Ib afferent achieving a frequency limit of 40 Hz, from a limit \leq 10 Hz with the muscle relaxed. Likewise, Fallon et al. [652] reported that while Ib afferents were almost entirely insensitive to vibration at amplitudes \leq 1.0 mm when the target muscle was relaxed, contractions of just 5% of maximum voluntary contraction lead to 100% of Ib afferents responding to vibration at amplitudes of less than 0.1 mm.

Summary

In summary, microneurography studies in humans have shown that in a relaxed muscle, Ia afferents have a greater sensitivity to tendon vibration compared to both group II and Ib muscle afferents, with many Ia afferents firing in a phase-locked manner with vibration at frequencies of 80 - 220 Hz [654, 655]. In contrast, ~30-40% of group II and 30-100% of Ib afferents appear to be completely insensitive to tendon vibration [650-653, 655]. Those group II and Ib afferents that are stimulated by vibration rarely fire beyond a frequency limit of 50 Hz with many firing at upper limits of 10-20 Hz [650, 651, 653, 655]. Thus, while it cannot be said that tendon vibration is a selective stimulus for primary muscle spindles, the overall afferent response to vibration is likely to be strongly dominated by a large increase in the firing of Ia afferent nerve fibres.

The mechanical properties of the vibration stimulus can have a notable effect on the afferents recruited. Based on the available evidence, tendon vibration with a rectangular shaped waveform, at an amplitude of 0.5 mm and a frequency of 80-100 Hz may be the most effective method of stimulating a large number of Ia afferents as selectively as possible [655]. When using sinusoidal vibration, Ia afferents are more effectively driven with an amplitude of > 1 mm and a force of ≥ 8 N [651, 652, 654]. Even low levels of voluntary muscle contraction greatly enhance the sensitivity of muscle afferents to vibration including Ia, group II and Ib afferents [652, 654, 655]. Thus, during voluntary muscle contraction tendon vibration appears to be a far less specific stimulus for primary muscle spindles.

Prolonged vibration

As discussed above, short bursts (< 10 seconds) of tendon vibration greatly enhance the discharge of primary muscle spindles, producing a corresponding rise in Ia afferent output. However, when vibration is maintained for ≥ 10 seconds Ia afferent excitability is progressively diminished [659]. In this way, prolonged vibration can be used to experimentally induce a temporary γ -loop dysfunction. Following prolonged vibration, the subsequent reduction in Ia afferent discharge has been documented using direct microneurographic recordings [660] and indirect measures such as the H-reflex [659, 661, 662] and short latency stretch reflex [663, 664], which are strongly influenced by Ia afferent excitability. Prolonged vibration is thought to

suppress Ia afferent output by enhancing presynaptic inhibition [665], increasing the recruitment threshold of Ia afferent fibres and/or by enhancing neurotransmitter depletion at the Ia afferent terminal ending [661, 666]. Importantly, prolonged vibration does not lead to postsynaptic depression of α -motoneuron excitability in the vibrated muscle [667-669].

Methodological considerations

There is evidence that both the frequency and duration of the prolonged vibration stimulus strongly influence the depression of Ia afferent excitability. In this regard, Van Boxtel [659] examined the effects of Achilles tendon vibration (100 Hz) of differing durations (10-120 seconds) on the time it took for the Soleus H-reflex to recover to its original size. It was found that as the duration of vibration increased the recovery time of the H-reflex increased, taking ~5 minutes to return to its original size after a 2 minute period of tendon vibration. Similarly, Abbruzzese et al. [661] found a strong relationship between the duration of Achilles tendon vibration (40 Hz, 0.6 mm amplitude) and Soleus H-reflex depression. These authors showed that 20 minutes of vibration suppressed the H-reflex for ~11 minutes after the vibration had ceased. Finally, Heckman [662] reported that 20 minutes of Achilles tendon vibration (100 Hz, ≤ 3 mm amplitude) was capable of depressing the size of the Soleus H-reflex response by 55-100% and that the reflex did not return to control values until ~50 minutes after the vibration had ceased. The notably slower recovery observed by Heckman [662] after an identical period of

vibration (20 minutes) may relate to differences in the amplitude and frequency of vibration when compared to the parameters used by Abbruzzese et al. [661]. Given the findings from microneurography studies that Ia afferent discharge is enhanced at higher frequencies [650, 655] and greater amplitudes [652] of vibration, it is not unreasonable to suggest that Ia afferent recruitment was more robust with the vibration parameters used in Heckman's study. Presumably, greater Ia afferent activation would lead to greater presynaptic inhibition, afferent fibre refractoriness and/or neurotransmitter depletion, which would in turn manifest in a longer Ia afferent (and thus H-reflex) recovery time. Evidence to support this hypothesis is provided by Abbruzzese et al. [661] who examined the effects of varying vibration frequency on H-reflex recovery time. For a given duration of vibration, these authors found a progressive increase in H-reflex recovery time as the vibration frequency was increased.

Effects on maximum effort quadriceps activation

Tonic excitatory input from Ia afferents to the homonymous α -motoneuron pool is necessary to achieve full muscle activation [276]. Thus, prolonged vibration can be expected to impair muscle activation during subsequent maximum voluntary contractions. Such a decrease in maximal activation has been observed across a number of different muscles with prolonged vibration consistently suppressing peak torque [157, 180-182], EMG amplitude [157, 180, 181] and single motor unit firing rates [180]. A number of studies

have examined the effects of prolonged vibration on quadriceps muscle activation specifically, and are discussed below.

Kouzaki et al. [181] were the first authors to explore the effects of prolonged vibration on quadriceps activation during maximum effort isometric contractions. These authors applied sinusoidal vibration directly to the muscle belly of the rectus femoris in healthy controls at a frequency of 30 Hz, amplitude of 2-3 mm, force of 5-10 N and for a duration of 30 minutes. Participants performed maximum effort contractions of the quadriceps before and after vibration and knee extensor peak torque production and rectified EMG were compared. In a control experiment on a separate day, Kouzaki et al. [181] asked participants to perform maximum effort quadriceps contractions before and after sitting in the chair for 30 minutes, without any vibration. The control condition had no effect on knee extensor torque or muscle activation. In contrast, prolonged vibration led to a ~10% reduction in knee extensor peak torque and rectus femoris EMG amplitude was found to decrease significantly more than in the vastus medialis or vastus lateralis, which remained largely unaffected.

These findings were replicated by Jackson and Turner [182] who applied 30 minutes of vibration to the rectus femoris of healthy controls at an amplitude of 1.5–2 mm and two different frequencies of 30 Hz and 120 Hz. The force of

application was not described. Both 30 Hz and 120 Hz vibration were found to reduce knee extensor isometric torque but 30 Hz vibration appeared to have a greater disfacilitatory effect than 120 Hz, particularly on rectus femoris EMG. Similar to the findings of Kouzaki et al. [181] the effects were localised to the vibrated rectus femoris muscle, with neither vibration frequency having a significant effect on vastus lateralis EMG amplitude.

A series of studies by Yu Konishi and his colleagues [157, 160, 183, 501, 670-672] have examined the effects of prolonged vibration on knee extensor isometric torque and integrated EMG in knee injured, elderly and young healthy populations. The parameters of vibration have been similar in each study, with sinusoidal wave forms applied to the infrapatellar tendon at a frequency of 50 Hz, amplitude of 1.5 mm, application force of 25-30 N and duration of 20 minutes. Using these parameters, in a total of 79 young healthy control participants, changes in quadriceps activation were remarkably similar, with knee extensor peak torque decreasing 9-13 %, vastus medialis integrated EMG decreasing 15-18% and vastus lateralis integrated EMG decreasing by 18-20% [78, 157, 183, 501, 670-672].

Using identical vibration parameters to Konishi and his colleagues, Richardson et al. [83] examined the effects of prolonged vibration on knee extensor torque and quadriceps EMG amplitude in a group of 14 young individuals

and a group of 14 older individuals, both without a history of knee injury. Similar to previous findings, Richardson observed a 7% reduction in knee extensor peak torque and 15% and 11% reductions in vastus medialis and vastus lateralis EMG amplitude respectively, in the younger group. However, vibration failed to suppress quadriceps activation in the older group (mean age 66 ± 1 year); suggesting that ageing per se may have an influence on quadriceps Ia afferent transmission. This conclusion is clouded somewhat by the findings of Konishi et al. [670], who compared the effects of prolonged vibration on quadriceps activation in a young healthy (mean age 29 ± 5 years) and elderly healthy group (mean age 76 ± 6 years), both without history of knee injury. The decline in knee extensor isometric torque was significantly lower in the elderly group compared to the young group but no age related differences could be found in vastus medialis, vastus lateralis or rectus femoris EMG responses to vibration, which were depressed by a similar amount in both groups.

Finally, Konishi et al. [671] examined the effects of prolonged vibration on maximum effort concentric and eccentric quadriceps contractions. They found similar declines in torque and EMG compared to earlier studies examining isometric contractions (Table 6.1), suggesting that Ia afferent input is necessary to achieve full muscle activation across all types of contraction.

Table 6.1. The effects of prolonged vibration on knee extensor peak torque and integrated EMG across different modes of muscle contraction*.

Mode of Contraction	Peak torque (Nm)			VM EMG (mV)			VL EMG (mV)		
	Pre	Post	% Δ	Pre	Post	% Δ	Pre	Post	% Δ
Isometric	235	211	-10%	0.220	0.190	-15%	0.250	0.200	-19%
Concentric	192	162	-16%	0.074	0.071	-5%	0.071	0.062	-13%
Eccentric	300	247	-18%	0.130	0.105	-19%	0.126	0.105	-17%

*Data are mean values and percentage change and are calculated from studies involving the same lead author [157, 671] using identical vibration parameters (50 Hz, 1.5 mm amplitude, 30 N force and 20 minutes duration). MVC = Maximum voluntary contraction. Nm = Newton metres. EMG = Electromyography. mV = Millivolts. VM = vastus medialis. VL = vastus lateralis. % Δ = percentage change.

Summary

Prolonged vibration (~10 seconds - 30 minutes) using frequencies ≥ 15 Hz has been shown to suppress Ia afferent excitability for an extended period of time. Twenty minutes of tendon vibration (40-100 Hz, 0.6-3.0 mm amplitude) impairs Ia afferent transmission for ~10-50 minutes after the vibration has ceased. There may be multiple mechanisms by which vibration achieves these effects, including an increase in presynaptic inhibition, increased refractoriness of the Ia afferent nerve fibres and increased neurotransmitter depletion at the Ia afferent terminal ending [661, 665, 666]. The frequency

and duration of the vibration stimulus influence the extent of Ia afferent suppression, with higher frequencies (up to 100 Hz) and longer durations (up to 30 minutes) shown to suppress Ia afferent excitability for longer periods [659, 661, 662]. Existing data for the quadriceps muscle has consistently shown that in young healthy control participants, 20-30 minutes of 30-50 Hz muscle or tendon vibration with a sinusoidal wave form, an amplitude of ≥ 1.5 mm and an application force of 5-30 N suppresses knee extensor torque and EMG during subsequent maximum voluntary contractions, usually by a magnitude of 7-20% [78, 83, 157, 181-183, 501, 670-672]. Importantly, tendon vibration appears to be more effective than muscle belly vibration at inducing widespread reductions in quadriceps EMG activation, affecting all portions of the muscle [157]. In contrast, muscle belly vibration appears to maximally disfacilitate the vibrated portion of the quadriceps (e.g. rectus femoris) without strongly influencing its synergists (e.g. vastus medialis, vastus lateralis) [181, 182]. Furthermore, there is evidence that normal Ia afferent discharge is essential to achieve full quadriceps activation across all types of contraction, with similar post vibration declines in peak torque and EMG amplitude in isometric, concentric and eccentric contractions [157, 671]. The effects of ageing on quadriceps Ia afferent drive remain unclear. One study has reported that prolonged vibration fails to suppress quadriceps activation in healthy older controls [83] and one study has presented equivocal evidence [670]. Nevertheless, these findings suggest that age may be an important factor to take into account when comparing the effects of prolonged vibration across groups.

METHODS

Participants

Four groups of participants volunteered to take part in this study. Twelve participants who had had an ACL reconstruction in the past 24 months (range: 5-22 months) and 15 participants with OA of the knee joint (Kellgren Lawrence Score ≥ 2) were recruited. These groups were matched in age and gender to corresponding groups of 12 and 15 healthy control participants with no history of knee joint injury or pathology (Table 6.2). Participants from all groups responded to advertisements requesting volunteers for research examining muscle weakness in people with knee joint injury and pathology. Participants in the ACL reconstruction group were included if they had a hamstring or a hamstring/gracilis graft. All volunteers in the OA group had ongoing knee pain and had previously been diagnosed with OA by their General Practitioner. Volunteers in all groups were excluded if they had a previous history of lower limb or spinal surgery (apart from ACL reconstruction), back pain in the last 6 months with associated neurological signs or symptoms or any pathology that precluded their participation in maximum effort strength testing. Participants provided written informed consent for all experimental procedures. Ethical approval for this study was granted by the Auckland University of Technology Ethics Committee in accordance with the principles set out in the Declaration of Helsinki.

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Table 6.2. Participant characteristics.

	ACLR group	Younger controls	OA group	Older controls
Age in years, mean (SD)	25.4 (9.0)	26.1 (8.4)	63.0 (9.7)	62.4 (10.5)
Height in metres, mean (SD)	1.71 (0.10)	1.73 (0.08)	1.69 (0.10)	1.70 (0.07)
Mass in kilograms, mean (SD)	69.1 (11.5)	65.7 (9.8)	77.4 (16.9)	70.0 (9.1)
Female, number (%)	8 (66.7)	8 (66.7)	8 (53.3)	8 (53.3)
Dominant limb tested, number (%)	3 (25.0)	3 (25.0)	9 (60.0)	9 (60.0)
Time since surgery in months, mean (SD)	10 (5.4)	-	-	-
Radiographic knee OA, number (%) *				
Grade II			4 (26.7)	-
Grade III			6 (40.0)	-
Grade IV			5 (33.3)	-
Medial compartment			13 (86.7)	-
Lateral compartment			9 (60.0)	-
Patellofemoral compartment			11 (73.3)	-
Bilateral knee OA, number (%)			6 (40.0)	-

ACLR = Anterior cruciate ligament reconstruction. OA = Osteoarthritis. SD = Standard deviation. * = more symptomatic knee in patients with bilateral OA. No significant between group differences were found for age, height or mass in ACL reconstruction and younger control groups (all $p > 0.05$). No significant between group differences were found for age, height or mass in OA and older control groups (all $p > 0.05$)

Radiographic assessment

Participants in the OA group were required to have a radiograph of the affected knee joint in the 2 weeks prior to data collection. Weight-bearing, fixed flexion radiographs of the knee were taken in the posteroanterior and lateral views [673] and scored by a single radiologist according to the Kellgren Lawrence scale [674]. Only participants with a Kellgren Lawrence Score ≥ 2 were included in the study.

Part A: Prolonged vibration

Experimental setup

All participants performed a standardised, 5 minute warm-up on an exercycle. Thereafter, participants were seated in a custom designed chair with the hips and knees flexed to 90°. Straps were firmly secured over the distal third of the thigh and across the chest to limit extraneous movement. A rigid strap was secured around the ankle, slightly superior to the malleoli. This was coupled to a metal attachment that was connected in series to a uniaxial load cell (Precision Transducers, Auckland, New Zealand), aligned horizontally with the ankle joint.

Knee extensor and knee flexor peak torque

Peak torque was measured in the injured limb of the ACL reconstruction

participants, the (most) affected limb of the OA participants and the matched limb (dominant/non-dominant) of the healthy controls. All participants were asked to perform maximum voluntary contractions of their knee extensors and knee flexors by pushing or pulling as hard as possible against the ankle strap. A series of 4 submaximal knee extension and 4 submaximal knee flexion contractions (25%, 50%, 50% and 75% of perceived maximum effort) were performed, with a 1 minute rest given between each contraction. Thereafter, a 2 minute rest was given before a set of three (6 second) maximum voluntary contractions of the knee extensors were performed followed by 3 (6 second) maximum voluntary contractions of the knee flexors. Participants received a consistent level of verbal encouragement [320] during each contraction. A 2 minute rest period was given between each maximum effort contraction. In the event that the peak force (N) produced during the maximum voluntary contractions continued to increase with each subsequent trial, a 4th, and in some cases a 5th, contraction was performed until force plateaued or decreased. This was done in an effort to elicit a true maximum effort from each individual [339]. Force (N) signals were recorded from the load cell during each contraction, where they were amplified (x100), sampled (1000 Hz) and displayed in real-time on a computer monitor placed in front of the participant using a customised software programme (Testpoint 7, Measurement Computing Corporation, Norton, USA).

Electromyography

During each maximum voluntary contraction, surface EMG signals were collected from the vastus medialis, vastus lateralis, semitendinosus and biceps femoris muscles. Prior to the placement of electrodes, the skin was shaved, abraded and cleaned with alcohol to reduce signal impedance. Bipolar Ag-AgCl disc electrodes (Norotrode 20, Myotronics Inc., Kent, USA) with an interelectrode distance of 2.2cm were positioned over the target muscles in accordance with surface electromyography for the non-invasive assessment of muscles guidelines [365]. A ground electrode (Red Dot, 3M, St Paul, USA) was positioned over the proximal tibia. All EMG signals were amplified (x1000), filtered (10 Hz – 1000 Hz) (AMT-8, Bortec Biomedical, Alberta, Canada), and sampled at 2000 Hz (Micro 1401, Cambridge Electronic Design, Cambridge, UK).

Prolonged vibration

Following the initial set of knee extensor and knee flexor maximum voluntary contractions, vibration was applied to the infrapatellar tendon using an electrodynamic shaker (Ling Dynamic Systems, Herts, UK) controlled by a customised software programme (Signal 3, Cambridge Electronic Design, Cambridge, UK) (Figure 6.1). Vibration was maintained for 20 minutes at a frequency, amplitude and force of 50 Hz, 1.5 mm and 25-30 N respectively [83, 157]. Participants were asked to remain as still as possible during the

application of vibration. The leg was clamped in place for the duration of the vibration period to prevent movement of the tendon relative to the vibration probe. Immediately after vibration, participants performed another set of at least three knee extensor maximum voluntary contractions and three knee flexor maximum voluntary contractions, in an identical manner to that described above. To avoid potential bias, participants were kept unaware of the hypothesis of the study and the purposes of the vibration until the end of the experimental procedures.

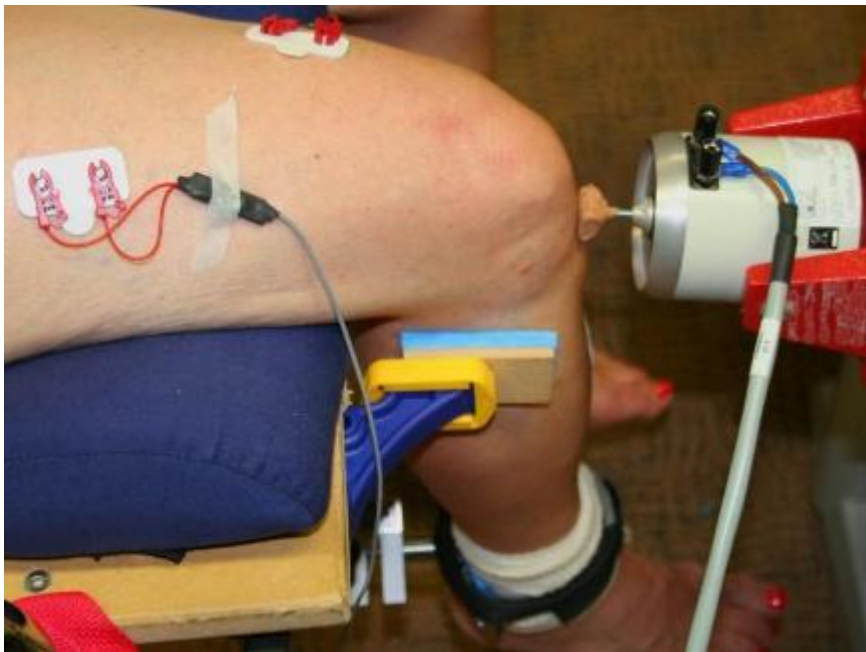


Figure 6.1. Experimental set up used during vibration of the infrapatellar tendon.

Data analysis

At each measurement interval, peak isometric knee extensor and knee flexor strength were calculated as the highest force (N) produced during any of the 3-5 maximum voluntary contractions performed for each muscle group. The length of the lever arm was measured from the lateral epicondyle of the femur to the centre of the ankle strap, which was parallel to the load cell. Lever arm length (m) was then multiplied by peak isometric force (N) to calculate peak torque (Nm). Using specialised software (Signal 3, Cambridge Electronic Design, Cambridge, UK) the root mean square (RMS) of the EMG signals from each muscle were calculated from a one second period corresponding to the time of maximum activation for each contraction.

Statistical analysis

To assess whether the dependent variables conformed to a normal distribution, Shapiro-Wilk tests were completed. Independent t-tests were used to analyse differences in baseline characteristics between the ACL reconstruction and younger control group and OA and older control group respectively. Between group differences in previbration knee extensor and knee flexor peak torque (Nm) were analysed using an analysis of covariance, with body mass (kg), age and gender as the covariates [675]. One sample t-tests were undertaken to analyse whether percent changes in knee extensor and knee flexor torque and EMG amplitude differed from zero after vibration

in each group. In addition, ANCOVAs were undertaken to analyse between group differences in the percent change in knee extensor and knee flexor torque and EMG amplitude following vibration. The covariates were age, gender and mass. The alpha level for all statistical procedures was set to 0.05.

Part B: Short duration vibration

Experimental setup

The testing session for part B took place an average of 6 ± 3 days after the initial testing session undertaken in part A and the experimental setup was identical.

Knee extensor peak torque

Knee extensor peak torque was collected in a similar manner to part A for all participants. Following the same submaximal warm up, a set of 12 (6 second) maximum voluntary contractions of the knee extensors were performed with (5 Hz, 80 Hz, 200 Hz) and without (0 Hz) superimposed vibration of the infrapatellar tendon. A 2 minute rest period was given between each maximum effort contraction and participants received a consistent level of verbal encouragement [320]. Force (N) signals were recorded from the load cell, where they were amplified (x100), sampled (1000 Hz) and displayed in real-time on a computer monitor placed in front of the participant using a

customised software programme (Testpoint 7, Measurement Computing Corporation, Norton, USA).

Electromyography

During each maximum voluntary contraction, surface EMG signals were collected from the vastus medialis and vastus lateralis muscles. Skin preparation, the electrode type and electrode placement was identical to part A. All EMG signals were amplified (x1000), filtered (10 Hz – 1000 Hz) (AMT-8, Bortec Biomedical, Alberta, Canada), and sampled at 2000 Hz (Micro 1401, Cambridge Electronic Design, Cambridge, UK).

Short duration vibration

Participants began each maximum voluntary contraction with the 1 cm² probe of an electrodynamic shaker (Ling Dynamic Systems, Herts, UK) lightly touching their infrapatellar tendon. As the participant contracted their quadriceps, the probe was manually pushed against the infrapatellar tendon. The force of application was controlled to be between 20 and 30 N, as measured using a hand held dynamometer. Participants were asked to ignore the vibration probe and focus on generating as much force as possible during each contraction. Rectangular shaped vibration pulses with an amplitude of 0.5 mm [653, 655] were applied to the infrapatellar tendon for ~5 seconds during each quadriceps contraction. Vibration frequency was

varied to allow 4 different conditions to be tested across the 12 maximum voluntary contractions (3 repetitions of each condition). The frequencies used were 0 Hz (no vibration), 5 Hz (sham condition), 80 Hz and 200 Hz. During the 0 Hz condition, the probe of the electrodynamic shaker was pushed into the infrapatellar tendon in an identical manner to each of the other conditions except that no vibration was applied. The order of the vibration conditions was randomised for each participant. To avoid potential bias, both the participant and the researcher delivering the vibration were blinded to the upcoming condition (i.e. 0 Hz, 5 Hz, 80 Hz or 200 Hz).

Data analysis

For each condition, peak isometric knee extensor strength was calculated as the highest force (N) produced during any of the three maximum voluntary contractions performed. The length of the lever arm was measured from the lateral epicondyle of the femur to the centre of the ankle strap, which was parallel to the load cell. Lever arm length (m) was then multiplied by peak isometric force (N) to calculate peak torque (Nm). Using specialised software (Signal 3, Cambridge Electronic Design, Cambridge, UK) the RMS of the EMG signals from each muscle were calculated from a one second period corresponding to the time of maximum activation for each contraction. Knee extensor peak torque and RMS values were normalised to express them as a percentage of the values recorded during the no vibration (0 Hz) condition.

Statistical analysis

To assess whether the dependent variables conformed to a normal distribution Shapiro-Wilk tests were completed. Separate ANCOVAs were undertaken for the (normalised) dependent variables to analyse whether any group by condition interactions were present during vibration (5 Hz, 80 Hz, 200 Hz) in the younger control vs. older control groups and OA vs. ACL reconstruction groups respectively. The covariates were age, gender and mass. As no group by condition interactions were found, the younger and older controls were combined into a single group (control group, $n = 27$), as were the OA and ACL participants (patient group, $n = 27$). One sample t-tests were used to assess whether the peak torque and EMG amplitude differed from the no vibration (0 Hz) condition for each group. The significance level for all statistical procedures was set to 0.05.

RESULTS

Participant characteristics

Baseline characteristics for each group are provided in Table 6.2. There were no statistically significant differences in age, height or mass (all $p > 0.05$) between the ACL reconstruction and younger control group or between the OA and older control group.

Baseline peak torque

Previbration knee extensor ($p < 0.05$) and knee flexor ($p < 0.01$) peak torque were significantly lower in the ACLR group compared to the younger control group (Figure 6.2). Previbration knee extensor peak torque was significantly lower in the OA group compared to the control group ($p < 0.01$). The difference in knee flexor peak torque did not reach statistical significance ($p > 0.05$) (Figure 6.2).

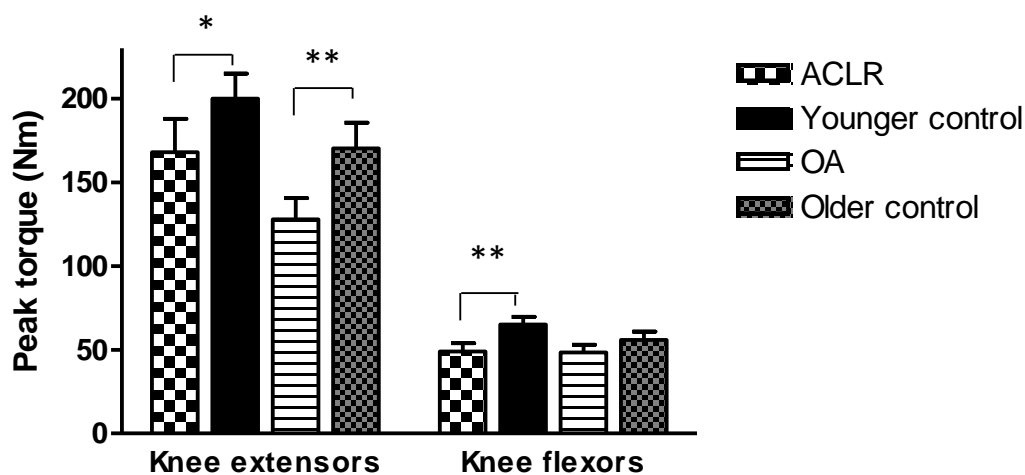


Figure 6.2. Baseline knee extensor and knee flexor peak torque (Nm) in all groups. ACLR = anterior cruciate ligament reconstruction. OA = osteoarthritis.

* = significant difference between groups ($p < 0.05$). ** = significant difference between groups ($p < 0.01$). Data are means and one standard error of the mean.

Part A: Prolonged vibration

Knee extensor and flexor peak torque

A summary of peak torque values at each measurement interval is presented in Table 6.3. Following tendon vibration, a statistically significant decrease in knee extensor peak torque was observed in both control groups (both $p < 0.001$) but not in the ACLR or OA groups (both $p > 0.05$) (Figure 6.3). The change in knee extensor torque was significantly different between the ACL reconstruction and younger control groups ($p < 0.05$) and the OA and older control groups ($p < 0.05$) respectively.

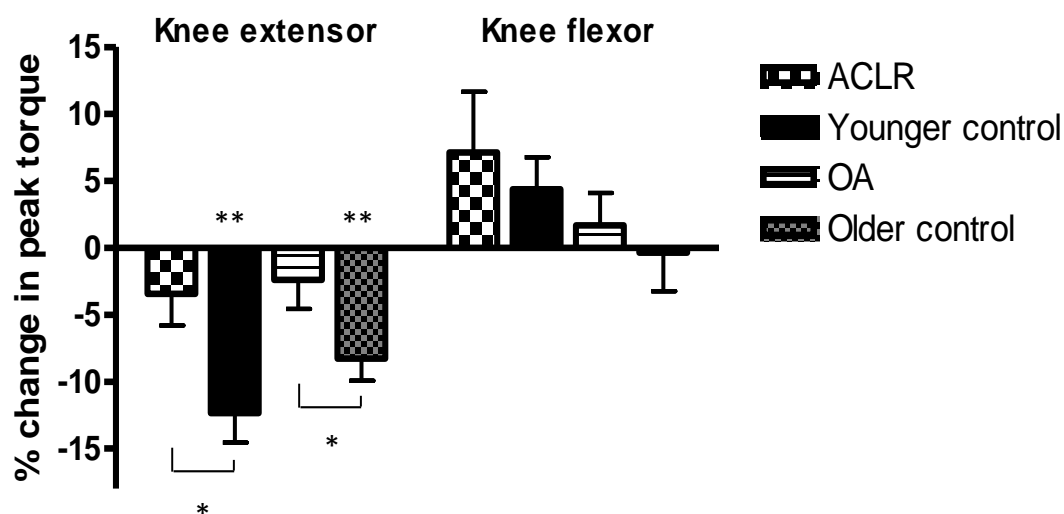


Figure 6.3. Percentage change in knee extensor and knee flexor peak torque (Nm) following prolonged vibration. ACLR = anterior cruciate ligament reconstruction. OA = osteoarthritis. * = significant difference between groups ($p < 0.05$). ** = significant change from zero ($p < 0.001$). Data are means and one standard error of the mean.

After vibration, the change in knee flexor peak torque did not differ from zero in any of the groups (all $p > 0.05$). The change in knee flexor peak torque was not significantly different between the ACL reconstruction and younger control groups ($p > 0.05$) or the OA and older control groups ($p > 0.05$).

EMG amplitude

A summary of RMS values at each measurement interval is presented in Table 6.3. After vibration, a statistically significant decrease in vastus medialis RMS was observed in both control groups ($p \leq 0.001$) but not in the ACL reconstruction ($p > 0.05$) or OA groups ($p > 0.05$) (Figure 6.4). Similarly, vastus lateralis RMS decreased after vibration in both control groups ($p \leq 0.001$), but not in the ACL reconstruction ($p > 0.05$) or OA groups ($p > 0.05$). The change in vastus medialis ($p < 0.01$) and vastus lateralis ($p < 0.05$) RMS was significantly different between the ACL reconstruction and younger control groups. The change in vastus medialis ($p < 0.01$) and vastus lateralis ($p = 0.001$) RMS was significantly different between the OA and older control groups. After vibration, the change in semitendinosus and biceps femoris RMS values did not differ from zero in any of the groups (all $p > 0.05$) and the changes did not differ between the ACL reconstruction and younger controls groups or the OA and older control groups (both $p > 0.05$).

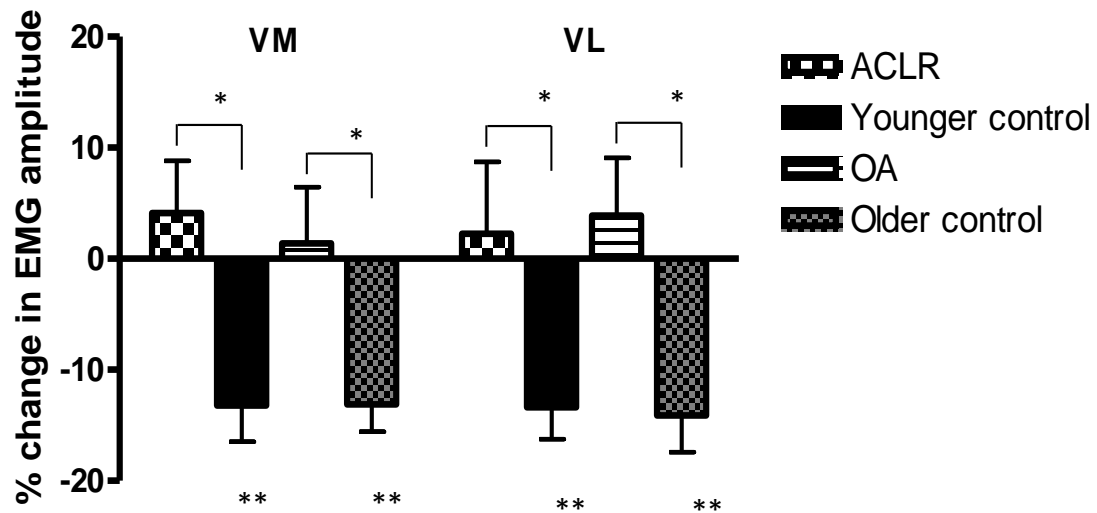


Figure 6.4. Percentage change in quadriceps EMG amplitude following prolonged vibration. ACLR = anterior cruciate ligament reconstruction. OA = osteoarthritis. VM = vastus medialis. VL = vastus lateralis. * = significant difference between groups ($p < 0.05$). ** = significant change from zero ($p \leq 0.001$). Data are means and one standard error of the mean.

Table 6.3. Summary of dependent variables before and after vibration.

Variable	Group	Pre	Post	% change
Knee extensor PT * ψ	ACLR	168 \pm 68	160 \pm 61	-3.4%
	Younger controls	200 \pm 54	174 \pm 44	-12.4% **
	OA	128 \pm 49	124 \pm 44	-2.4%
	Older controls	170 \pm 59	156 \pm 55	-8.2% **
VM RMS * ψ	ACLR	0.32 \pm 0.25	0.32 \pm 0.24	4.1%
	Younger controls	0.40 \pm 0.17	0.34 \pm 0.13	-13.2% **
	OA	0.13 \pm 0.06	0.13 \pm 0.06	1.4%
	Older controls	0.27 \pm 0.19	0.24 \pm 0.18	-13.3% **
VL RMS * ψ	ACLR	0.31 \pm 0.27	0.31 \pm 0.26	2.2%
	Younger controls	0.32 \pm 0.15	0.29 \pm 0.16	-13.4% **
	OA	0.13 \pm 0.06	0.13 \pm 0.06	3.9%
	Older controls	0.22 \pm 0.13	0.19 \pm 0.13	-14.1% **
Knee flexor PT	ACLR	49 \pm 18	52 \pm 19	7.1%
	Younger controls	65 \pm 16	67 \pm 18	4.4%
	OA	48 \pm 18	49 \pm 16	1.7%
	Older controls	56 \pm 19	55 \pm 19	-0.4%
ST RMS	ACLR	0.19 \pm 0.09	0.19 \pm 0.08	4.7%
	Younger controls	0.35 \pm 0.15	0.33 \pm 0.13	-0.8%
	OA	0.17 \pm 0.09	0.18 \pm 0.10	7.1%
	Older controls	0.22 \pm 0.12	0.23 \pm 0.15	3.8%
BF RMS	ACLR	0.14 \pm 0.06	0.13 \pm 0.05	-5.2%
	Younger controls	0.23 \pm 0.10	0.22 \pm 0.07	1.5%
	OA	0.12 \pm 0.10	0.10 \pm 0.08	-2.5%
	Older controls	0.16 \pm 0.07	0.16 \pm 0.07	2.6%

Data are means \pm SD. PT = peak torque (Nm). RMS = root mean square. VM = vastus medialis. VL = vastus lateralis. ST = semitendinosus. BF = biceps femoris. ACLR = anterior cruciate ligament reconstruction. OA = osteoarthritis. * = significant difference between ACL reconstruction and younger controls ($p < 0.05$). ψ = significant difference between OA and older controls ($p < 0.05$). ** = significant change from zero ($p < 0.05$).

Part B: Short duration vibration

Knee extensor peak torque

Short duration vibration did not lead to a significant increase in knee extensor peak torque for either group (all $p > 0.05$) (Figure 6.5).

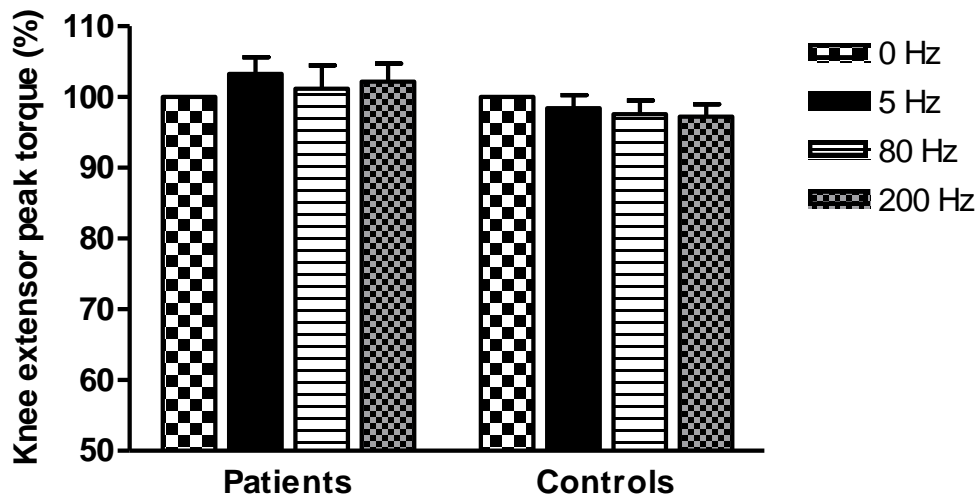


Figure 6.5. Knee extensor peak torque [normalised to a percentage of peak torque in the no vibration (0 Hz) condition] during short duration vibration of the infrapatellar tendon at different frequencies. Data are means and one standard error of the mean.

EMG amplitude

Short duration vibration did not lead to a significant increase in vastus medialis EMG amplitude for either group (all $p > 0.05$) (Figure 6.6). Similarly, there was no significant increase vastus lateralis EMG amplitude with short duration vibration (all $p > 0.05$) (Figure 6.7).

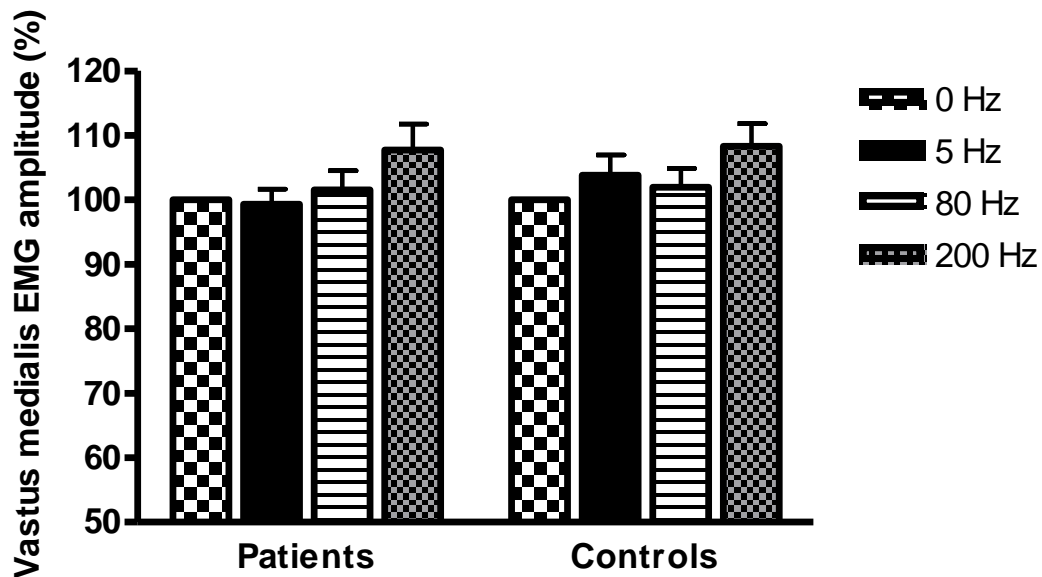


Figure 6.6. Vastus medialis (VM) EMG amplitude [normalised to a percentage of VM EMG amplitude in the no vibration (0 Hz) condition] during short duration vibration at different frequencies. Data are means and one standard error of the mean.

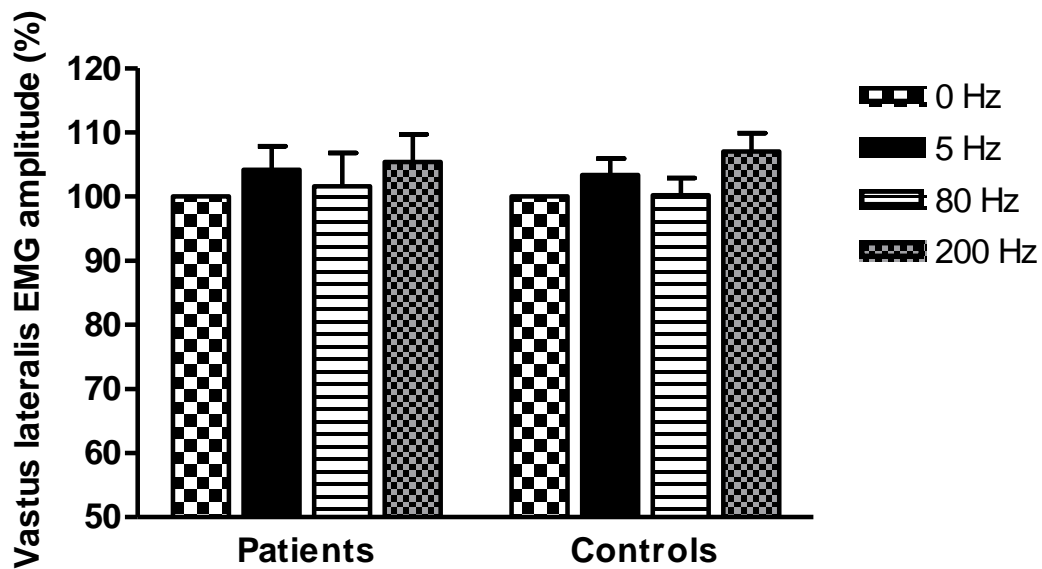


Figure 6.7. Vastus lateralis (VL) EMG amplitude [normalised to a percentage of VL EMG amplitude in the no vibration (0 Hz) condition] during short duration vibration at different frequencies. Data are means and one standard error of the mean.

DISCUSSION

In part A of this study, prolonged vibration failed to impair quadriceps activation in participants who had recently undergone an ACL reconstruction. This is in agreement with previous findings from populations with a ruptured but unrepaired ACL [157] and following ACL reconstruction [83, 501]. For the first time, the current study also showed that γ -loop dysfunction may contribute to quadriceps AMI in individuals with knee joint OA. Prolonged tendon vibration

induces a temporary γ -loop dysfunction by impairing the afferent transmission from Ia fibres to the homonymous α -motoneuron pool [179]. The subsequent loss of excitatory sensory input reduces α -motoneuron excitability, preventing full activation of the muscle. Thus, a decrease in knee extensor peak torque and quadriceps EMG amplitude is expected after vibration, as observed in both the younger and older healthy control groups. In contrast, the lack of change in quadriceps activation seen in the ACL reconstruction and OA groups suggests that Ia afferent transmission was already impaired in these individuals, thus torque and EMG amplitude were unaffected by vibration.

While prolonged vibration is a useful neurophysiological tool to explore the function of the γ -loop, it does not allow us to accurately determine the contribution of γ -loop dysfunction to the overall magnitude of AML, or quadriceps weakness. AML can be severe in individuals with knee OA and following ACL injury, with quadriceps voluntary activation deficits of 25-35% observed [13, 74, 676, 677]. While the ~8-13% reduction in postvibration knee extensor torque seen in the control groups may suggest that the γ -loop makes a relatively small contribution to the overall level of AML, this is not necessarily true. Microneurography studies have demonstrated that in relaxed muscles, the firing rate of most Ia afferent fibres is depressed following vibration and the spindle response to stretch is reduced by ~25% [660]. Furthermore, H-reflex amplitude,

which is partly determined by Ia afferent transmission, is reduced by ~30-40% following prolonged vibration [678]. However, we cannot be sure what portion of the Ia afferent drive is impaired in pathological populations with γ -loop dysfunction. We can simply observe that prolonged vibration has no additional effect on OA or ACL reconstructed participants' ability to activate their quadriceps, which suggests that these individuals have a pre-existing impairment in Ia afferent drive that is at least at the same level as that produced by 20 minutes of vibration. For example, it may be that ~30% of the effective Ia afferent drive is impaired by prolonged vibration but that in a knee injured group with γ -loop dysfunction, ~80% of the effective Ia afferent drive is impaired. In this case, prolonged vibration may have no additional effect on quadriceps activation but neither would the change in quadriceps activation observed in healthy controls represent the true effect of γ -loop dysfunction on quadriceps activation in a pathological population (which would be greater). Furthermore, as AMI is caused by activity in multiple inhibitory pathways [66, 679], the influence of γ -loop dysfunction may be underestimated in individuals with OA. This is due to spatial facilitation and the all-or-nothing nature of α -motoneuron depolarisation. While firing of a discrete number of α -motoneurons may be completely prevented by a given inhibitory input, others will only be partially inhibited and are still able to depolarise [229]. However, when two (or more) forms of inhibitory/disfacilitatory input are present, the partial inhibition produced by each input is often sufficient to prevent depolarisation of a greater

number of α -motoneurons, so that the total inhibition is greater than the algebraic sum of the individual inhibitory/disfacilitatory inputs [521]. In this way, even if prolonged vibration exactly mimicked the loss of Ia afferent drive produced by γ -loop dysfunction, the effects of γ -loop dysfunction on quadriceps activation may be far greater in a pathological population than the effects of prolonged vibration on quadriceps activation in healthy controls, where there are no additional sources of inhibition/disfacilitation.

While the findings from part A of this study suggest that γ -loop dysfunction contributes to AMI after ACL reconstruction and in knee joint OA, the mechanisms leading to γ -loop dysfunction are uncertain and it is not yet clear how this problem could be addressed in a rehabilitation setting. In part B of this study, we investigated the effects of short duration tendon vibration on quadriceps muscle activation. As expected, short duration vibration failed to augment knee extensor peak torque and EMG amplitude in healthy controls. Presumably, Ia afferent drive is already sufficient to drive quadriceps α -motoneurons maximally or near maximally in these individuals and any additional Ia afferent input is unlikely to have an appreciable effect on muscle activation [276, 680]. However, contrary to our hypothesis, short duration tendon vibration failed to enhance knee extensor peak torque and EMG amplitude in the OA and ACL reconstruction groups, despite these groups having an

established γ -loop dysfunction. There are a number of possible explanations for these findings, each of which will be discussed below.

Firstly, it should be emphasised that even during isometric muscle contractions, extrafusal muscle fibres still undergo a degree of shortening. In the absence of effective intrafusal muscle fibre contraction (e.g. due to inefficient γ -efferent drive), mechanical unloading or “slackening” of the spindles may occur as the extrafusal fibres shorten [654]. This has previously been shown to reduce the sensitivity of muscle spindles to vibration [654]. Thus, it could be that impaired γ -efferent drive in the ACL reconstruction and OA participants in the current study led to spindle unloading during maximum effort isometric contractions. In turn, this would reduce the vibration sensitivity of the quadriceps muscle spindles and may prevent an effective increase in Ia afferent output. We attempted to account for spindle unloading in two ways. First, we used rectangular rather than sinusoidal waveforms when applying tendon vibration, as primary muscle spindles are thought to have a greater sensitivity to rectangular shaped pulses [652, 653]. In addition, we included a “supramaximal” 200 Hz vibration condition in order to compensate for the potential loss of vibration sensitivity in the unloaded spindles [276]. Despite these countermeasures, vibration was still unable to enhance quadriceps activation in patients with an established γ -loop dysfunction.

Another possible explanation for a lack of improvement in quadriceps activation is that vibration may simultaneously enhance the output of other afferent fibres (e.g. Ib afferents) that have a competing inhibitory effect on the quadriceps α -motoneuron pool. While tendon vibration is a relatively specific stimulus for primary muscle spindles in a quiescent muscle, this is not the case during voluntary muscle contraction. Microneurography studies [652, 654, 655] have shown that even weak muscle contractions greatly enhance the vibration sensitivity of Ib muscle afferents, with many firing in a 1:1 relationship with the vibration frequency, even at frequencies > 100 Hz. Thus, during maximum effort isometric contractions, tendon vibration can be expected to enhance Ib as well as Ia afferent discharge. Such an increase in Ib afferent discharge may have an autogenic inhibitory effect on the quadriceps α -motoneuron pool [166, 681] that would counteract the facilitatory effect of enhanced Ia afferent input. A lack of effective γ -efferent drive may compound this problem as the increase in vibration sensitivity of the spindle that usually occurs with α - γ coactivation [657, 658, 682] would be diminished, biasing the sensory output in favour of Ib afferents. Thus, it may be that muscle spindle unloading and/or enhanced autogenic inhibition explains the inability of short duration vibration to augment quadriceps activation the in OA and ACL reconstruction groups.

Against these arguments, Hagbarth et al. [276] provided clear evidence that short duration vibration is able to strongly enhance muscle activation in the presence of an experimentally induced γ -loop dysfunction. This study was performed in healthy control participants without a history of lower limb injury. Local anaesthetic was infused around the common peroneal nerve to experimentally block γ -efferent drive to the dorsiflexors. As expected, this led to a significant impairment in tibialis anterior maximum force production, EMG amplitude, and single motor unit firing rates. Crucially, Hagbarth et al. [276] showed that tendon vibration was able to augment single motor unit firing rates and increase maximum effort force production by 50-100%, despite a residual block in γ -efferent transmission. It is important to highlight that these results were obtained during maximum effort voluntary contractions (i.e. when the vibration sensitivity of Ib afferents is likely to be enhanced) and in the presence of a γ -efferent block (and thus spindle unloading). These findings suggest that neither spindle unloading nor an increase in Ib inhibition can fully explain our observation that vibration failed to augment quadriceps activation in participants with an established γ -loop dysfunction. Rather, it may be that the afferent portion of the γ -loop was impaired in the OA and ACL reconstruction groups and/or that the vibration induced increase in Ia afferent discharge was insufficient to overcome the inhibition produced by other spinal reflex pathways (e.g. group I non-reciprocal (Ib) inhibition, flexion reflex). These possibilities will be discussed below.

Dysfunction in the afferent portion of the γ -loop may be related to changes in synaptic efficacy at the Ia afferent α -motoneuron synapse or changes in the sensitivity of the muscle spindles themselves [187]. The synaptic efficacy of the Ia afferent α -motoneuron synapse is partly controlled by presynaptic inhibition. The interneurons mediating presynaptic inhibition can be modulated in a bidirectional manner and receive input from many different afferent fibres, including knee joint afferents [185, 186, 683]. Thus, it is theoretically possible that a change in joint afferent discharge associated with knee injury or pathology could increase presynaptic inhibition of quadriceps Ia afferents, at least partially accounting for the γ -loop dysfunction observed in individuals with OA and following ACL reconstruction [187]. The magnitude of quadriceps Ia afferent presynaptic inhibition has not yet been assessed in individuals with an established joint pathology. In healthy control participants, experimental manipulation of joint afferent discharge has led to disparate conclusions regarding the likely effects of joint damage on presynaptic inhibition. Palmieri et al. [187] found that paired reflex depression of the quadriceps H-reflex increased following experimental joint infusion, which the authors interpreted as evidence that quadriceps Ia afferent presynaptic inhibition is increased by joint swelling. In contrast, Marchand-Pauvert et al. [188] found no evidence that electrical stimulation of knee joint afferents modifies the initial, purely monosynaptic component of the quadriceps H-reflex, suggesting that joint afferent discharge does not have an appreciable effect on quadriceps Ia afferent presynaptic

inhibition. Similarly, in the cat, even strong electrical stimulation of the major nerve innervating the knee joint fails to produce a P-wave in the dorsal horn, suggesting that knee joint afferents are not particularly effective in generating presynaptic inhibition in Ia afferents [620]. Thus, it remains uncertain whether an increase in quadriceps Ia afferent presynaptic inhibition may contribute to γ -loop dysfunction. Furthermore, the excitability of the interneurons mediating presynaptic inhibition is typically suppressed by supraspinal pathways during voluntary muscle contractions and the stronger the contraction, the greater the reduction in presynaptic inhibition [665, 684]. This argues against a significant role for this mechanism in γ -loop dysfunction.

Alternatively, a change in the sensitivity of the muscle spindle may help to explain vibration's lack of efficacy. In this respect, independent to their γ -efferent drive, muscle spindles have been shown to receive sympathetic input via adrenergic receptors [189, 190]. Extensive work in animals has shown that increased activity in the sympathetic nervous system can reduce the resting discharge and responsiveness of muscle spindles to stretch [191-193]. As sympathetic activity may be increased by chronic joint pathology [194-196], it is possible that spindle sensitivity is affected via this mechanism. Furthermore, muscle disuse due to hind limb unloading has been shown to suppress spindle sensitivity in rats [197]. It is unknown whether a reduction in spindle sensitivity due

to enhanced sympathetic activity and/or muscle disuse occurs in humans and could partially explain γ -loop dysfunction.

Finally, Hagbarth et al.'s [276] previous findings that vibration enhances muscle activation during maximum voluntary contractions relied on an experimentally induced model of γ -loop dysfunction and were obtained from a group of healthy control participants. In contrast, the participants in our study had sustained significant damage to their knee joints that was likely associated with various degrees of joint swelling, inflammation, instability and damage to articular sensory receptors. Joint damage is thought to lead to quadriceps AML via multiple, independent neural pathways [66]. Rather than being mutually exclusive, it has been suggested that it is the combined activity of these pathways that determines the overall magnitude of AML [66]. As such, in our OA and ACL reconstruction participants it may be tendon vibration was successful at enhancing primary muscle spindle discharge but that ongoing inhibition from other neural pathways mitigated its excitatory effects, preventing the recruitment of additional motor units by the increase in Ia afferent input.

A limitation of the current study is that we did not confirm the presence of a quadriceps activation deficit in the ACL reconstruction or OA groups using techniques such as burst superimposition or interpolated twitch. As such, it

could be argued that despite evidence of γ -loop dysfunction, the ACL reconstruction and OA participants in this study may have learnt to fully activate their quadriceps in the absence of full excitatory input from Ia afferents. While this is theoretically possible, we consider it unlikely. Firstly, there is no evidence that full activation is possible in the absence of normal Ia afferent input. On the contrary, attenuating Ia afferent discharge has consistently been shown to impair full muscle activation in a wide variety of studies, across a number of different muscles [179]. Secondly, Konishi and colleagues have observed a significant impairment in quadriceps force production per unit volume of muscle (an indirect measure of activation) in ACL injured [685] and ACL reconstructed [686] participants compared to age matched controls. Furthermore, using interpolated twitch, Urbach et al. [13] have shown that quadriceps activation deficits may still be apparent 2 years after ACL reconstruction. Similarly, the majority of studies in people with knee joint OA have found clear evidence of AMI [687]. Those studies where quadriceps activation deficits were equivocal [92, 675, 688-691] all used burst superimposition to calculate quadriceps central activation ratios. The central activation ratio has consistently been shown to overestimate quadriceps activation compared to interpolated twitch [88, 692-694], while even interpolated twitch has been suggested to overestimate true muscle activation [89], (thus underestimating AMI).

CONCLUSION

The results of this study show for the first time that γ -loop dysfunction contributes to quadriceps AML in individuals with knee joint OA and confirm previous findings that this pathway is involved in AML after ACL reconstruction. Dysfunction in the γ -loop pathway results in a loss of Ia afferent feedback during strong voluntary contractions that prevents full activation of the muscle and may partially explain the marked quadriceps weakness and atrophy that is often observed in these populations. Quadriceps weakness is clinically important in these individuals as it associated with physical disability [47, 48, 51, 74], an increased rate of loading [53, 54] and has been identified as a risk factor for the initiation and progression of joint degeneration [55-57].

Contrary to our original hypothesis, short duration tendon vibration failed to enhance quadriceps muscle activation in the OA and ACL reconstruction groups, despite an established γ -loop dysfunction. Based on these findings, short duration vibration cannot be recommended as an intervention strategy to overcome the effects of quadriceps AML. The reasons for vibration's lack of efficacy are unclear but may be related to an underlying dysfunction in the afferent portion of the γ -loop or an inability of the increased excitatory input from muscle spindles to overcome inhibition of the quadriceps α -motoneuron pool mediated by other neural pathways.

Chapter Seven: Summary and Conclusions

AMI is a significant hindrance to effective rehabilitation, leading to severe and often persistent quadriceps muscle weakness in patients with knee joint pathology, following acute knee injury and after knee joint surgery. AMI is caused by a change in the discharge of sensory receptors in or around the damaged knee joint. Factors that may alter afferent discharge include swelling [107, 121], inflammation [115, 145, 175], joint laxity [155] and damage to articular sensory receptors [157]. Abnormal sensory output from knee joint changes the excitability of multiple neural pathways that combine to reduce quadriceps α -motoneuron excitability, preventing full activation of the muscle. However, the specific neurophysiological pathways involved in AMI, and their relative contribution across different types of joint pathology, are only partially understood. Furthermore, it has been argued that without targeting AMI directly, clinicians will be unable to restore quadriceps strength in an optimal and timely manner following joint damage [34, 65, 75]. To date, clinically useful interventions to counter AMI have been underdeveloped [64]. This thesis aimed to address some of these issues.

KEY FINDINGS

In Chapter 3, healthy participants with an uninjured knee had their joint infused saline dextrose solution to produce an experimental model of joint injury. For each participant the joint infusion was standardised to an intraarticular pressure of 50 mmHg. This is an important methodological advancement over previous studies [71, 123, 124, 128, 132, 205] and was done in order to reduce the inter-individual variability in afferent discharge due to differences in the size of the joint cavity and capsular elastance [121, 134]. As shown in previous research [68, 98, 124, 125, 132, 640], experimental joint swelling led to a significant decrease in knee extensor peak torque and EMG amplitude. In addition, we demonstrated for the first time that experimental joint swelling reduces quadriceps muscle fibre conduction velocity during subsequent maximum voluntary contractions. This likely reflects a preferential inhibition of high threshold motoneurons innervating large diameter, type II muscle fibres and is an important finding as such a mechanism could help to explain the greater type II atrophy that has been observed in some studies after knee injury [695] and in chronic pathologies such as knee OA [696, 697].

Following experimental joint infusion, participants were randomised into an experimental or a control group. The experimental group had three plastic bags of crushed iced cubes wrapped around the swollen knee joint for 20 minutes.

After icing the knee, knee extensor peak torque and muscle fibre conduction velocity increased significantly compared to the control group, who did not receive an intervention but remained seated in an identical position for the same 20 minute period. These findings provide the first evidence that cryotherapy can significantly reduce the severity of AML during strong voluntary contractions, when AML is clinically important. The magnitude of this effect is illustrated by the fact that knee extensor peak torque returned to within ~6% of baseline (preinfusion) values following the cryotherapy intervention. Since publishing our findings from this study [402], subsequent research has been conducted in patients with knee joint OA exploring the effects of cryotherapy on quadriceps muscle activation. In this study [81], a significant increase in quadriceps central activation ratio was observed after 20 minutes of knee joint cryotherapy. This was associated with an immediate increase in knee extensor peak torque of ~15%. The changes in quadriceps central activation were maintained for at least 30 minutes after the ice was removed [81]. Taken together with the results from Chapter 3, these findings have important clinical implications, suggesting that cryotherapy can be used to augment traditional resistance training in patients with knee joint injury, surgery or pathology. Applying 20 minutes of cryotherapy to the damaged knee joint immediately before quadriceps resistance training may provide a window of opportunity for clinicians, where greater neural activation of the quadriceps musculature can occur, enhancing gains in muscle hypertrophy and strength.

In order to determine which patients will benefit most from this intervention, it is important to understand the mechanisms by which cryotherapy reverses AML. Cryotherapy has previously been shown to slow nerve conduction velocity [243] and impair the firing of articular sensory receptors [233]. Thus, it is likely that cryotherapy enhances quadriceps activation, in part, by attenuating the abnormal joint afferent output that is responsible for causing AML. Moreover, there is evidence that cryotherapy also has a direct excitatory effect on the central nervous system. We explored this possibility further in Chapter 4, utilising TMS to examine the effects of cryotherapy on quadriceps corticomotor and intracortical excitability. A novel finding was that 20 minutes of cryotherapy applied to an uninjured knee joint enhances quadriceps corticomotor excitability. We found no evidence for a change in intracortical excitability with icing, suggesting a subcortical mechanism of action. Given previous findings that the H-reflex: M-wave ratio is enhanced by cryotherapy [130, 262], an excitatory effect at the spinal level seems likely, perhaps mediated by the release of monoamines from descending brainstem pathways [262, 269]. Importantly, the fact that icing has a direct excitatory effect on the central nervous system suggests that cryotherapy is likely to be at least partially effective across a broad range of knee joint pathologies, as quadriceps muscle activation should be enhanced regardless of whether AML is primarily caused by an increase or a decrease in sensory output from the damaged knee joint.

Also in Chapter 4, we examined the acute effects of swelling on quadriceps corticomotor and intracortical excitability in healthy individuals with a previously uninjured knee joint. This was the first time such a study has been undertaken. Using TMS, we observed a significant increase in vastus lateralis MEP area following experimental infusion of saline dextrose into the knee joint. As in Chapter 3, the level of effusion was standardised for each participant to an intraarticular pressure of 50 mmHg. While somewhat surprising, our finding of an increase in quadriceps corticomotor excitability is similar to previous observations of an increase in quadriceps MEP amplitude compared to healthy controls [204] and a decrease in MEP threshold on the injured side [203] in patients with chronic knee joint pathology.

These observations have been interpreted as evidence for increased corticospinal drive from the primary motor cortex to the quadriceps [203, 204]; perhaps in an attempt to counteract the strong reflex inhibition at a spinal cord level. However, this contention is purely speculative without a more detailed examination of changes in excitability at the level of the primary motor cortex. The study in Chapter 4 was the first to take such an approach by utilising paired-pulse TMS to allow the specific measurement of inhibitory (SICI) and facilitatory (ICF) interneuron excitability in the motor cortex. In addition we explored changes in intermuscular coherence in the β -band, providing an indirect

measure of common cortical drive to the quadriceps muscle. Despite a significant increase in quadriceps MEP area with experimental swelling, we found no evidence of a change in SICI or ICF, arguing against a change in motor cortex excitability. While we cannot absolutely rule out changes in the excitability of other cortical interneurons, such as that underlying short interval intracortical facilitation (SICF) or long interval intracortical inhibition (LICI), the lack of change in β -band coherence suggests that this is less likely. This interpretation is strengthened by recent findings that TMS combined with maximum effort voluntary contractions may partially reverse quadriceps AMI following total knee joint arthroplasty [226] and partial meniscectomy [227]. Despite a very low dose of TMS (three single pulses), knee extensor torque [226] and central activation [227] increased significantly following combined TMS and maximum voluntary contractions but not in controls who only performed maximum voluntary contractions [227]. If cortical drive to the quadriceps was already enhanced in these patients it seems unlikely that TMS would increase knee extensor torque and activation, particularly at such a low dose.

An alternative explanation for the observed increase in MEP area in Chapter 4 is that acute swelling (and possibly chronic joint pathology) enhances the excitability of subcortical structures that transmit a portion of the corticospinal volley to the quadriceps α -motoneuron pool. One such possibility is the lumbar

propriospinal interneurons, which are known to receive input from knee joint afferents [499] and have been shown to transmit a large portion of the corticospinal volley to the quadriceps a-motoneuron pool [431]. Importantly, if a greater *portion* of the TMS induced corticospinal volley is relayed via these propriospinal interneurons when compared to the *overall* voluntary motor command (which will reflect the sum of input from multiple descending pathways, relayed via multiple neuronal systems) this could explain the paradoxical increase in quadriceps MEP area despite no evidence of a change in cortical excitability and the putative inhibitory effects of swelling on quadriceps a-motoneuron excitability [71, 123, 205, 698]. Regardless of the mechanism, it appears that increased quadriceps corticomotor excitability is insufficient to overcome inhibition of the quadriceps a-motoneuron pool as several studies have shown that experimental joint infusion strongly reduces quadriceps EMG amplitude [68, 98] and peak torque production [98, 124, 125, 132] during maximum effort voluntary contractions and quadriceps activation deficits are common in patients with clinical joint effusion [82, 99, 143, 641]. Furthermore, using an identical joint infusion procedure in Chapter 3, we observed strong inhibition of knee extensor peak torque, EMG amplitude and muscle fibre conduction velocity during subsequent maximum voluntary contractions.

While swelling is clearly an important factor in AML, recent evidence [149] suggests that nociception alone may be sufficient to induce quadriceps activation deficits. Furthermore, following joint aspiration in patients with chronic arthritis, additional increases in knee extensor torque have been demonstrated following the infusion of local anaesthetic [143] or corticosteroid [142] into the knee joint. Animal studies [127, 161, 175, 504] provide strong evidence that joint inflammation facilitates flexion reflex excitability. As the flexion reflex produces a general pattern of flexor muscle facilitation and extensor muscle inhibition, it has been suggested [66, 169] that this pathway could contribute to AML. Several recent studies [79, 176, 177] in humans have found a lower flexion reflex threshold in patients with knee joint pathology compared to healthy controls, providing indirect evidence of a change in excitability in this pathway. However these studies were all cross-sectional in nature, with small sample sizes. Given the marked heterogeneity of the flexion reflex threshold in the normal population [178, 503], further exploration was warranted. Furthermore, while knee joint swelling has been shown to enhance group I non-reciprocal (Ib) inhibition of the quadriceps α -motoneuron pool [70], it is not clear whether it may affect the excitability of other pathways such as the flexion reflex. Chapter 5 aimed to address these issues by exploring the effects of knee joint aspiration and intraarticular corticosteroid injection on flexion reflex threshold and knee extensor peak torque in individuals with chronic knee joint arthritis. To our knowledge, this is the first study in humans to manipulate joint afferent discharge

and examine changes in flexion reflex excitability; although it has previously been reported that flexion reflex excitability was enhanced in 1 out of 2 subjects tested following experimental knee joint infusion (unpublished observations cited from [66]). In Chapter 5, we observed a significant decrease in flexion reflex excitability following knee joint aspiration and corticosteroid injection. This provides the strongest evidence to date that inflammation of the knee joint enhances flexion reflex excitability in humans, as has previously been shown in animals. Furthermore, we observed a small but significant increase in flexion reflex threshold following knee joint aspiration alone. This is a novel finding, suggesting that intraarticular swelling may enhance flexion reflex excitability as well as increasing the excitability of other spinal reflex pathways.

The change in flexion reflex threshold observed in Chapter 5 was accompanied by a notable increase (~25%) in knee extensor peak torque production after just 15 ± 2 days, suggesting a parallel reduction in quadriceps AML. Interestingly, this increase in quadriceps strength is of a similar magnitude to the reported change in knee extensor torque produced by 5-6 months of high intensity resistance training in patients with chronic arthritis [638, 639]. Recent work in patients with OA suggests that combining transcutaneous electrical nerve stimulation, an intervention known to reduce AML [81, 205], with resistance training produces greater improvements in knee extensor peak torque than resistance training

alone [25]. Furthermore, in patients with knee joint OA, the combination of daily glucosamine sulphate and progressive resistance training or daily NSAIDs and progressive resistance training has been shown to lead to greater increases in quadriceps muscle strength compared to resistance training and placebo tablets [24]. Taken together, these findings suggest that joint aspiration and corticosteroid injection prior to a period of resistance training may lead to greater quadriceps strength gains than resistance training alone. The clinical advantages of aspiration and corticosteroid injection are that it requires a single intervention (rather than multiple applications) and may have fewer systemic side effects than NSAIDs, particularly in an elderly arthritic population.

AMI often persists in the absence of clinically detectable swelling, inflammation and pain [11, 62, 80]. This suggests that mechanisms unrelated to swelling and inflammation may also contribute to AMI [62]. One such mechanism may be a dysfunction in the quadriceps γ -loop, resulting in disfacilitation of the homonymous α -motoneuron pool due to a lack of normal excitatory input from Ia afferents [157]. In Chapter 6, we used prolonged vibration of the infrapatellar tendon to examine quadriceps γ -loop function in healthy uninjured controls, individuals with OA of the knee joint and individuals who had recently undergone an ACL reconstruction. Prolonged vibration was shown to decrease knee extensor peak torque and EMG amplitude in healthy controls but have no

significant effect on quadriceps activation in individuals with OA or following ACL reconstruction. This suggests that the γ -loop was already dysfunctional in these groups and thus could not be impaired any further by prolonged vibration. Importantly, this is the first time such a dysfunction has been shown in an OA population, suggesting that quadriceps γ -loop dysfunction contributes to AMI in these individuals, as has previously been shown after ACL injury [157, 183] and ACL reconstruction [77, 78, 83].

The mechanisms underlying γ -loop dysfunction remain poorly understood. Konishi and his colleagues [157, 160] have provided indirect evidence that a loss of excitatory input from knee joint mechanoreceptors may lead to γ -loop dysfunction. This could occur due to damage or degeneration of joint soft tissue structures that simultaneously disrupts the normal afferent feedback from these tissues [155, 156, 644-646]. In turn, this is thought to disfacilitate the quadriceps γ -motoneuron pool and diminish γ -efferent drive to primary muscle spindles, thereby reducing excitatory input from Ia afferents to the quadriceps α -motoneuron pool. In Chapter 6, we reasoned that if a loss of γ -efferent drive is the major mechanism underlying γ -loop dysfunction, then short duration tendon vibration should provide an artificial stimulus to the primary spindles, temporarily restoring Ia afferent input to the quadriceps α -motoneuron pool and enhancing motor unit recruitment. Contrary to this hypothesis, we could provide no

evidence that short duration tendon vibration enhances quadriceps activation in OA and ACL reconstructed participants with an established γ -loop dysfunction. One explanation for this finding is that impaired transmission in the afferent portion of the γ -loop is at least partly responsible for γ -loop dysfunction in these individuals. This could be due to changes in the sensitivity of the muscle spindles themselves [197, 699] or changes in synaptic efficacy at the Ia afferent α -motoneuron synapse [187]. Alternatively, it may be that Ia afferent input was indeed increased by vibration but that this was not sufficient to overcome ongoing inhibition of the α -motoneuron pool due to other neural pathways. Given our findings in Chapter 6, short duration tendon vibration does not appear to be a useful therapeutic intervention to overcome quadriceps AML in individuals with γ -loop dysfunction.

RECOMMENDATIONS FOR FUTURE RESEARCH

The findings from this thesis highlight a number of areas that could be examined in future research:

1. Chapter 3 showed that icing the knee joint can temporarily reverse quadriceps AML caused by joint swelling. It would of interest to examine the long term effects of a combined cryotherapy and resistance training programme vs. resistance training alone on quadriceps muscle strength, hypertrophy and

central activation in a population affected by knee injury, knee surgery or pathology.

2. Given the paradoxical finding that quadriceps MEP area increased despite no changes in intracortical excitability and presumed inhibition of the quadriceps α -motoneuron pool, it would be of interest to examine changes in lumbar propriospinal neuron excitability following knee joint swelling using the technique outlined by Forget et al. [700]. In addition, the use of paired pulse TMS to explore differences in the excitability of various intracortical circuits in patients with chronic knee joint pathology (or pre and post knee surgery) would provide valuable additional information on the role of cortical excitability in AML.

3. Previous studies using experimental models of cutaneous and muscle pain [701, 702] have shown a change in short interval intracortical inhibition in response to nociceptive input. Thus, it is possible that even if experimental swelling does not influence motor cortex excitability, an increase in nociceptive output from the joint may contribute to AML via an intracortical mechanism. It would be of interest to examine the effects of joint pain on motor cortex excitability, particularly given the recent development of an experimental model of anterior knee pain using the injection of hypertonic saline into the infrapatellar fat pad [403].

4. Given the somewhat uncertain role of swelling on AMI in chronic joint pathology, it would be of interest to compare the effects of experimental joint infusion on quadriceps muscle activation in healthy controls and individuals with chronic arthritis in the same study, using a standardised intraarticular pressure (e.g. 50 mmHg) for all participants.

5. In Chapter 5 we observed a 25% increase in knee extensor peak torque ~15 days after knee joint aspiration and corticosteroid injection in individuals with chronic knee joint arthritis. It would be of interest to determine whether applying such an intervention prior to quadriceps resistance training would lead to greater long term gains in quadriceps strength, muscle hypertrophy and muscle activation than resistance training alone.

6. The findings from Chapter 6 provide indirect evidence that the afferent portion of the γ -loop may be impaired following joint damage. As such, it would be of interest to further examine the role of Ia afferent presynaptic inhibition in AMI using the D1 inhibition technique described by Hultborn et al. [703] , both at rest and during voluntary muscle contraction.

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