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NEUROMUSCULAR FACTORS AFFECTING STRETCH-INDUCED TORQUE LOSS

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ABSTRACT

The mechanisms underpinning the immediate torque loss induced by acute, static muscle stretching are still not clear. The current research was designed to examine the neuromuscular factors influencing this torque loss. In Study 1, the contributions of central versus peripheral factors to the stretch-induced torque loss were investigated. Measures of central drive, including the EMG amplitude normalised to the muscle compound action potential amplitude (EMG:M), percent voluntary activation (%VA) and first volitional wave amplitude (V:M), and measures of peripheral function, including the twitch peak torque and 20:80 Hz tetanic torque ratio were made before, and immediately and 15 min after a 5-min continuous plantar flexor stretch. There was a 15.7% ($p<0.05$) reduction in plantar flexor torque immediately after stretch that recovered by 15 min post-stretch. There were strong correlations between changes in measures of central of drive and both the torque loss immediately ($r=0.65$-0.93) and during the torque recovery ($r=0.77$-0.81; 15 min) after stretch, suggesting that central factors were strongly related to the loss of torque. Small (11%; $p<0.05$) changes in electrical-elicited muscle torque were not associated with the voluntary torque reduction.

Alternatively, intermittent (i.e. repeated) stretching commonly performed by athlete and clinical populations causes cycles of ischaemia-reperfusion, increasing the likelihood of contractile failure. Therefore, Study 2 was designed to determine whether intermittent stretch might cause greater torque loss when compared to continuous stretch, and to quantify the potentially greater peripheral effect. The main findings were that

intermittent stretch induced a greater torque loss (-23.8%; $p<0.05$) for longer (30 min) than continuous stretch (-14.3%; 15 min; $p<0.05$), however the torque losses were related to central drive depression rather than peripheral factors in both conditions. Additionally, whilst reductions in central drive were observed only immediately after intermittent stretch (EMG:M, -27.7%; %VA, -15.9%;$p<0.05$), a prolonged (30 min) torque loss of ~5% ($p<0.05$) found after intermittent stretch could not be explained by changes in central drive or contractile failure and might thus be explained by peripheral factors other than those measured presently.

Central drive failure can clearly be of spinal origin, and it is reasonable to speculate that muscle stretch might affect the afferent-mediated motor neurone facilitatory system. Thus, in Study 3 a vibration-stimulation protocol (vib+stim) was used to elicit reflex-mediated muscular contractions during two experiments. In Experiment 1, vib+stim was imposed with the ankle joint plantar flexed (+10°), neutral (0°) and dorsiflexed (-10°). Torque and EMG amplitudes during vibration and during the self-sustained torque period after vib+stim were greater in dorsiflexion, providing method validation. In Experiment 2, vib+stim was imposed twice before (Control) and immediately, 5, 10 and 15 min after a 5-min intermittent stretch protocol. Torque and EMG amplitude were depressed immediately after stretching during both vibration (-60% and –41%, respectively; $p<0.05$) and the self-sustained torque period (65% and 44%; $p<0.05$) but recovered within 5 min. This suggests that motor neurone disfacilitation is a possible mechanism affecting torque loss. Collectively, the current results point to a central drive depression underpinning the stretch-induced torque loss, which likely involves effects on the motor neurone facilitatory system.

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LIST OF PUBLICATIONS INCLUDED AS PART OF THE THESIS

Original papers

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Conference Presentation

17th annual Conference of the European College of Sports Science, 26-29 June 2013, Barcelona – Spain. Oral presentation: Trajano GS, Seitz L, Nosaka K, Blazevich AJ. Neuromuscular Factors affecting plantar flexor torque after intermittent vs. constant stretch.

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CHAPTER 1

Introduction and Overview

Stretching exercises are commonly utilised in pre-exercise preparatory routines (Smith, 1994). It is purported that stretching exercises could increase range of motion and decrease injury incidence, especially in sports with high-intensity stretch-shortening cycle, by increasing muscle-tendon unit's compliance (Witrvouw, Mahieu, Danneels & McNair, 2004). Several randomised controlled studies (Ekstrand, Gillquist, & Liljedahl, 1983; Bixler & Jones, 1992; Amako et al., 2003) and a systematic review (Small, McNaughton & Matthews, 2008) have reported a positive effect of pre-exercise stretch on soft tissue injury risk. Also, it has been reported that pre-exercise stretch is still a common practice amongst coaches, and that coaches usually recommend, on average, 13 min of stretch prior to the exercise (Shehab et al, 2006). Moreover, in order to promote a transient increase in muscle-tendon unit compliance it seems necessary that the stretch exercise lasts for at least 4 min (McHugh & Cosgrave, 2010). However, the use of stretching exercises applied before physical activity for the purpose of enhancing the performance or preventing injuries has been criticised (McHugh & Cosgrave, 2010; Pope, Herbert, Kirwan, & Graham, 2000; Rubini, Costa, & Gomes, 2007; Shrier, 2004; Thacker, Gilchrist, Stroup, & Kimsey, 2004). Importantly, it has been often shown that acute muscle stretching lasting more than 60 s reduces maximal contractile force and power production (Avela, Kyrolainen, & Komi, 1999; Cramer et al., 2007; Fowles, Sale, & MacDougall, 2000; Kay & Blazevich, 2009a; Nelson, Guillory, Cornwell, & Kokkonen, 2001). For example, stretching exercise performed for 60 s or longer elicits an average force reduction of 7.5% (Kay & Blazevich, 2011) and longer duration (e.g.

29 min) stretching has been shown to reduce force for up to one hour (Fowles et al., 2000). This decrease in contractile capacity may compromise functional performance. Thus, there is a need to understand the factors influencing, and mechanisms underpinning, this effect with a view to developing strategies that mitigate against it.

An original hypothesis offered to explain force loss was that stretch could change the mechanical characteristics of the muscle itself, resulting in a decrease in muscle-tendon complex stiffness or shifting the muscle length-tension relationship to a less optimal point (Cramer et al., 2007; Fowles et al., 2000). For instance, an increase in tendon compliance after passive muscle stretching might negatively affect force transmission and certainly cause the muscle to operate at a shorter length, which could ultimately affect maximal force production. However, it has been consistently shown that acute static stretching has little or no effect on tendon stiffness, especially when a warm-up is performed prior to the stretching (i.e. the muscle-tendon unit has been pre-conditioned) (Kay & Blazevich, 2009a, 2009b, 2010; Morse, Degens, Seynnes, Maganaris, & Jones, 2008).

A second hypothesis is that contractile force decrements result from a reduced efferent neural drive during voluntary activation, which has been typically been measured as a decrease in muscle activity recorded by electromyography (Behm, Button, & Butt, 2001; Cornwell, Nelson, & Sidaway, 2002; Cramer et al., 2005; Fowles et al., 2000; Kay & Blazevich, 2009b). In fact, the reduction in electromyogram (EMG) amplitude may more broadly indicate both a decrease in efferent neural drive to the muscle or an inability to conduct muscle post-synaptic potentials within the muscle, which typically results in a reduced force production (Gandevia, 2001). Thus, mechanisms associated

with the transmission of potentials, ultimately influencing excitation-contraction (E-C) coupling, may be implicated in addition to changes in efferent neural drive based on the available EMG data. Furthermore, these EMG data do not provide information regarding the specific site at which neuromuscular activation might be compromised (e.g. supra-spinal, spinal or muscular). Another important consideration is that these post-stretch decreases in EMG amplitude are not always seen (Herda et al., 2011; Power, Behm, Cahill, Carroll, & Young, 2004; Ryan et al., 2008), and it is not known whether this results from sensitivity and reliability issues associated with EMG measurements, or indicates that other mechanisms must be at least as important as neural drive modification (Arabadzhiev, Dimitrov, Dimitrova, & Dimitrov, 2010; Christie, Inglis, Kamen, & Gabriel, 2009; Dimitrova & Dimitrov, 2003; Farina, Merletti, & Enoka, 2004). Thus, the mechanisms underpinning the stretch-induced force loss are not clear.

Given that changes in tendon properties (and therefore muscle length during contraction) do not appear to explain the loss of force, two main neuromuscular mechanisms that might therefore explain the force loss are: 1) a reduction in the efferent neural drive to the muscle mediated by spinal (Avela et al., 1999) or supra-spinal mechanisms (Gandevia, 2001), or 2) an impairment in the muscle's E-C coupling mechanism (Allen, 2004). However, there is no consensus as to the location of the changes leading to the force decline because no research has examined, in detail, neuromuscular function at different sites within the system after a period of acute stretching that is sufficient to lead to force loss. In the following review of literature, the potential for these mechanisms to influence the stretch-induced force loss is considered with a view to developing specific testable hypotheses for future research.

1.1. Voluntary muscle force

Voluntary muscle contractions are elicited when excitatory postsynaptic potentials activate the motor neurone pool. This process is induced by activity in the motor cortex acting via descending pathways and can be modulated by afferent feedback, inter-neurone activity and motor neurone intrinsic properties (Heckman, Gorassini, & Bennett, 2004). Once the motor neurone is depolarised and the action potential reaches the neuromuscular junction, a postsynaptic action potential travels along the sarcolemma to the T-tubules (Gandevia, 2001). T-tubule depolarisation activates the voltage-sensitive dihydropyridine receptors (DHPR) and stimulates an interaction with the calcium-release ryanodine receptors (RyR), resulting in calcium release from the sarcoplasmic reticulum into the myoplasm. This process stimulates actomyosin interaction and causes sarcomere shortening (assuming sarcomeric force exceeds internal and external opposing forces) in a process known as E-C coupling (Balog, 2010). The magnitude of the resulting muscular force depends on the number of motor units recruited and the motor neurone discharge rate, which shifts the Ca^{2+} release-Ca^{2+} uptake balance (into the sarcoplasmic reticulum) towards release and an increase in myoplasmic Ca^{2+} concentration. According to "Henneman's size principle", motor units are recruited in order of increasing peak twitch force (Henneman, 1985). This theory is grounded in the strong correlations found between the level of resistance of the motor neurone, which determines its recruitment threshold, and motor unit force (Heckman & Enoka, 2012). Importantly, both motor unit recruitment and firing rate are determined by supra-spinal and spinal facilitation/inhibition balance.

1.1.1 Reduction in supra-spinal drive

Alterations in supra-spinal drive can noticeably affect muscular force production. Reductions in muscular force during continued activation (i.e. muscle fatigue) have been shown to result from an inability of the descending supra-spinal drive to maximally activate the muscle's motor neurone pool (Gandevia, 2001). Reductions in supra-spinal drive have been clearly demonstrated during and after exercise leading to muscle fatigue, through repeated maximal contractions (Taylor & Gandevia, 2008), submaximal sustained contractions (Søgaard, Gandevia, Todd, Petersen, & Taylor, 2006) and long-duration (endurance) efforts (Lepers, Maffiuletti, Rochette, Brugniaux, & Millet, 2002). For instance, an 18% decrease in maximal voluntary force was reported after running a marathon (Ross, Middleton, Shave, George, & Nowicky, 2007). This force loss was attributed at least in part to a reduction of motor cortical outflow, measured as the amplitude of the motor-evoked potential (MEP) during cortical transcranial magnetic stimulation (TMS) (Ross et al., 2007). Moreover, reductions in MEP amplitude were taken as evidence of a reduced efficiency of transmission along the corticospinal tract for 12 min after a 2-min maximal isometric elbow flexor contraction (Gandevia, Petersen, Butler, & Taylor, 1999). Taken together, these results suggest that acute reductions in muscular force output can occur through alterations in supra-spinal drive.

The mechanisms underpinning this reduced input from motor cortex to the motor neurone are still unclear and need further investigation. However, changes in the behaviour of cortical neurones and/or the influence of afferent fibres inhibiting the descending volley should be considered as potential mechanisms (Taylor & Gandevia,

2008). Although there is considerable evidence supporting the existence of a sub-optimal supra-spinal output to motor neurones after fatiguing contractions, very little is known about how muscle stretch might affect supra-spinal drive to the muscle. Thus, at this time there is no clear evidence as to whether a supra-spinal depression might influence muscular force production subsequent to a bout of muscle stretching.

It is well known that motor cortical outflow may be influenced by sensory inflow (Matthews, 1991), and it is interesting to note that changes in limb position for example can acutely influence the organisation of the primary motor cortex (Gellhorn & Hyde, 1953; Scott, Sergio, & Kalaska, 1997). In 1953, Gellhorn and Hyde clearly demonstrated that changes in muscle length could affect the extent of the cortical area from which a specific muscle could be activated via surface electrical stimulation. Moreover, evidence from animal and human experiments provide convincing evidence that stretch-sensitive afferent fibres project to the cerebral cortex. Studies using animal (primate) models have shown that muscle spindle (i.e. stretch-activated) type I and II afferents fibres project to cortical areas 3a (somato-sensory cortex) and 4 (motor cortex), which provide evidence for the possibility that muscle stretch could influence cortical activity especially in those areas (Hore, Preston, & Cheney, 1976; Phillips, Powell, & Wiesendanger, 1971). In particular, area 3a is purported to be involved in somato-motor-vestibular integration (Huffman & Krubitzer, 2001). The neurones in this cortical region can project both mono- and poly-synaptically (via inter-neurones) to the spinal motor neurones of the stretched muscles (Matthews, 1991; Rathelot & Strick, 2009), as well as the primary motor cortex (Avendaño, Isla, & Rausell, 1992; Huerta & Pons, 1990; Murray & Coulter, 1981), suggesting a possible contribution to control motor output. Human experiments have also consistently demonstrated the possible

involvement of cortical structures in response to the stimulation of stretch-sensitive afferents. For instance, it has been demonstrated that muscle stretch could evoke cortical potentials in humans (Cohen, Starr, & Pratt, 1985; Starr, McKeon, Skuse, & Burke, 1981). Additionally, prolonged muscle vibration (which preferentially activates Ia afferent fibres) (Marconi et al., 2008) and changes in muscle length (i.e. towards longer muscle length) (Coxon, Stinear, & Byblow, 2005) have been shown to reduce the excitability of the primary motor cortex as assessed using TMS, suggesting that input from stretch-sensitive afferents can modulate motor cortical excitability. In light of the above-mentioned evidence, it seems reasonable to speculate that passive muscle stretch could acutely and directly affect motor cortical outflow. Nonetheless, this assumption has not been explicitly examined.

1.1.1.2. Limitations to previous research measuring neural (central) drive after muscle stretch

It is commonly argued that prolonged muscle stretches (e.g. >60 s) result in a reduced muscle activity measured by EMG (Avela et al., 1999; Cramer et al., 2007; Kay & Blazevich, 2009b). Specifically, a strong correlation has been shown between the reduction in muscle force after acute plantar flexor muscle stretching and the reductions in EMG amplitude measured during maximal voluntary contraction (Kay & Blazevich, 2009b). However, a reduction in EMG amplitude does not unquestionably indicate a reduced supra-spinal (motor cortical) drive, as changes in spinal reflex loops, motor neurone intrinsic properties and muscle sarcolemmal action potential propagation can affect it (Arabadzhiev et al., 2010; Farina, Merletti, et al., 2004). Moreover, caution should be exercised when inferring changes in neural input (i.e. supra-spinal and spinal)

to the muscle through EMG measurements. This is because changes in EMG amplitude can occur in response to factors other than changes in neural drive, such as amplitude cancellation (Christie et al., 2009; Farina, Cescon, Negro, & Enoka, 2008; Keenan, Farina, Maluf, Merletti, & Enoka, 2005; Keenan, Farina, Merletti, & Enoka, 2006) and motor unit synchronisation (Farina, Merletti, et al., 2004; Yao, Fuglevand, & Enoka, 2000), changes in muscle length (Farina, Merletti, Nazzaro, & Caruso, 2001; Frigon, Carroll, Jones, Zehr, & Collins, 2007; Yao et al., 2000) and alterations in intracellular action potential amplitude and velocity (Arabadzhiev et al., 2010; Dimitrova & Dimitrov, 2003). Thus, reductions in EMG amplitude *per se* cannot be taken as evidence for reductions in neural drive to the muscle.

In addition to EMG alterations researchers have also reported decreases in voluntary muscle activation (%VA), as measured using the interpolated twitch technique (ITT), after acute passive stretch (Behm et al., 2001; Fowles et al., 2000), possibly indicating a reduction in neural drive to the muscle. However, these changes are not always seen (Power et al., 2004; Ryan et al., 2008). The principle of the ITT is to apply an electrical stimulus to the muscle, or its nerve, on top of a maximal voluntary contraction (MVC) in order to increase the firing frequency of the fibres above that obtained volitionally, theoretically allowing for maximal muscle contractile capacity to be achieved (Merton, 1954). The torque produced during 'maximal' muscle activation is then compared to the torque produced by an electrical twitch immediately after the MVC, producing a ratio that reflects the extent of voluntary muscle activation (Shield & Zhou, 2004). However, this measurement has been shown to be influenced by supra-spinal, spinal and/or peripheral structures (De Haan, Gerrits, & de Ruiter, 2009; Millet & Lepers, 2004; Taylor, 2009a) and is therefore not solely a measure of neural drive. For instance,

measures of %VA obtained by using ITT have been reported to be affected by several factors such as muscle length (Arampatzis, Mademli, De Monte, & Walsh, 2007), force transmission by the series elastic components (Taylor, 2009b) and changes in intracellular calcium concentration (Place, Yamada, Bruton, & Westerblad, 2008). Another problem affecting the interpretation of previous data is that different muscles were targeted and stretch protocols used so it is not possible to reconcile the inconsistent findings. Thus, data obtained using EMG and ITT data have been inconsistent, and the use of these techniques has not allowed for accurate delineation of the site at which muscle activation might be modified by stretching.

Alternatively, other methods have been used to assess changes in neural drive. Firstly, when the muscle, or its motor nerve, is electrically stimulated the excitability of the muscle membrane can be non-invasively assessed by measuring the amplitude of the compound muscle action potential (M-wave). Normalising the surface EMG (root-mean-squared) signal to the M-wave maximal amplitude (i.e. EMG:M) eliminates the effect of peripheral changes in membrane excitability and indicates if there is a change in central drive to the muscle (Millet & Lepers, 2004). However, this measurement can still be affected by factors such as changes in motor unit synchronisation and amplitude cancellation. Secondly, the first volitional wave (V-wave), which is an electrophysiological variant of H-reflex elicited with a supra-maximal stimulus intensity during maximal voluntary contraction (Upton, McComas, & Sica, 1971), can be used to assess changes in neural drive. The H-reflex is a monosynaptic reflex evoked when the homonymous Ia afferent is electrically stimulated at a sub-maximal intensity on a mixed nerve (Pierrot-Deseilligny, 1997). When the nerve is stimulated, a descending action potential (M-wave) causes muscle contraction and the Ia afferent fibres projecting back

on the spinal cord excite the α-motor neurone pool to create another action potential in the innervated skeletal muscle (H-reflex). Its amplitude is usually utilised as a measure of spinal excitability, also reflecting the efficiency in Ia afferent synapses (Aagaard, Simonsen, Andersen, Magnusson, & Dyhre-Poulsen, 2002a; Knikou, 2008; Pierrot-Deseilligny, 1997; Pierrot-Deseilligny & Burke, 2005). However, when supra-maximal nerve stimulation is applied during a maximal voluntary contraction, together with the direct M-wave, the H-reflex reappears (i.e. V-wave) as the antidromic impulses (i.e. opposite direction of normal impulse) in the motor neurones collide with the efferent nerve impulses caused by the voluntary contraction (Aagaard, 2003; Duclay & Martin, 2005; Gondin, Duclay, & Martin, 2006; Pensini & Martin, 2004; Solstad, Fimland, Helgerud, Iversen, & Hoff, 2011). The supra-maximal intensity used during nerve stimulation to evoke V-wave promotes massive excitation of all Ia afferent axons in the peripheral nerve, subsequently recruiting both large and small motor neurones (Aagaard, Simonsen, Andersen, Magnusson, & Dyhre-Poulsen, 2002b). The V-wave is purported to be indicative of changes in motor unit firing frequency and may be considered a useful measure of central drive obtained during MVC. Nonetheless, it can be directly affected by activity in descending pathways (e.g. pre-synaptic inhibition at the spinal level), so it may not be reliable under some experimental conditions. Despite this caveat, V-wave measurements could provide substantial evidence for/against central drive modifications and can be used to more clearly determine whether acute static stretching influences central drive. However, no researchers have measured changes in V-wave amplitudes after an acute bout of muscle stretching. In fact, based on the information presented above, the concurrent measurement of V-wave amplitude, EMG:M and %VA might provide the best evidence of central changes in neural drive after muscle stretching. A consistent change in all three tests could be taken as excellent

evidence for an influence of muscle stretch on efferent neural drive, and motivate more detailed examinations of the neuromuscular pathway to identify the site of change.

1.1.2. Inhibition or disfacilitation at the spinal Level

The spinal circuitry is a complex network of sensory neurones, inter-neurones and motor neurones that can inhibit or facilitate descending volitional signals and it is possible that muscle stretching might cause inhibition or disfacilitation of this circuitry. In order to achieve maximal discharge frequency, and thus to produce high levels of muscular force, spinal motor neurones rely upon a facilitatory system that increases the gain of synaptic input (Hultborn, Denton, Wienecke, & Nielsen, 2003). This facilitatory modulation is mediated by persistent inward currents (PICs), which are a voltage-dependent characteristic of spinal motor neurones that, when activated, amplify and prolong synaptic inputs (Heckman et al., 2004). PIC development changes the input-output relationship and may produce sustained depolarisations (i.e. plateau-potentials) more specifically in low-threshold motor neurones (Heckmann, Gorassini, & Bennett, 2005). PICs occur largely at the motor neurone dendrites and are controlled by the interaction between descending monoaminergic drive (specifically the neurotransmitters noradrenaline and serotonin in the spinal cord) and afferent feedback, especially including the length-sensitive muscle spindle Ia afferents (Heckman et al., 2004). For instance, changes in muscle length directly affect the level of dendritic amplification to the motor neurone (Hyngstrom, Johnson, Miller, & Heckman, 2007), so prolonged increases in muscle length caused by muscle stretch may reduce Ia afferent input onto α-motor neurones. Indeed, a reduction in Ia afferent efficiency (measured as a decrease in H-reflex amplitude) concomitant with a decrease in force has previously been

reported immediately after prolonged (1 hour) passive stretching (Avela et al., 1999); a reduction in Ia afferent input could affect the motor neurone facilitatory process, preventing maximal discharge rates being attained during force production. However, the H-reflex is a very specific measurement that when measured at a relaxed muscle cannot provide information regarding motor neurone facilitation and its resulting force modulation.

PICs have been investigated more completely in decerebrate cats preparations using a steady synaptic input mediated by tendon vibration, which selectively activates Ia afferents (Frigon et al., 2011). Despite the fact that animal models offer a better platform to study PICs, it is possible to gain some understanding of it, and its force modulatory effects, by using tendon vibration reflexes (TVR) in humans. TVRs are purported to be reflective of PIC manifestation and have been used to investigate the possible influence of Ia afferent on alpha motor neurone output (McPherson, Ellis, Heckman, & Dewald, 2008; Mottram, Suresh, Heckman, Gorassini, & Rymer, 2009; Suresh, Wang, Heckman, & Rymer, 2011). Thus, TVR methods may provide an ideal tool to investigate the potential effect of passive muscle stretch on the amplification (or lack) of steady synaptic input to the motor neurone. However, to best of the author's knowledge no previous research has investigated the effects of acute muscle stretch on PIC development.

1.1.3. Impairment of the excitation-contraction (E-C) coupling process

Information regarding E-C coupling efficiency (i.e sarcoplasmic reticulum's ability to release calcium to muscle contraction) can be obtained by comparing the peak torque

produced during high- (e.g. 80 Hz) and low- (e.g. 20 Hz) frequency electrical nerve (or muscle) stimulation (Edwards, Hill, Jones, & Merton, 1977; Jones, 1996; Martin, Millet, Martin, Deley, & Lattier, 2004). This phenomenon, commonly referred to as low-frequency fatigue (LFF), is characterised by a greater force loss in response to low vs. high frequency stimulation (Keeton & Binder-Macleod, 2006). It has been suggested that LFF may be caused by a reduction in calcium release from the sarcoplasmic reticulum due to impairment in the interaction between voltage-sensitive dihydropyridine receptors (DHPR) and calcium-release ryanodine receptors (RyR) (Balog, 2010). One of the possible factors that could disrupt this interaction between receptors is an increased sarcoplasmic calcium concentration, mediated by stretch activated channels (SAC), which reduce the calcium release by the RyR during muscle contraction (Allen, 2004; Balog, 2010). Long-lasting LFF and increases in intracellular calcium concentration are commonly observed as a consequence of lengthening (i.e. eccentric) contractions (Jones, 1996; Keeton & Binder-Macleod, 2006; Martin et al., 2004). However, this examination has not been completed after passive muscle stretch in humans, although it has been previously shown that SACs can be activated by passive muscle stretch in animal models (Armstrong et al., 1993). Additionally, an increase in muscle inflammatory cell (neutrophil) concentration was observed after passive muscle stretching, again suggesting a potential role for calcium infiltration in muscle cells (Pizza, Koh, McGregor, & Brooks, 2002). Thus, the possibility exists that acute passive muscle stretch could activate SACs, increase the sarcoplasmic calcium concentration, and ultimately impair calcium release and the E-C coupling process. Evidence for this mechanism has not been gathered, but might be observed by examining the torque produced by low-frequency vs. high-frequency stimulation after passive stretch (Martin et al., 2004). Hence, a study of the effect of stretching on high

vs. low-frequency force production using electrical stimulation might indicate whether calcium-related E-C failure is a factor affecting force loss.

1.1.3.1. Muscle "catch-like" properties

Skeletal muscle tends to produce a greater contractile force and rate of force development (slope of the force-time curve) when two action potentials (i.e. a doublet) arrive at the muscle with a very short (e.g. 10 ms) delay at the onset of a voluntary contraction (Binder-Macleod & Kesar, 2005; Burke, Rudomin, & Zajac, 1970). This phenomenon is usually termed the skeletal muscle catch-like property, and it is possible to simulate this by delivering two rapid electrical pulses to a muscle or its nerve followed by a low-frequency (e.g. 20 Hz) train of pulses (Binder-Macleod & Kesar, 2005; Burke et al., 1970). It has been hypothesised that the arrival of doublets at the muscle membrane could increase the myofibrillar affinity for calcium (Abbate, Bruton, De Haan, & Westerblad, 2002). However, more recent data obtained in isolated mouse fibres indicate that doublets occurring at the onset of a contraction might also increase sarcoplasmic reticulum calcium release via the RyR receptor (Cheng, Place, Bruton, Holmberg, & Westerblad, 2013). The increase in rate of force development and peak contractile torque are often more pronounced when the muscle is fatigued, suggesting that it might be an essential mechanism for counteracting the loss of contractile force (Bentley & Lehman, 2005; Binder-Macleod & Russ, 1999). As described previously it is possible that passive stretch could negatively affect sarcoplasmic calcium release kinetics (Armstrong et al., 1993; Pizza et al., 2002), however it is not yet known whether passive muscle stretching can influence the catch-like behaviour of muscle or whether the addition of a doublet at contraction onset could counteract the subsequent force loss. By comparing the peak torque elicited by a standard train of electrical pulses

(e.g. 20 Hz) versus a train prefixed with a doublet pulse (i.e. catch-induced train) (Bentley & Lehman, 2005; Binder-Macleod & Russ, 1999) it may be possible to better understand the effect of acute passive muscle stretching on calcium handling and the utilisation of muscle's catch-like properties.

1.2. Muscle ischaemia

An adequate level of blood supply is important for muscle force production, both for oxygen delivery and the removal of metabolites. Thus, impairment in blood flow has been suggested to be an important factor influencing muscle fatigue (Kent-Braun, 2009; Murthy, Hargens, Lehman, & Rempel, 2001). It has also been shown that a high level (i.e. nearly at the level induced by cuff occlusion) of ischaemia can occur during static muscle stretching (McCully, 2010). Given that skeletal muscle is isovolumetric, a reduction in muscle oxygenation during passive stretch could result when the muscle's cross-sectional area is reduced, pennation decreases and intramuscular pressure increases during muscle stretch, which ejects blood from the muscle and prevents arterial in-flow (Kagaya & Muraoka, 2005; Otsuki, Fujita, Ikegawa, & Kuno-Mizumura, 2011). This increase in intra-muscular pressure would be influenced by stretch intensity (i.e. tissue strain) and the muscle's viscoelastic properties (Magnusson, 1998). Stretching protocols commonly utilise multiple sets of stretch separated by rest intervals, which results in a cycle of ischaemia and reperfusion and potentially causing symptoms of local muscle fatigue. Additionally, the reperfusion process may also be problematic. During reperfusion muscular production of xanthine oxidase, an enzyme with an important function in the generation of reactive oxygen species (ROS), increases and may result in ROS-dependent muscle fatigue (Granger, 1988; Powers & Jackson, 2008). Furthermore, it has been shown in animal models that passive muscle

stretch itself can increase nitric oxide release (Tidball et al., 1998) and ROS production (Chambers, Moylan, Smith, Goodyear, & Reid, 2009; Palomero, Pye, Kabayo, & Jackson, 2012). ROS accumulation can reduce muscle force production by impairing RyR-mediated calcium release and affecting the E-C process (Powers & Jackson, 2008). Hence, comparing stretches of the same intensity and duration, differing only in the number of intervals (i.e. reperfusions), could provide information regarding the influence of the ischaemia-reperfusion process on the loss of force results from static stretch regimens. Such examination has not been done, so it is not clear whether the potential influence of acute muscle stretching on perfusion-reperfusion-dependent muscle force loss varies between continuous vs. intermittent stretch protocols.

1.3. Summary

This review provides evidence that both central and peripheral mechanisms might contribute to the muscle stretch-induced force loss. Regarding central mechanisms, reduced spinal and supra-spinal drive and/or impaired motor neurone facilitation processes are potential candidates to the explain force loss. Regarding peripheral mechanisms, acute muscle stretch might impair the E-C coupling process due to SAC channel activation or increasing ROS production. However, presently no research has investigated these mechanisms in detail. It is therefore necessary to clarify whether these mechanisms contribute to stretch-induced force losses, thus strategies to mitigate the force loss can be developed.

1.4. Aim of the thesis

The major aims of the present thesis are to 1) determine the contribution of central vs. peripheral mechanisms to the force loss after a 5-min continuous muscle stretch; 2)

examine whether intermittent stretch might cause greater force loss when compared to continuous stretch, and to quantify the potentially greater peripheral effect; and 3) complete a third study, according to the results of studies 1 and 2, to more specifically determine whether spinal motor neurone facilitatory mechanisms (i.e central) or muscle contractile processes (i.e. peripheral) might be negatively affected by acute passive muscle stretching.

CHAPTER 2

Study 1: Contribution of Central vs. Peripheral Factors to the Force Loss Induced by Passive Stretch of the Human Plantar Flexors

2.1 Introduction

Prolonged (≥60 s) passive muscle stretch reduces maximal force production in human muscles (Behm & Chaouachi, 2011; Kay & Blazevich, 2012). However the mechanisms underpinning this loss have not been fully elucidated and effective strategies for minimising the force loss have not been developed. A post-stretch decrease in central (efferent) drive to the muscles has been considered to affect force production, evidenced by the reductions in electromyogram (EMG) amplitudes that are often observed (Fowles et al., 2000; Kay & Blazevich, 2009b). However the EMG signal can be affected by peripheral factors, including changes in muscle fibre action potential amplitude and propagation velocity (Arabadzhiev et al., 2010; Farina, Merletti, et al., 2004), so factors other than central drive limitations could also explain these results.

To better quantify changes in central drive other techniques could be used, including normalisation of EMG amplitudes to the maximal muscle compound action potential (M_{max}) amplitude (Arabadzhiev et al., 2010), the use of the interpolated twitch technique to estimate 'per cent voluntary activation' (Merton, 1954; Taylor, 2009b) and the measurement of V-wave amplitudes during maximum voluntary contractions (Upton et al., 1971). On the other hand, each of these measures is also considered potentially imperfect in some way (Aagaard et al., 2002b; Arabadzhiev et al., 2010; Farina,

Merletti, et al., 2004; Taylor, 2009b), so strong evidence for a central drive limitation subsequent to muscle stretch might only be indicated when a depression is observed in several simultaneously-obtained measures, and these depressions are related to (i.e. correlated with) the loss of force. As yet, such a detailed examination has not been completed so it is not clear whether a reduction in central drive is a key mechanism underpinning the force loss.

In addition to central factors, peripheral factors might influence the loss of force after stretch. For example, research using animal models has shown that passive muscle stretch can increase intracellular calcium concentration via stretch-activated channel activation and disturb calcium homeostasis (Armstrong et al., 1993). Such a disturbance can negatively affect the synergistic interaction between the calcium-release ryanodine receptor and voltage-sensitive dihydropyridine receptors, impairing excitation-contraction (E-C) coupling (Balog, 2010). In humans it is possible to estimate the efficiency of this process by comparing the torque produced during low- vs. high-frequency electrical motor nerve stimulation trains (Jones, 1996; Martin et al., 2004). In fact, it is also reasonable to expect changes in a muscle's response to short-interval double-spike stimuli when they precede a train of constant-frequency stimuli (i.e. a 'catch-inducing' train) (Binder-Macleod & Kesar, 2005; Burke et al., 1970) if calcium homeostasis is disrupted, because this response is thought to be influenced by the Ca^{2+} binding sensitivity to troponin (Abbate et al., 2002; Nielsen, 2009). Thus, decreases in force production might occur even if no significant changes in central drive are produced and no metabolic disturbances are elicited. To date, the effect of static muscle stretch on muscle contractile properties remains relatively unexplored, so it is not clear if these are potential targets for intervention.

Given the above, the purpose of the present study was to establish the relative contribution of central vs. peripheral factors to the stretch-induced force loss after a 5-min continuous passive plantar flexor muscle stretch. It was tested the hypothesis that impairments would be detected at both central and peripheral levels, and that these changes would be similarly correlated with changes in muscle force production. Three different examinations of central drive were completed (EMG:M ratio, per cent voluntary activation [interpolated twitch technique; ITT] and V-wave amplitude) in order to more robustly quantify potential central changes, whilst muscle and nerve stimulation procedures were used to gain information with regards to peripheral changes.

2.2 Methods

2.2.1 Subjects

Thirteen healthy men (mean ± SD: age, 26.5 ± 5.0 y; height, 1.72 ± 0.9 m; body mass, 71.1 ± 13.8 kg) with no previous neuromuscular impairment volunteered for the study. Sample size estimation was based on the previous data where the same neuromuscular tests were applied in fatigued muscle (Martin et al, 2004). The sample size of 12 gave an effect size of 0.79 with power of 80% at the alpha level of 0.05 (G*Power 3.0.10 software). The subjects reported not being engaged in flexibility training for at least 6 months prior to the study and refrained from such training during data collection period. The procedures performed during this research were approved by the Edith Cowan University Human Research Ethics Committee and were in agreement to the Declaration of Helsinki. All participants read and signed an informed consent document.

2.2.2 Study design and overview

Subjects visited the laboratory on three occasions at the same time of day separated at least by 48 hours. A full familiarisation of the stretch protocol and test procedures was provided in the first session whilst the subsequent two visits were used for the completion of the following experimental conditions in a randomised order: 1) control (or no stretch), and 2) 1 set of 5 min passive plantar flexor stretching. The subjects were assessed immediately before, and immediately and 15 minutes after each intervention (Figure 1). During familiarisation the muscle stimulation intensities for all electrically evoked measurements were determined and both the maximum tolerable passive torque during stretch and the maximal voluntary contraction torque (MVC) were measured. In the experimental trials, the subjects performed a warm-up on a Monark cycle for 5 min by cycling at 60 rpm with a 1-kg load to produce a power output of 60 W. The subjects were then seated upright in the chair of an isokinetic dynamometer (Biodex System 3 Pro, Biodex Medical System, Shirley, New York, USA) with the knee fully extended (0º), the ankle in the neutral position (90º) with the sole of the foot perpendicular to the shank, and the lateral malleolus aligned to the centre of rotation of the dynamometer. The knee was placed in an extended position to better assess the full plantar flexor torque (Cresswell, Löscher, & Thorstensson, 1995), minimise the risk of muscle cramp during muscle stimulation and to allow for muscles to be more completely stretched during the muscle dorsiflexion rotations (i.e. plantar flexor stretches).

2.2.3 Muscle stretching protocol

All stretch procedures were performed on an isokinetic dynamometer with the muscles relaxed. The plantar flexors were stretched for 5 min by rotating the ankle into dorsiflexion at $5^{o} \cdot s^{-1}$ until a passive resistance equal to 90% of the maximal tolerable stretch torque (assessed during familiarisation) was achieved. Muscle stretch elicits a viscoelastic stress relaxation response, resulting in a rapid reduction in stretch intensity when the joint position is held constant (Magnusson, 1998). In order to avoid this response, the joint angle was continually adjusted (increase of dorsiflexion) so that the passive torque always remained within 5 Nm of the initial stretch torque level. Standard stretching practices are somewhat analogous to the 'constant torque' design so the present results should be of practical significance.

2.2.4 Voluntary and evoked torque measurements

Peak isometric plantar flexor torque (T_{Peak}) was assessed during MVCs with the ankle in a neutral position (90º). Maximal force is usually reduced by the anticipation of discomfort caused by the electrical stimulation (Button & Behm, 2008). To avoid the effect of stimulus anticipation two MVCs were performed at each time point: the first MVC was used to calculate T_{Peak} and measure muscle activity (EMG; see below), the second MVC included tibial nerve stimulation in order to measure muscle activation (ITT) and V-wave amplitude (Figure 2.1). Participants were instructed to produce torque against the dynamometer by rotating the ankle as fast and as hard as possible. Verbal encouragement and visual feedback were provided during all MVCs.

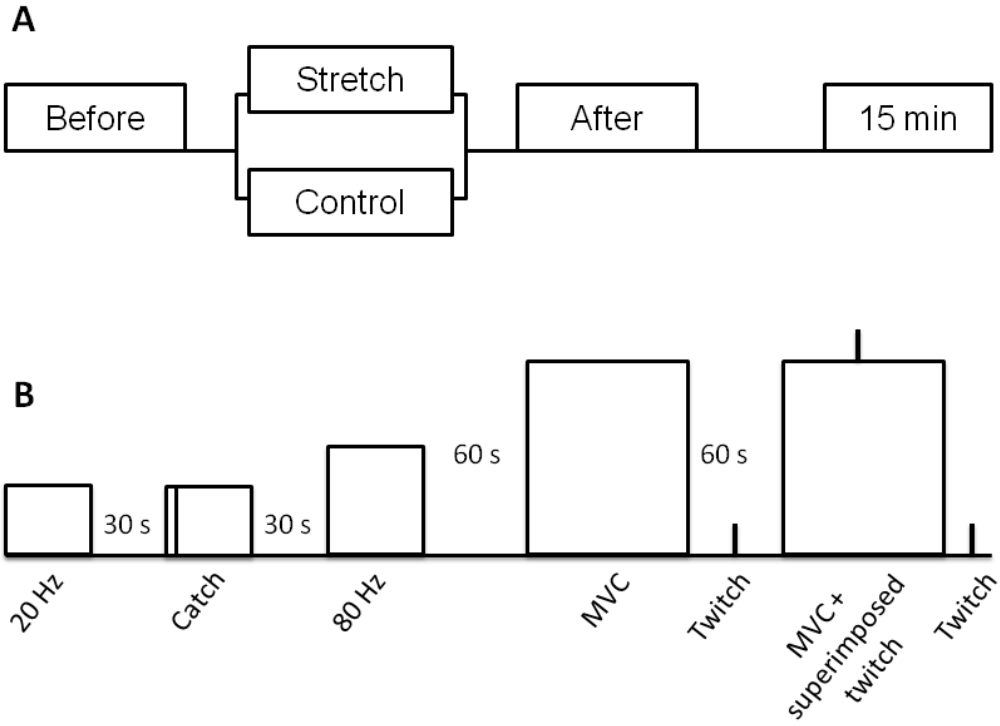

Figure 2.1. A: The experimental design and time course of measurements: subjects were assessed before, and immediately and 15 minutes after the stretch or control (rest) period. B: the order of measurements (20 Hz, 20 Hz tetanic stimulation; catch, catch-inducing tetanic stimulation; 80 Hz, 80 Hz tetanic stimulation; MVC, maximal voluntary contraction; Twitch, single pulse stimulation)

2.2.5 Stimulation procedures

2.2.5.1 Muscle stimulation (20:80 ratio, constant vs. catch-inducing train stimulation)

A constant current electrical stimulator (DS7, Digitimer Ltd, Welwyn Garden City, UK) was used to deliver an electrical square-wave stimulus (0.5-ms pulse width) to the plantar flexor muscle belly through two self-adhesive electrodes (9 × 5 cm, Dura-Stick® II, Chattanooga Group, Hixon, USA). The cathode was placed distal to the popliteal crease and the anode over the distal myotendinous junction of the soleus. For all tetanic stimulations, the intensity necessary to reach 50% of MVC with a 0.5-s 80 Hz tetanic stimulation was used as suggested by previous studies (Martin et al., 2004).

Three tetanic stimulations with the same duration were delivered to test for E-C coupling efficiency: 1) 20 Hz train of 11 pulses (0.05 s interpulse interval); 2) catch-inducing train (i.e. 2 pulses at 0.01 s plus 10 pulses at 0.05 s interpulse interval); and 3) 80 Hz train of 36 pulses (0.0125 s interpulse interval). The peak torque produced by the 20 Hz and 80 Hz stimulations were used to calculate the 20:80 ratio, which was used as a measure of E-C coupling efficiency (Martin et al., 2004). The catch-inducing train was used to assess the muscle's capacity to increase torque production under this specific condition, when compared to a constant-frequency train (20:catch ratio).

2.2.5.2 Nerve stimulation (Twitch, ITT, V-wave)

The same electrical stimulator was used to deliver the electrical square wave 1-ms pulse width stimuli to the posterior tibial nerve via a cathode electrode (Ag-AgCl, 10 mm) fixed to the popliteal fossa and an anode electrode of large size (9 × 5 cm, Dura-Stick® II, Chattanooga Group, Hixon, USA) placed on the anterior surface of the knee. ITT was used to estimate the percentage voluntary activation (%VA) of the muscle. The intensity for a single twitch was set at 120% of the intensity required to elicit M_{max}, to ensure a supramaximal current stimulus was used. Supra-maximal twitches were elicited before, during and 2 s after an isometric plantar flexor MVC (Merton, 1954). The interpolated twitch was manually elicited when the subjects reached maximal force. A comparison of the interpolated twitch to the resting potentiated (i.e. post-MVC) twitch was completed, with %VA being calculated using the equation (Shield & Zhou, 2004):

$$\%VA = [1-(\text{superimposed twitch}/\text{potentiated twitch})] \times 100.$$

The superimposed twitch was also used to capture the V-wave; decreases in amplitude were considered as evidence of a decrease in efferent drive to the motor neurone (Aagaard et al., 2002b). Although, multiple assessments are ideal to improve the method's reliability, only one stimulation was provided at each time point in order to minimise the effects of fatigue. Thus, the balance between an optimum number of stimulations and the minimisation of muscle fatigue was considered. The V-wave peak-to-peak amplitude was measured and then normalised to the M-wave amplitude measured prior to the MVC (V:M ratio).

2.2.6 Measurement of muscle activity (EMG)

Surface EMG was recorded from soleus and lateral gastrocnemius using a bipolar electrode configuration at a 4000 Hz analogue-digital conversion rate (bandwidth 10 to 500 Hz) using the Bagnoli-8 Main Unit EMG system (DelSys, Inc., MA, USA). The inter-electrode distance was 1 cm and a reference electrode was placed on the lateral malleolus. Further, to obtain clearer M- and V-wave data, surface EMG was recorded from soleus in a pseudo-monopolar configuration (sample rate 4000 Hz) using the BioAmp EMG system (PowerLab System, ADInstruments, NSW, Australia), with one electrode placed on the medial aspect of soleus below the distal gastrocnemius junction and the other placed at the Achilles tendon-soleus muscle-tendon junction ~3 cm superior to the malleolus (Blazevich, et al., 2012). The skin under the electrodes was shaved, abraded and cleaned with alcohol to reduce the inter-electrode resistance below 5 kΩ. EMG data were also recorded during the stretching maneuvers to ensure that muscle activation remained below 5% of the maximal value; a small activity response is often seen even when the subjects are asked to remain completely relaxed (Blazevich, et al., 2012). Muscle activity was expressed as root mean square EMG amplitude (500-ms

averaging window) and normalised to the M-wave amplitude measured before the contraction (EMG:M) to account for the possible influence of peripheral factors. The EMG:M quantified from SOL (EMG:M_{SOL}) and LG (EMG:M_{LG}) were summed and considered as a measure of neural efferent drive to the triceps surae (EMG:M_{TS}). Ankle joint torque, joint angle and EMG data were simultaneously recorded using LabChart v.6.1.3 Software (PowerLab System, ADInstruments, NSW, Australia).

2.2.7 Statistical analysis

Separate two-way repeated measures ANOVAs were performed to compare changes in all variables between conditions (stretch vs. control) over time (before, after and 15 min after). Pairwise comparisons with Bonferroni corrections were performed when significant interaction effect was detected. Pearson's product-moment correlation coefficient were computed to determine the relationships between changes in torque and changes in central (EMG:M; %VA; V-wave) and peripheral (20:80 ratio, 20:catch ratio, peak twitch torque) mechanisms. Statistical significance was set at an α level of 0.05. Intra-class correlations (ICC) were computed to evaluate reliability of central drive (; %VA; V-wave; EMG:M) and torque (T_{peak}; twitch; 20 Hz, catch-inducing and 80 Hz) measurements during control condition.

2.3 Results

ICC values describing the reliability of central drive and torque measurements during control conditions were between 0.83–0.93 and 0.98-0.99, respectively, suggesting that these measures were reliable.

There was a significant interaction (time × condition) effect for T_{peak} ($p<0.05$). Post-hoc analyses revealed a significant reduction of 15.7% immediately after stretch with no significant difference from the baseline being found at 15 minutes, and no changes in the control condition (Figure 2.2).

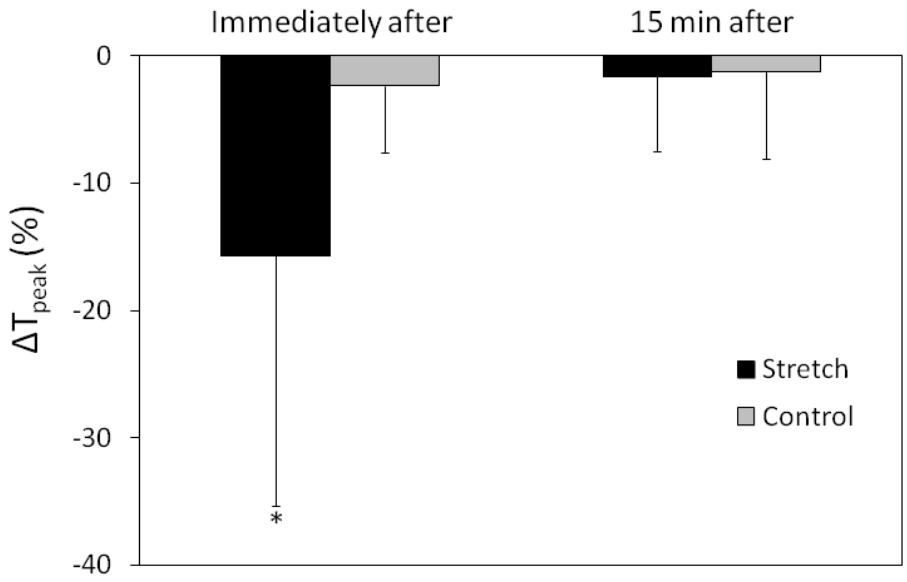

Figure 2.2. Torque loss immediately and 15 min after stretch. * Significantly greater change compared to the control condition ($p<0.05$).

Similarly, a significant interaction effect was found for EMG:M_{SOL} ($p<0.05$) and EMG:M_{TS} ($p<0.05$). Post-hoc analyses revealed a reduction of 13.2% for EMG:M_{SOL} and 8.2% for EMG:M_{TS} immediately after stretch but no difference at 15 min,

indicating that EMG:M was fully recovered by 15 min. Both %VA and V:M ratio were not significantly different to the control condition at any time point. .

There were moderate-strong correlations between changes in torque and changes in central drive measurements, including EMG:M_{SOL} (r=0.93, p<0.001), EMG:M_{LG} (r=0.82, p=0.001), EMG:M_{TS} (r=0.88, p<0.001), as well as %VA (r=0.76, p=0.002) and V:M ratio (r=0.65, p=0.017) immediately after stretch (Figure 2.3). Thus, greater decrements in torque were observed in subjects who had greater reductions in measures of central drive. Interestingly, the recovery of peak torque within 15 min of stretch (Figure 2.3) was also correlated strongly with EMG:M_{SOL} (r=0.81, p=0.001), EMG:M_{LG} (r=0.79, p=0.001), EMG:M_{TS} (r=0.80 p=0.001) and %VA (r=0.77, p=0.003) recovery. These results indicate that a similar temporal response occurred in torque production and central drive.

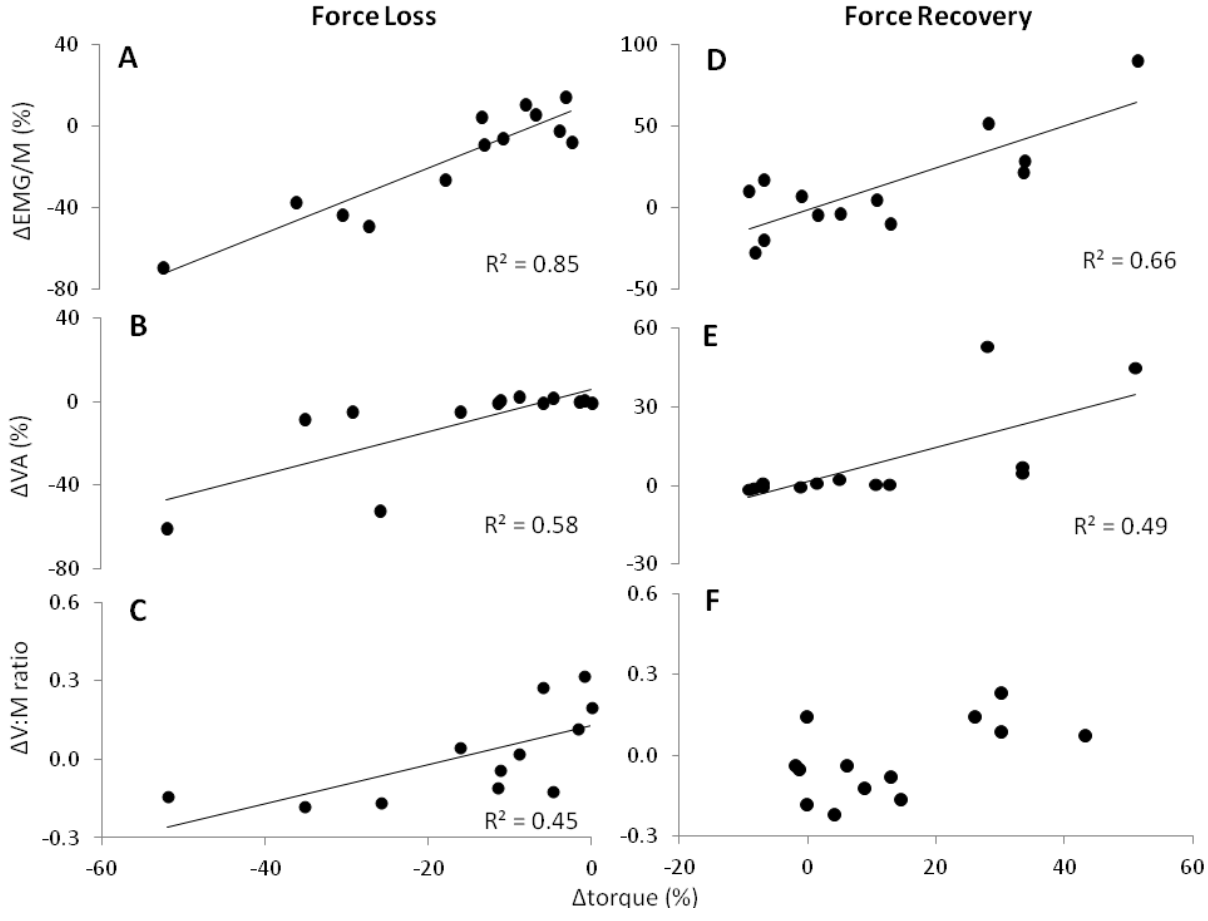

Figure 2.3. Relationship between changes in torque and changes in indicators of central drive. A strong correlation was found between the reduction in torque and decreases in A) the soleus EMG:M ratio (r=0.93), B) percent voluntary activation (%VA; r=0.76), and C) V-wave amplitude (V:M ratio; r=0.65). Force recovery was also strongly correlated with recovery of D) the soleus EMG:M ratio (r=0.81) and E) %VA (r=0.77). The correlation between the change in torque during recovery and changes V-wave amplitude (F) was not statistically significant. Force loss = changes in torque from baseline to immediately after stretch; Force recovery = changes in torque from immediately to 15 min after stretch.

There was a significant interaction effect for torque elicited by the 20 Hz, catch-inducing train and twitch peak torque (T_{peak}) ($p<0.05$). Post-hoc analyses revealed that reductions in peak torque in response to 20 Hz (11.5%), catch-inducing (10.8%) and twitch (9.4%) stimulations occurred only immediately after stretch, with no further change to 15 min. There was a trend towards a reduction in torque elicited by 80 Hz stimulation ($p<0.1$), but no significant interaction effect was found. In addition, no interaction effect or correlation was found for 20:80 ($p>0.05$) or 20:catch ($p>0.05$) ratios, suggesting that muscle force production was affected somewhat by the stretch protocol, but it could not be explained by changes in E-C coupling efficiency. There were no changes in M_{max} amplitude detected, when compared to the control condition ($p>0.05$), indicating that muscle excitability was not affected by the stretch protocol.

2.4 Discussion

The present research examined the contributions of central vs. peripheral factors to the stretch-induced torque loss in the human plantar flexors. The main findings were that: 1) decreases in EMG:M, voluntary activation and V-wave amplitude (i.e. central factors) were strongly related to both the torque reduction after stretch and the torque

recovery, indicating that central drive modification influenced the loss and recovery of muscle force; and 2) the muscle's contractile capacity (i.e. electrical-elicited contractions) was moderately reduced, but these changes were not associated with the loss or recovery of torque and there was no evidence of a change in E-C coupling efficiency after the stretch protocol utilised in the present study.

The present data provide the clearest evidence of a reduced central drive influencing force production after stretch. As shown in Figure 2.4, three different parameters (EMG:M, %VA and V-wave) were investigated in order to detect central changes, and reductions in these parameters were strongly correlated with reductions in torque after stretch. Moreover, recovery of EMG:M and %VA were strongly correlated with force recovery, suggesting that recovery of efferent drive may have been important in the return of muscle force to baseline. It has been suggested that the force reduction might be caused by a decrease in central drive because decreases in EMG amplitude have been reported and relationships between changes in EMG amplitudes and changes in torque have been demonstrated (Fowles et al., 2000; Kay & Blazevich, 2009b). However, EMG amplitude can be influenced by peripheral, in addition, to central, factors so some caution was exercised in the interpretation of these results. Although the EMG:M ratio, as measured in the present study, can still be influenced by factors other than the absolute magnitude of central drive (e.g. motor unit synchronisation), the simultaneous depression of voluntary activation and V-wave amplitude is strongly suggestive of a central depression, and the correlations between EMG:M, voluntary activation and the torque recovery to 15 min provides substantial additional support for the hypothesis (Figure 2.3). Thus, the findings of the present study strongly support the proposition that a reduced central drive is a major factor contributing to the voluntary torque loss caused by acute passive muscle stretch.

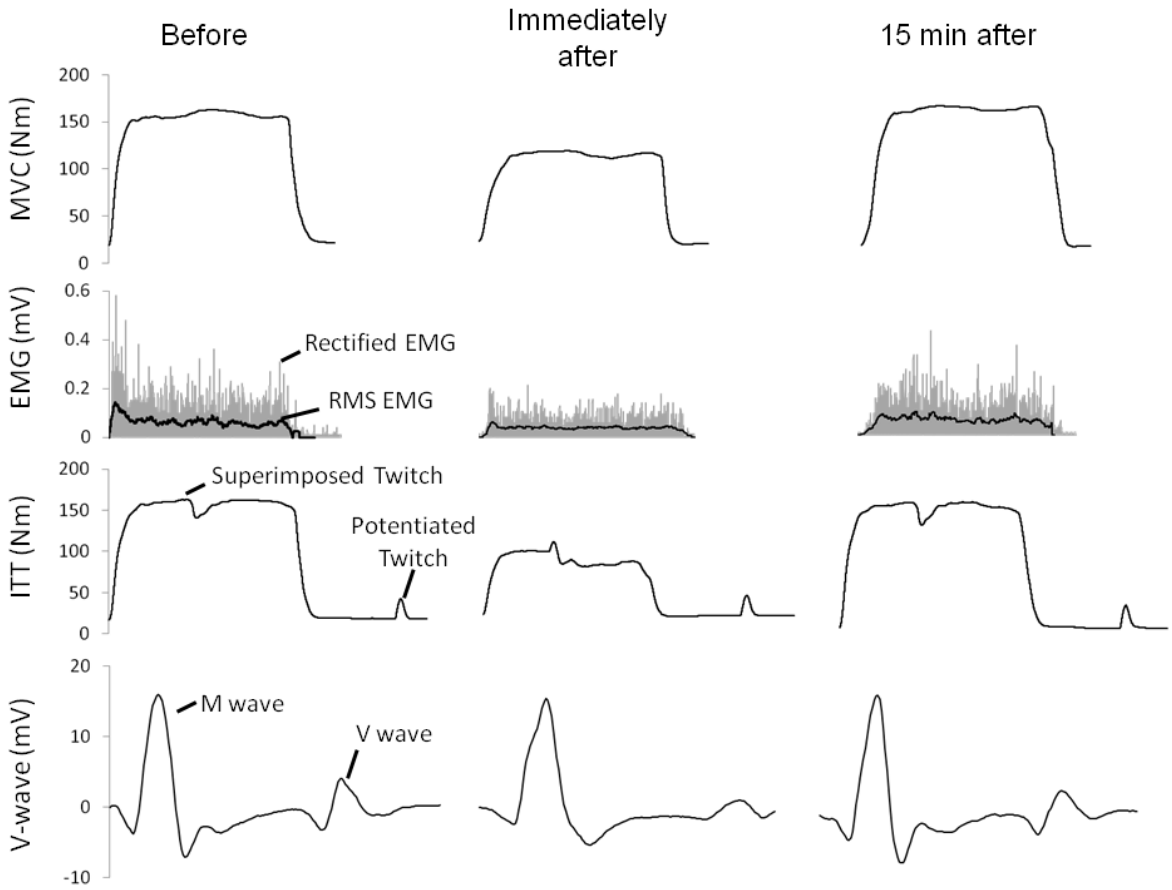

Figure 2.4. Example of data obtained from one subject before (left column), and immediately (middle column) and 15 min after (right column) the stretch protocol. A decrease in maximal voluntary contraction (MVC) torque (first row), EMG amplitude (second row) and V wave amplitude (last row), and an increase in the superimposed twitch torque (i.e. decreased voluntary activation; third row) are visible immediately after stretch. (ITT; interpolated twitch technique)

From the current data it is not possible to determine the site/s of origin of the central drive limitation. Descending output from the motor cortex can exert significant executive control over muscle force so changes in supra-spinal command are clearly a potentially important factor. To the best of my knowledge there is no clear evidence that muscle stretching can affect supra-spinal outflow, however this possibility is particularly worthy of exploration because the mild pain response elicited by the stretch

might have been sufficient to reduce motor cortical drive to the muscle (Le Pera et al., 2001; Schabrun & Hodges, 2012) and thus reduce motor unit firing frequency (Farina, Arendt-Nielsen, & Graven-Nielsen, 2005; Farina, Arendt-Nielsen, Merletti, & Graven-Nielsen, 2004). Studies imposing muscle stretch whilst pharmacologically blocking the pain response may be useful in testing this hypothesis. Spinal-level inhibition is also a candidate site for examination. Spinal interneurones can modulate both Ia afferent feedback and motor neurone excitability through inhibitory and excitatory mechanisms (Jankowska & Hammar, 2002). In particular, the soleus Ia inhibitory interneurone is thought to be excited by both agonist and synergist Ia afferents (Fetz, Jankowska, Johannisson, & Lipski, 1979; Schieppati, Romano, & Gritti, 1990). Thus, the stretch protocol used in the present experiments may have promoted an autogenic inhibition of soleus and a subsequent decrease in its activity level. Finally, motor neurone disfacilitation is a possible mechanism. Alpha motor neurones are strongly dependent upon facilitatory inputs to achieve maximal discharge frequency, and thus to produce high levels of muscular force (Hultborn et al., 2003). This facilitatory modulation occurs at the motor dendrites and is controlled by the interaction between descending monoaminergic drive and spinal circuits, especially including the Ia afferents (Heckman et al., 2004). For instance, changes in muscle length directly affect the level of dendritic amplification to the motor neurone (Hyngstrom et al., 2007), so the prolonged increase in muscle length during the stretch may have reduced Ia afferent input onto α-motor neurone. Indeed, a reduction in Ia afferent efficiency (measured as decrease in H-reflex amplitude) concomitant with a decrease in plantar flexor torque has previously been reported immediately after prolonged passive stretching (Avela et al., 1999); a reduction in Ia afferent input could affect the motor neurone facilitatory process preventing maximal discharge rates being attained during voluntary torque production.

Interestingly, both the H-reflex amplitude and maximal force production were shown to recover within 15 minutes of the stretch (Avela et al., 1999). The temporal match with our data is suggestive that central drive might be reduced immediately after stretch, and then recover relatively rapidly and simultaneously with torque.

In the present study it was also tested, for the first time, the hypothesis that passive stretch could affect E-C coupling efficiency by comparing the torque produced during low- and high-frequency tetanic stimulation. Reductions in tetanic torque were evident in 20 Hz and catch-inducing tetanic stimulation conditions, suggesting that the muscle's contractile capacity was compromised. However, these reductions were relatively small and were not correlated with the changes in voluntary peak torque. Moreover, the lack of changes in 20:80 and 20:catch ratios suggests that calcium homeostasis was not affected significantly by the present stretch protocol and that any small changes in muscle or tendon mechanical properties also did not specifically influence torque induced by the high-frequency pair of pulses during the catch-inducing train. Additionally, no change in M_{max} amplitude was found, indicating that sarcolemmal and t-tubular function was not significantly compromised. One might speculate that the moderate changes in muscle torque could result from mechanical changes within the parallel elastic component. It has been proposed that parallel elastic components are responsible for epimuscular force transmission, which is an important factor contributing to maximal force production (Maas, Meijer, & Huijing, 2005; Maas & Sandercock, 2010), and connective tissues such as the perimysium might be affected by static muscle stretch (Borg & Caulfield, 1980; Purslow, 1989). Clearly, changes in the series elastic components, and particularly the Achilles tendon, are unlikely to have had a substantial influence (Kay & Blazevich, 2009a, 2009b, 2010; Morse et al., 2008).

Regardless, the moderate changes in muscular function did not appear to have a notable effect on voluntary torque production in this study.

With respect to the current data, some limitations should be highlighted. This study was designed to investigate the mechanisms underpinning the torque loss, so a relatively long stretch duration was employed (5 min). However, short duration stretches (< 45 s), which are commonly performed in pre-exercise routines, are unlikely to negatively affect force production. Thus, it is possible that the neuromuscular changes observed in the present study would not be observed under shorter stretch conditions. Nonetheless, further research is needed to clarify the 'dose-response' relationship between stretch duration and central drive depression. Second, mechanical properties of the muscle (e.g. changes in parallel elastic components) could not be measured, but may have influenced muscle force production without affecting the E-C coupling process. Further research is required to clearly determine the effects of changes in the mechanical properties of, in particular, the parallel elastic components on neuromuscular measurements and whether muscle stretch might influence these. Third, using the present methodology it was not possible to determine the site/s of origin of the central drive limitation. Research using brain imaging and cortical brain stimulation techniques could provide a clearer picture in this regard.

In summary, the present data indicate that the torque decrement elicited by passive plantar flexor muscle stretch was strongly associated with a reduction in central (efferent) drive. This conclusion is based on the significant decrease and recovery of the EMG:M ratio, the strong correlation between the torque loss and decreases in EMG:M, %VA and V:M, and the association of torque recovery with EMG:M and %VA recovery; further research is required to determine the specific location of the central drive modification. The stretch protocol may have induced a deficit at the muscular

level, however changes were not substantial and were not correlated with the torque loss or recovery. Notwithstanding the clear loss of force induced by the stretch, it is also important to note, from a practical perspective, that torque recovered quickly and certainly within 15 minutes. These changes occurred in response to 5-min muscle stretch and future studies should determine if shorter durations of stretch impair maximal force by the same mechanisms.

CHAPTER 3

Study 2: Intermittent Stretch Reduces Force and Central Drive More Than Continuous Stretch

3.1 Introduction

The detrimental effect of passive muscle stretching on maximal force production has been well documented (Behm & Chaouachi, 2011; Kay & Blazevich, 2012), yet the mechanisms underpinning this stretch-induced torque loss are not completely understood and therefore strategies cannot be developed to minimise its impact. It was recently shown that torque depression subsequent to a single 5-min constant-torque plantar flexor stretch was largely explained by a reduction in central drive to the muscle, with a minor effect at the muscle level (Study 1). However, most studies investigating the torque loss caused by stretch have utilised intermittent (i.e. repeated) stretch protocols (Kay & Blazevich, 2012). Such protocols are commonly performed in clinical and sports environments and the possibility exists that intermittent protocols elicit different changes in central drive and muscle mechanical properties than continuous stretches. In particular, intermittent stretch has been reported to be more efficient in reducing muscle stiffness when compared to continuous stretch (McNair, Dombroski, Hewson, & Stanley, 2001; Nordez, McNair, Casari, & Cornu, 2007), which might be associated with the cyclic strain reducing muscle viscosity and/or the thixotropic behaviour of the musculo-articular system (McNair et al., 2001). Additionally, intermittent and continuous stretches potentially elicit different changes in tissue oxygenation kinetics.

A significant ischaemic response (i.e. nearly at the level induced by cuff occlusion) can occur during a continuous passive muscle stretch (McCully, 2010), as a result of a prominent increase in intramuscular pressure (Otsuki et al., 2011). Alternatively, intermittent stretch is characterised by repeated stretches separated by rest intervals, which results in a cycle of ischaemia and blood reperfusions. Ischaemia-reperfusion cycles have been shown to cause damage in a variety of tissues, including skeletal muscle, through reactive oxygen species-dependent mechanisms (Blaisdell, 2002; Gute, Ishida, Yarimizu, & Korthius, 1998). During the blood reperfusion phase there is the possibility of reactive oxygen species formation through xanthine oxidase and nitric oxide pathways (Powers & Jackson, 2008); indeed passive muscle stretch has been shown to increase nitric oxide (Tidball et al., 1998) and reactive oxygen species production (Chambers et al., 2009; Palomero et al., 2012) in animal models. Importantly, an increase in reactive oxygen species production can affect the calcium-release ryanodine receptor and thus impair the excitation-contraction coupling process (Bruton et al., 2008; Powers & Jackson, 2008). It is therefore possible that intermittent stretch might impede functioning of the contractile apparatus more than continuous stretch, influence both the magnitude and the temporal profile of the torque loss differently to continuous stretch, and increase the torque deficit attributable to muscular rather than neural mechanisms. Thus, rather than the decrease in oxygenation levels elicited by stretch protocol alone that may be problematic, it is the independent (and possibly additive) effect of multiple reperfusion events and muscle stretch cycles on the contractile apparatus. Despite the potentially disparate effects of intermittent and continuous stretching, their relative effects on muscle torque depression, and the mechanisms that underpin it, have not been explicitly studied.

Given the above, the purpose of the present study was to compare the effects of continuous and intermittent muscle stretch protocols on the stretch-induced force loss, and to determine the relative contributions of central versus peripheral factors to these losses. It was hypothesised that 1) intermittent and continuous plantar flexor stretches would confer similar acute improvements in ankle joint range of motion, and that 2) central (neural) drive would be equally depressed after both stretch protocols. However, It was also hypothesised that 3) whilst continuous muscle stretch would cause a greater magnitude of tissue oxygenation reduction than intermittent stretch, substantial cyclic variations in tissue oxygenation (i.e. ischaemia-reperfusion cycles) would result from the intermittent stretch, and thus 4) intermittent stretch would cause a greater magnitude of, and more prolonged reduction in, muscle torque resulting from impairments in the contractile apparatus.

3.2 Methods

3.2.1 Subjects

Eighteen healthy men (mean ± SD: age, 26.8 ± 4.5 y; height, 1.75 ± 0.1 m; body mass, 72.7 ± 12.6 kg) with no previous neuromuscular impairment volunteered for the study. The subjects had not engaged in flexibility training for at least 6 months prior to the study and refrained from such training during data collection period. The subjects refrained from vigorous exercise and alcohol consumption for 24 h, and stimulant (e.g. caffeine) use for 6 hours, prior to testing. They read and signed an informed consent document, and the research was approved by the University Human Research Ethics Committee.

3.2.2 Study design and overview

Subjects visited the laboratory on four occasions at the same time of day separated by at least 48 h. In the first session they were fully familiarised with the test procedures whilst the subsequent three visits were used for the completion of the following experimental conditions in a counterbalanced order: 1) control (or no stretch); 2) 1 set of 5 min (continuous stretch); and 3) 5 sets of 1 min (intermittent stretch; 15 s rest) passive plantar flexor stretching. The subjects were assessed immediately before, and immediately, 15, and 30 minutes after each intervention. During the familiarisation session the intensities of all electrically evoked muscle and nerve stimulation measurements were determined and both the maximum tolerable passive torque during stretch and the maximal voluntary contraction torque (MVC) were measured. In the experimental sessions, performed at the same time of day as the familiarisation session, the subjects warmed up on a Monark cycle for 5 min by cycling at 60 rpm with a 1-kg load. The subjects were then seated upright in the chair of an isokinetic dynamometer (Biodex System 3 Pro, IPRS, Suffolk, UK) with the knee in full extension (0º), the ankle in the neutral position (90º; plane of foot relative to tibia) with the sole of the foot perpendicular to the shank, and the lateral malleolus of the fibula aligned to the centre of rotation of the dynamometer.

3.2.3 Muscle stretching protocol

All stretch procedures were whilst seated (0^{o} knee angle) performed on an isokinetic dynamometer with the subjects instructed to keep their muscles relaxed. The plantar flexors were stretched by rotating the ankle into dorsiflexion at $5^{o} \cdot s^{-1}$ until the passive resistance reached 90% of the maximal tolerable stretch torque, as measured during the familiarisation session. Passive torque typically decreases during the stretch (i.e. stress

relaxation (Magnusson, 1998)), so the joint angle was continually adjusted toward dorsiflexion during the stretch to maintain the passive torque within 5 Nm of the initial stretch torque level. With this design stretches in both conditions were of the same intensity and volume, with the only difference being that the intermittent stretch had 15-s resting interval after each 1-min stretch.

3.2.4 Voluntary and evoked torque measurements

Peak isometric plantar flexor torque (T_{Peak}) was assessed during MVCs with the ankle in a neutral position (90º). Maximal torque is commonly reduced by the anticipation of discomfort caused by the supra-maximal nerve stimulation (Button & Behm, 2008). Thus, to avoid the effect of stimulus anticipation, two MVCs were performed at each time point: the first MVC was used to calculate T_{Peak} and measure muscle activity (EMG; see below), and the second MVC was performed concurrently with tibial nerve stimulation in order to measure voluntary activation (interpolated twitch technique; ITT) and V-wave amplitude. The subjects were instructed to produce a force against the dynamometer foot plate by rotating the ankle as fast and as hard as possible. Verbal encouragement and visual feedback were provided during all MVCs.

3.2.5 Stimulation procedures

3.3.5.1 Muscle stimulation (20:80 ratio, constant vs. catch-inducing train stimulations)

A constant current electrical stimulator (DS7, Digitimer Ltd, Welwyn Garden City, UK) was used to deliver an electrical square-wave stimulus (0.5-ms pulse width) to the plantar flexor muscle belly through two self-adhesive electrodes (9×5 cm, Dura-Stick® II, Chattanooga Group, Hixon, USA). The cathode was placed on the medial and lateral gastrocnemius muscle bellies, where greatest motor response was elicited (i.e. assumed

motor point) and the anode was placed over the distal myotendinous junction of soleus. For all tetanic stimulations, the intensity necessary to reach 50% of MVC with a 0.5-s 80 Hz tetanic stimulation was used. Three tetanic stimulations with the same duration (0.5 s) were delivered to test for excitation-contraction (E-C) coupling efficiency: 1) 20 Hz train; 2) catch-inducing train (i.e. 20 Hz train with the first two pulses at 100 Hz); and 3) 80 Hz train. The peak torque produced by the 20 Hz and 80 Hz stimulations were used to calculate the 20:80 ratio, which was used as a measure of E-C coupling efficiency (Martin et al., 2004). The catch-inducing train was used to assess the muscle's catch-like properties, which is thought be affected by changes in calcium release, when compared to a constant-frequency train (20:catch ratio) (Binder-Macleod & Kesar, 2005; Burke et al., 1970).

3.2.5.2 Nerve stimulation (Twitch, ITT, V-wave)

The same electrical stimulator was used to deliver the electrical square wave 1-ms pulse width stimuli to the posterior tibial nerve via a cathode electrode (Ag-AgCl, 10 mm) fixed to the popliteal fossa and an anode electrode of large size (9×5 cm, Dura-Stick® II, Chattanooga Group, Hixon, USA) placed on the anterior surface of the knee. ITT was used to estimate the percentage voluntary activation (%VA) of the muscle. The intensity for a single twitch was set at 120% of the intensity required to elicit M_{max}, to ensure that a supramaximal current stimulus was used. Supra-maximal twitches were elicited before, during and 2 s after an isometric plantar flexor MVC (Merton, 1954). A comparison of the interpolated twitch to the resting potentiated (i.e. post-MVC) twitch was completed, with %VA being calculated using the following equation (Shield & Zhou, 2004): %VA = [1-(superimposed twitch/potentiated twitch)] × 100. The superimposed twitch was also used to elicit the first volitional wave (V-wave), which is

an electrophysiological variant of the H-reflex (Upton et al., 1971) and has been extensively used and validated in the literature to determine changes in efferent drive to the muscle (Aagaard et al., 2002b; Duclay & Martin, 2005). Although multiple V-wave assessments are ideal to improve the method's reliability (Aagaard et al., 2002b), a single stimulation was performed at each time point to minimise the effects of fatigue (Trajano et al., 2013). Thus, the balance between an optimum number of stimulations and the minimisation of muscle fatigue was taken into consideration. The V-wave peak-to-peak amplitude was measured and then normalised to the M-wave amplitude measured prior to the MVC (V:M ratio) and reductions in the V:M ratio were interpreted as evidence of a reduction in central drive (Aagaard et al., 2002b).

3.2.6 Measurement of muscle activity (EMG)

Surface EMG was recorded from soleus (SOL) and lateral gastrocnemius (LG) using a bipolar electrode configuration at a 4000 Hz analog-digital conversion rate (bandwidth 10 to 500 Hz) using the Bagnoli-8 Main Unit EMG system (DelSys, Inc., MA, USA). The inter-electrode distance was 1 cm and a reference electrode was placed on the fibula's lateral malleolus. Further, to obtain clearer M- and V-wave data, surface EMG was recorded from SOL in a pseudo-monopolar configuration (sample rate 4000 Hz) using the BioAmp EMG system (PowerLab System, ADInstruments, NSW, Australia), with one electrode placed on the medial aspect of SOL below the distal gastrocnemius junction and the other placed at the Achilles tendon-soleus muscle-tendon junction ~3 cm superior to the malleolous (Blazevich, et al., 2012). The skin under the electrodes was shaved, abraded and cleaned with alcohol to reduce the inter-electrode resistance below 5 kΩ. During the stretch protocols EMG data were also recorded to ensure that

muscle activation remained below 10% of the maximal value; a small activity response is normally detected even when the subjects are asked to remain completely relaxed (Blazevich, et al., 2012). Muscle activity was expressed as root mean square (RMS) EMG amplitude (500-ms averaging window) and normalised to the M-wave amplitude measured before the contraction (EMG:M) to account for the possible influence of peripheral factors. The EMG:M quantified from SOL (EMG:M_{SOL}) and LG (EMG:M_{LG}) were summed and considered as a measure of central drive to the triceps surae (EMG:M_{TS}) (Kay & Blazevich, 2009b; Trajano et al., 2013). Ankle joint torque, joint angle and EMG data were simultaneously recorded using LabChart v.6.1.3 Software (PowerLab System, ADInstruments, NSW, Australia).

3.2.7 Measurement of muscle oxygenation

Near-infrared spectroscopy (NIRS) (NIRO-200, Hamamatsu Photonics K.K., Hamamatsu, Japan) was used to estimate the changes in muscle oxygenation continuously during muscle stretching. A probe holder, consisting of 2 silicon photodiodes as photodetectors on one side and three light-emitting diodes on the other side (separated by 4 cm), was firmly attached to the skin at the mid-belly of the medial gastrocnemius and was covered with a dark tape to eliminate other light interference. Oxy-haemoglobin concentration (HbO_2), was assessed in a baseline condition of 5 min of rest before testing. Haemoglobin oxygenation status was quantified as the change from the baseline value of HbO_2. NIRS data were obtained from the medial gastrocnemius and was assumed to be indicative of the triceps surae oxygenation status during stretch (See example Figure 3.1). This was prompted by the space limitations imposed by the simultaneous placement of EMG and muscle stimulation electrodes and

NIRS probes on lateral gastrocnemius and, more specifically, the soleus muscle belly. (Hamaoka, McCully, Quaresima, Yamamoto, & Chance, 2007).

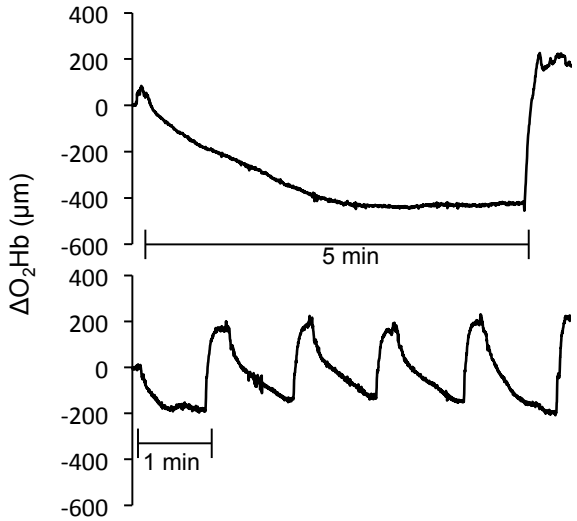

Figure 3.1. Muscle oxygenation response in one subject during continuous stretch (A; prolonged ischemic response) and intermittent stretch (B; cycles of ischemia and reperfusion).

3.2.8 Statistical analysis

Data are presented as mean ± SD. A two-way repeated measured ANOVA was used to compare ROM, passive torque and oxygenation status between protocols (continuous and intermittent) over time (1, 2, 3, 4 and 5 min of stretch). Separate two-way repeated measures ANOVAs were performed to compare changes in T_{peak}, and central and peripheral function variables between conditions (control, continuous and intermittent) over time (before, immediately after, and 15 and 30 min after). Pairwise comparisons with Bonferroni corrections were performed when a significant interaction effect was detected. Pearson's product-moment correlation coefficients were computed to

determine the relationships between changes in torque and changes in central (EMG:M; %VA; V-wave) and peripheral (20:80 ratio, 20:catch ratio, peak twitch torque) mechanisms. Statistical significance was set at an α level of 0.05.

3.3 Results

There was a significant interaction (time × condition) effect for range of motion ($p=0.043$). Post-hoc analyses revealed an increase in range of motion at minutes 2 (3.9%), 3 (6.1%), 4 (8.7%), and 5 (10.2%) when compared to minute 1 in the continuous stretch condition only (Figure 3.2). Passive torque during stretch was not different between protocols. There was an interaction effect for changes in muscle oxygenation ($p=0.000$), with a greater decrease in muscle oxygenation being found during the stretch in the continuous stretch condition from minutes 2 to 5 (Figure 3.1 and 3.3).

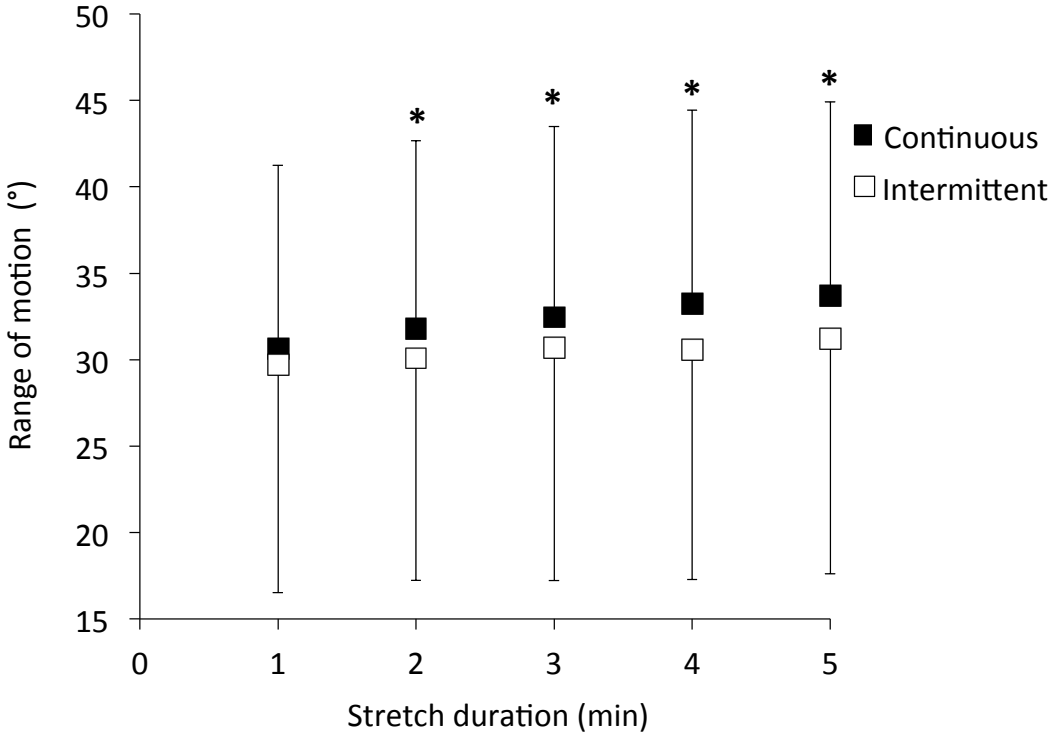

Figure 3.2. Range of motion achieved from minutes 1 to 5 during continuous and intermittent stretch protocols. The range of motion only increased during continuous stretching * Significantly different from the first minute ($p \leq 0.05$). Data are presented as mean ± SD.

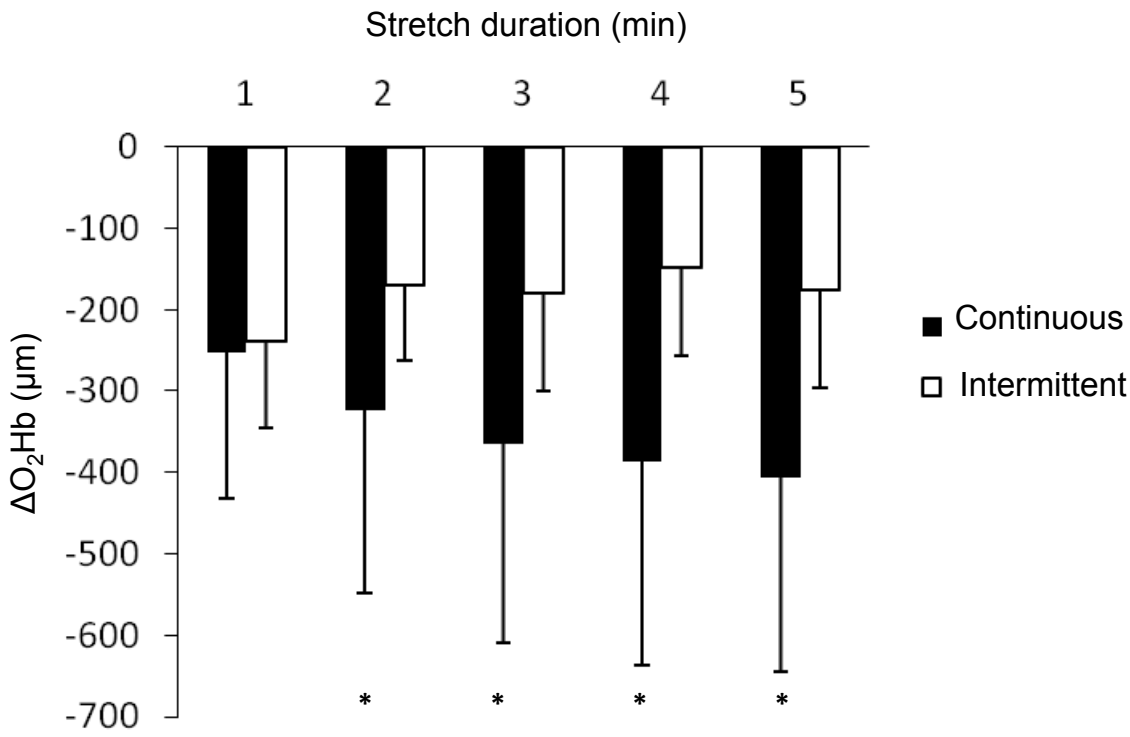

Figure 3.3. Muscle oxygenation change from baseline during continuous and intermittent stretch (mean ± SD). O_2Hb was reduced more during continuous than intermittent stretch from minute 2. Also, during the continuous stretch there was a reduction in muscle oxygenation at minutes 2, 3, 4 and 5 when compared to minute 1.* Significant difference between conditions and significant different from minute 1 for the continuous stretch ($p \leq 0.05$).

A significant interaction effect was found for T_{peak} ($p=0.001$). Post-hoc analysis revealed that T_{peak} decreased more after intermittent (-23.8 ± 22.1%) than continuous stretch (-14.3 ± 17.2%). T_{peak} reduction was fully recovered by 15 min after continuous stretch, but remained depressed by -5.7 ± 5.1% and -5.6 ± 5.8% at 15 and 30 min, respectively, after intermittent stretch (Figure 4).

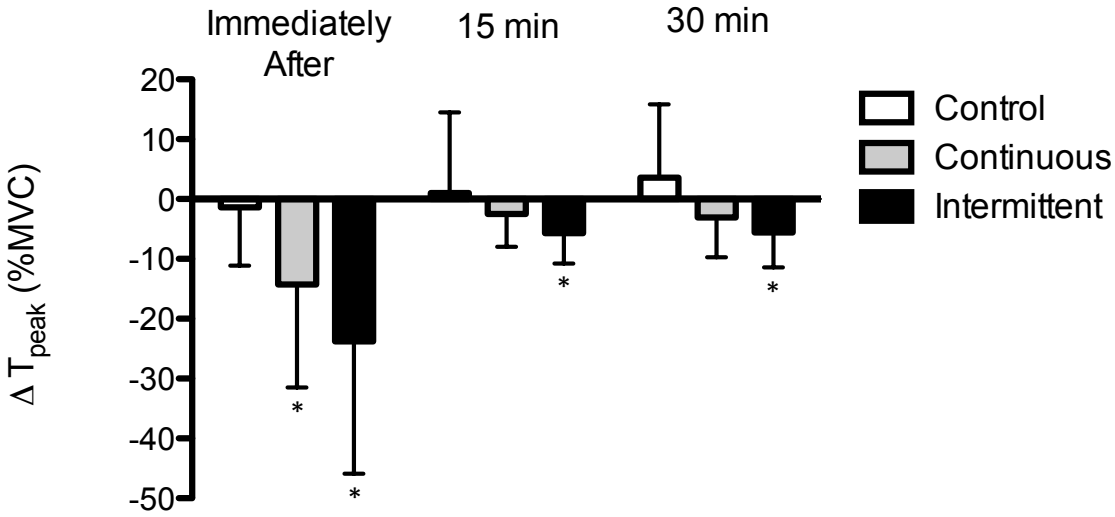

Figure 3.4. Torque changes immediately, and 15 min and 30 min after continuous stretch, intermittent stretch or passive rest (control). Peak torque was reduced immediately after both stretch and remained reduced until 30 min after intermittent stretch. * Significantly greater change compared to the control condition ($p<0.05$). Data are presented as mean ± SD.

Significant interaction effects were found for EMG:M_{SOL} ($p=0.013$), EMG:M_{LG} ($p=0.005$) and EMG:M_{TS} ($p=0.000$). Post-hoc analyses revealed greater reductions in EMG:M_{SOL} (-27.7 ± 31.6%), EMG:M_{LG} (-24.5 ± 35.7%) and EMG:M_{TS} (-27.1 ± 30%) immediately after intermittent stretch when compared to continuous stretch (-12.8 ± 24.8, -1.0 ± 38.5 and -7.9 ± 33.3 %, respectively). No significant reductions in EMG:M were found at 15 and 30 min after stretch. Moreover, there was an interaction effect for %VA, with a reduction in %VA (-15.9%) detected immediately after intermittent stretch

when compared to control and continuous stretch conditions, with no significant difference between conditions at 15 and 30 min post-stretch. Additionally, there was no significant interaction effect for V:M.

There were strong correlations between changes in torque and changes in central drive measurements, including EMG:M_{SOL} (r=0.91, p=0.000), EMG:M_{LG} (r=0.75, p=0.000), EMG:M_{TS} (r=0.81, p=0.000) and %VA (r=0.78, p=0.000) immediately after continuous stretch. Also, moderate-strong correlations were found between changes in torque and changes in EMG:M_{SOL} (r=0.88, p=0.000), EMG:M_{LG} (r=0.84, p=0.000), EMG:M_{TS} (r=0.89, p=0.000), %VA (r=0.93, p=0.000) and V/M (r=0.51; p=0.031) immediately after intermittent stretch. Thus, subjects who had greater reductions in measures of central drive also had greater reductions in peak torque. Interestingly, the full recovery of peak torque 15 min after continuous stretch was also strongly correlated with EMG:M_{SOL} (r=0.87, p=0.000), EMG:M_{LG} (r=0.72, p=0.001), EMG:M_{TS} (r=0.89 p=0.000) and %VA (r=0.72, p=0.001) (Figure 3.5). In contrast, the partial recovery of peak torque 15 min after intermittent stretch was moderately correlated with the recovery of EMG:M_{SOL} (r=0.58, p=0.012), EMG:M_{LG} (r=0.54, p=0.022), EMG:M_{TS} (r=0.54 p=0.01) and %VA (r=0.60, p=0.009) (Figure 3.6). These results indicate that a similar temporal response occurred in both torque production and central drive in both stretch conditions, however intermittent stretch caused a small (~5%) but long-lasting torque reduction that could not be attributed to reductions in central drive.

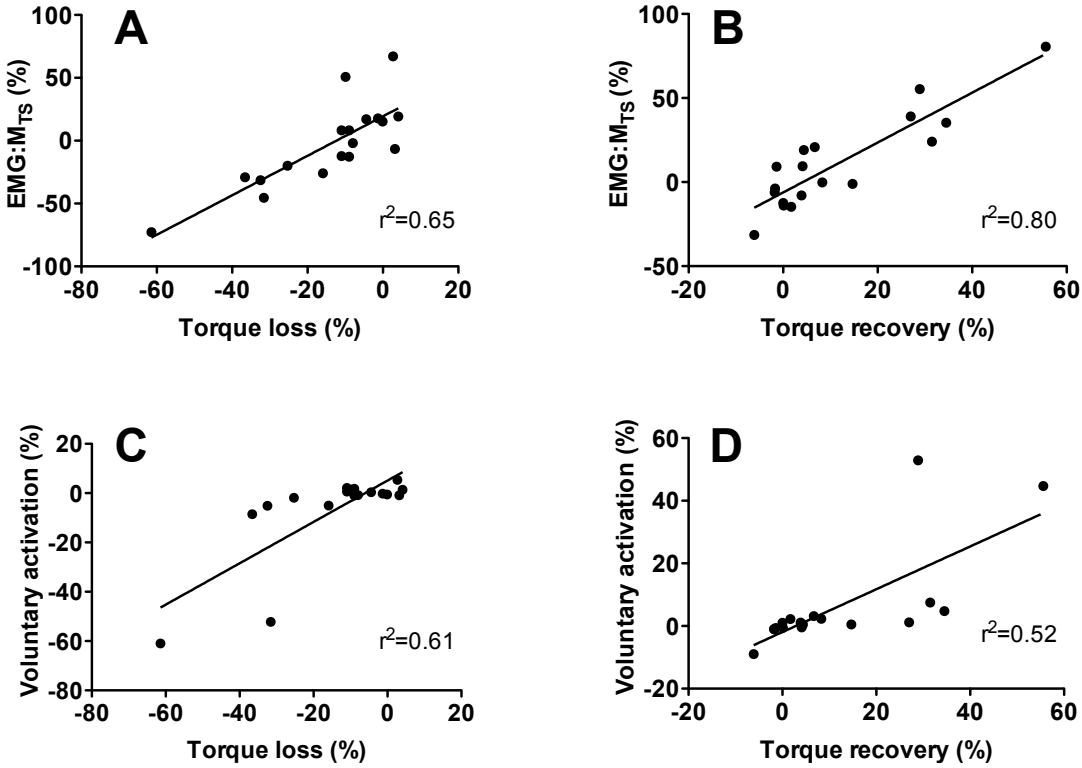

Figure 3.5. Relationship between changes in torque and changes in indicators of central drive after continuous stretch. A strong correlation was found between the reduction in torque and decreases in: A) the triceps surae EMG:M ratio (r=0.81) and B) percent voluntary activation (%VA; r=0.78). Force recovery was also strongly correlated with recovery of C) the triceps surae EMG:M ratio (r=0.81) and D) %VA (r=0.77). Force loss = change in torque from baseline; Force recovery = change in torque from immediately to 15 min after stretch.

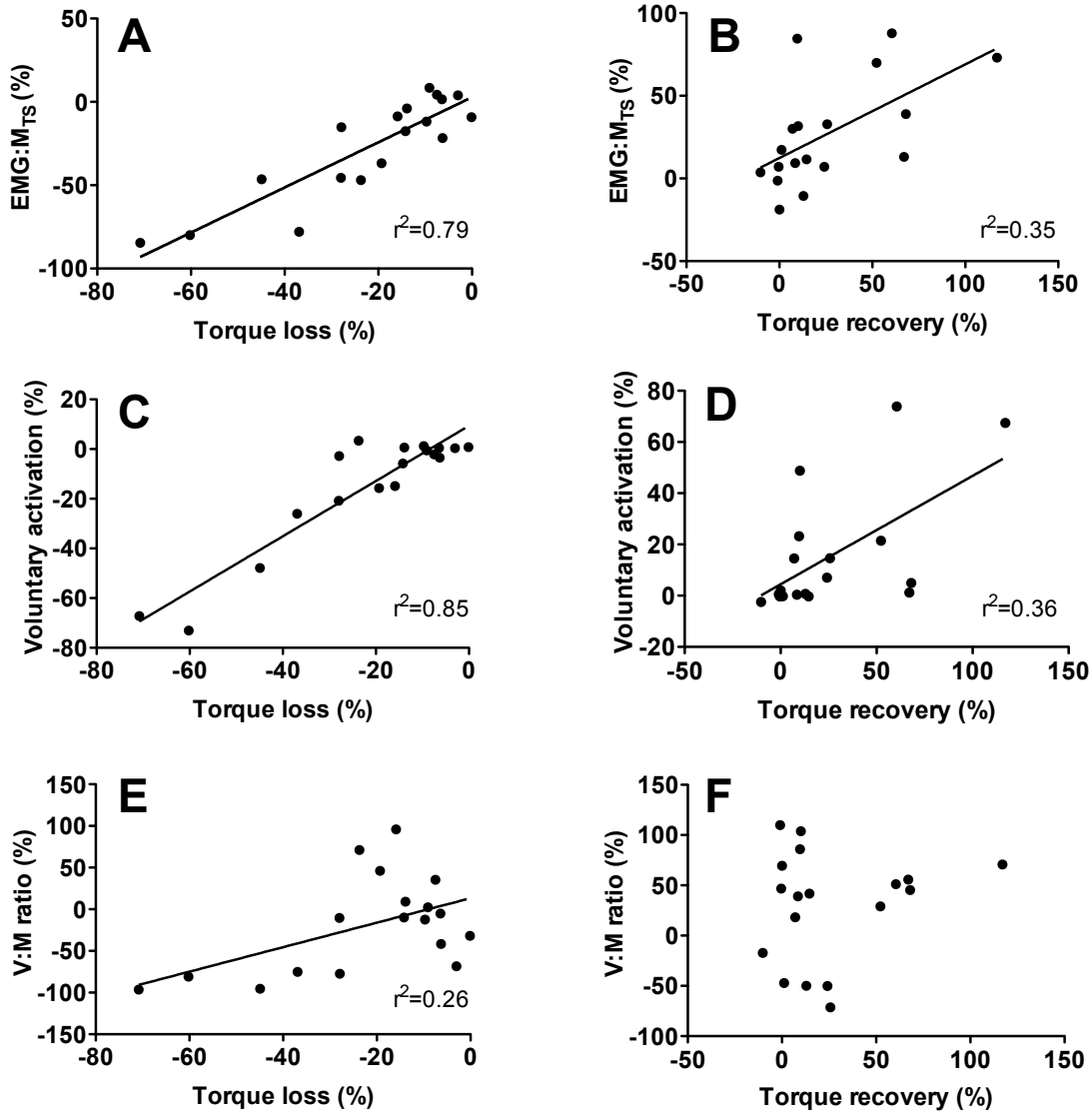

Figure 3.6. Relationship between changes in torque and changes in indicators of central drive after intermittent stretch. A strong correlation was found between the reduction in torque and decreases in: A) the triceps surae EMG:M ratio (r=0.89); B) percent voluntary activation (%VA; r=0.93) and V-wave amplitude (V:M; r=0.51). Force recovery was also moderately correlated with recovery of: D) the triceps surae EMG:M ratio (r=0.54) and E) %VA (r=0.60), but not F) V:M.

There was a significant interaction effect for torque elicited by the 20 Hz (p=0.01), catch-inducing (p=0.000) and 80 Hz (p=0.041) trains. Post-hoc analyses revealed reductions in peak torque in response to 20 Hz (-10.9 ± 8.7%), catch-inducing (-10.6 ±

8.1%) and 80 Hz (-6.7 ± 5.5%) stimulations immediately after continuous stretch, with no further change to 15 and 30 min. Likewise, the peak torque elicited by 20 Hz, catch-inducing and 80 Hz stimulations were reduced immediately after intermittent stretching (-13.1 ± 8.9%, -12.8 ± 7.5% and -6.4 ± 6.4%, respectively) with no difference in the control condition detected at 15 and 30 min post-stretch. In addition, no interaction effect or correlation was found for 20:80 ($p=0.46$) or 20:catch ($p=0.88$) ratios, suggesting that reductions in muscle force were not associated with changes in E-C coupling efficiency. There was no interaction effect detected for M_{max} amplitude ($p=0.21$), indicating that muscle excitability was not affected by the different stretch protocols. Likewise, there was no interaction effect detected for peak twitch torque ($p=0.09$).

3.4 Discussion

In Chapter 2 it was clearly identified a major contribution of central in comparison to peripheral mechanisms to the stretch-induced torque loss. However, whether the relative importance of these mechanisms might differ between various stretch protocols was still unknown. The novel findings of this study were that: 1) continuous stretch improved range of motion more than intermittent stretch; 2) continuous stretch lowered muscle oxygenation levels more than intermittent stretch; 3) the post-stretch torque loss in both conditions was associated with decreases in central drive; 4) intermittent stretch had a greater effect on torque loss magnitude and duration than continuous stretch; and 5) the prolonged (30 min) torque loss of ~5% subsequent to intermittent stretching could not be explained by central factors or changes in E-C coupling efficiency and could thus be explicable by peripheral factors other than those measured in the present study. These findings support the hypotheses that intermittent stretch can cause an acute torque

depression through central mechanisms, and there is a (small but) longer-lasting force loss possibly through peripheral mechanisms.

Surprisingly, only continuous stretch elicited a significant increase in range of motion during the stretch manoeuvre. This finding was unexpected since both protocols were performed to the same passive torque level and had the same total duration. One possible explanation is that the continuous stretch may have elicited a greater stress-relaxation response, requiring a greater increase in the range of motion to keep the passive torque constant during the stretch (Magnusson et al., 1996). To the best of our knowledge the comparison of equal intensity (i.e. with a continuous adjustment of passive tension) intermittent versus continuous stretch to acutely improve range of motion has never been investigated. The present data suggest that continuous stretch elicits a greater creep effect, and may improve range of motion more than intermittent stretch. Future studies investigating the effects of controlled-intensity continuous versus intermittent stretches on muscle mechanical properties and the time-course of potential changes are necessary to clarify these responses. Additionally, the continuous protocol caused a greater magnitude of reduction in muscle oxygenation, from the second to the fifth min of stretch, when compared to the intermittent protocol (Figure 3.3). Because muscle remains isovolumetric during stretch and contraction, the reduction in muscle oxygenation during muscle stretch results from the muscle circumference reduction causing an increase in intramuscular pressure, which both forces the blood from the muscle and prevents arterial in-flow (Otsuki et al., 2011). It has been previously reported that high levels of ischaemia and a subsequent dramatic reperfusion occur during passive muscle stretch (McCully, 2010), and the present data extend these findings by showing that muscle ischaemia levels were 2-fold higher during continuous stretch than in intermittent stretch in the lateral gastrocnemius. Clearly the intervals

after each minute of stretch during the intermittent protocol allowed the muscle time for blood reperfusion in the present study, which minimised the total decrease in HbO_2 when compared to continuous stretch. Given that greater changes in muscle torque were elicited by the intermittent stretch protocol, it was speculated that muscular responses associated with the ischaemia-reperfusion cycles might have had some influence. One important limitation of our data is that only the medial gastrocnemius muscle oxygenation status was monitored and assumed to be reflective of all the triceps surae. Although, it was expected an increase in intra-muscular pressure in all muscles, causing a reduction in blood flow in the whole triceps surae. Small variations are likely to occur between muscles because of architectural variation and thus intramuscular pressure differences. However, the lack of change in the 20:80 Hz torque ratio after both stretch protocols does not support the original hypothesis of an impairment in the E-C coupling process caused by the ischaemia-reperfusion cycles. Importantly, the influence on muscle torque production, at least, was minor when compared to the influence of central (neural) drive reduction.

Interestingly, despite the significant volume of literature reporting an acute effect of static stretch on muscle force production, a direct comparison between continuous and intermittent stretch protocols does not exist. Therefore, the finding that the force loss was greater and more prolonged after intermittent than continuous stretch is novel and has clear practical implications; constant stretch appears preferable for improving range of motion whilst affecting force to a lesser degree. Also of interest was that, while the force loss elicited by both stretch protocols was associated with significant reductions in measures of central drive, the reductions in central drive after intermittent stretch were more substantive. In fact, while the continuous stretch elicited a significant reduction in EMG:M_{SOL} only, the intermittent protocol caused significant decreases in EMG:M_{SOL}

(of greater magnitude than continuous stretch), EMG:M_{LG} and EMG:M_{TS} as well as a significant reduction in percent voluntary activation. These results suggest that the intermittent stretch protocol not only elicited a greater magnitude of reduction in central drive, but also influenced more muscles within the triceps surae. The association between torque depression and central drive limitation was further demonstrated by the finding that central drive recovery was strongly associated with the recovery of force 15 min after the continuous stretch, and moderately associated with recovery after intermittent stretch (Figure 3.6). For intermittent stretch, factors other than the recovery of central drive were possibly responsible for the prolonged torque loss. These findings are in agreement with previous studies showing that muscle torque and central drive recover rapidly after acute passive muscle stretching (Avela et al., 1999; Fowles et al., 2000; Kay & Blazevich, 2009b; Trajano et al., 2013). The results of the present study are similar to others who have investigated stretch-induced plantar flexor force loss during isometric contractions. For instance, Fowles et al (2001) reported a 28% (standard deviation was not reported) reduction in torque and Avela et al (1999) a 23.2 ±19.7% force loss. Indeed, using the data of Avela et al (1999), it was predicted force losses of up to 63% using the 2 standard deviation rule (i.e. 95% of results should fall within 2 standard deviations of the mean), which is substantial and in line with our data. However, the present study is one of the few to present individual data for the torque loss and therefore to clearly show the variable response of subjects. Although, from the present data it is not possible to determine the mechanisms that underpin the reduction in central drive; supra-spinal inhibition, interneurone inhibition and/or motor neurone disfacilitation are possibilities worthy of exploration in future studies (Trajano et al., 2013).

In order to test whether the ischaemia-reperfusion cycles caused impairment in the muscle contractile apparatus, 20 Hz, catch-inducing and 80 Hz tetanic stimulations were imposed after the stretches. Reductions in torque elicited by these stimulation protocols were seen after both stretch protocols, however the lack of change in 20:80 and 20:catch ratios suggest a lack of disturbance in the myoplasmic free Ca^{2+} concentration (Allen, 2004; Jones, 1996; Martin et al., 2004). In addition, the torque elicited by tetanic stimulations was recovered by 15 min, yet a prolonged reduction in the low-frequency stimulation (i.e. 20 Hz) torque would have been expected if there was impairment in the E-C coupling process (Jones, 1996; Martin et al., 2004). Thus, other changes within the muscle must have occurred after stretch. One possibility is that a viscoelastic deformation in the muscle may have affected lateral force transmission and ultimately maximal force production (Bojsen-Møller, Schwartz, Kalliokoski, Finni, & Magnusson, 2010). Muscle parallel elastic components such as the perimysium play a crucial role in the lateral transmission of force (Maas et al., 2005) and, since changes in Achilles tendon would likely have been negligible (Kay & Blazevich, 2009b; Morse et al., 2008), are the most likely component to be affected by the stretch protocol (Purslow, 1989). Nonetheless, lateral force transmission seems to be optimised when motor units are activated asynchronously and at a physiological frequency range (Brown, Cheng, & Loeb, 1999; Rack & Westbury, 1969; Roszek & Huijing, 1997). In the present study the muscle's ability to produce force using electrical stimulation, which results in synchronous activation of motor units, may not be ideal to detect these changes. The development of stimulation protocols that allow less-synchronous activation, such as wide pulse-width protocols or contractions evoked by tonic-vibration reflex induction (Bergquist, Clair, & Collins, 2011; Magalhaes & Kohn, 2010) might shed light on this in future studies. Although, there was no relationship between changes in peripheral

measures and the force loss caused by stretch, the prolonged small (~5%) force loss observed after intermittent stretch could not be explained by changes in central drive and may therefore be of peripheral origin. It must also be considered that the methods utilised in the present study were not sensitive enough to detect small changes in central or peripheral function that may each have contributed to the force loss.

In conclusion, the results of the present study support the hypothesis that a decrease in central drive is the major factor affecting maximal torque production after passive stretch. This was concluded based on the reduction of central parameters after continuous (EMG:M) and intermittent stretch (EMG:M, %VA), the correlation between torque loss and reductions in EMG:M, %VA and V:M, and the association of torque recovery with the recovery of EMG:M and %VA. Important new findings are that intermittent stretch caused a greater reduction in torque and central drive despite the fact that range of motion increased more and there was a greater level of muscle ischaemia during the continuous stretch. Although the decrease in central drive appeared to be most implicated as a factor causing the force depression, there is some evidence that the ischaemia-reperfusion cycles may have further affected force production. Nonetheless, the prolonged force loss (to at least 30 min) elicited by the intermittent protocol could not be explained by the central or peripheral mechanisms measured in the present research. Clinicians who deem increasing range of motion prior to exercise to be important, despite potential force losses, might consider the use of continuous rather than the intermittent stretching protocols in their programs, and should impose a time delay between stretch and exercise training. However, the relative effect of stretch at central vs. peripheral levels is not known in elderly and clinical populations (i.e. those with potential connective tissue limitations), so further research is required in order to make specific statements in this regard.

CHAPTER 4

Study 3: Can passive Stretch Inhibit Spinal Motor Neurone Facilitatory Mechanisms in the Human Plantar Flexors?

4.1 Introduction

It is well established that an acute bout of passive muscle stretching can acutely reduce maximal force production (Kay & Blazevich, 2012). Several lines of evidence support that a reduction in central drive to the muscle has a considerable involvement in this phenomenon (Avela et al., 1999; Fowles et al., 2000; Kay & Blazevich, 2009b; Trajano, Nosaka, Seitz, & Blazevich, 2013; Trajano et al., 2013). However, the mechanisms underpinning this reduced central drive after stretching remain unclear. Speculatively, stretch-sensitive muscle proprioceptive structures (i.e. group Ia/II muscle spindle afferents and free nerve endings) might be desensitised after prolonged passive stretching, which could ultimately affect motor neurone facilitatory processes. Facilitatory modulation is mediated by the development of persistent inward currents (PICs), which are a voltage-dependent characteristic of spinal motor neurones. When activated, PICs amplify and prolong synaptic input, changing the input-output relationship and producing sustained depolarisation especially in low-threshold motor neurones (Heckman et al., 2004). This amplification allows the motor neurones to fire at the higher frequencies necessary to produce maximal levels of muscular force (Hultborn et al., 2003).

The amplification of motoneuronal responses to excitatory postsynaptic potentials has been studied primarily in animal preparations using steady synaptic input imposed by tendon vibration, which selectively activates muscle spindle Ia afferents (Frigon et al., 2011). Despite the fact that animal preparations provide a more controlled environment to study PICs, tendon vibration reflexes (TVR) have been used in human experiments to improve our understanding of PICs and their influence on muscular force output (McPherson et al., 2008; Mottram et al., 2009; Suresh et al., 2011). When a high-frequency vibration is applied to the tendon it generates a train of Ia afferent impulses inducing progressive excitation of the homonymous motor neurones, and elicits PICs in these motor neurones (Heckman & Binder, 1988). The slow increase in isometric force during the vibration sequence and, even more, the visibly sustained force that persists after the vibration ceased, provide remarkable evidence for the presence of PICs (Heckman et al., 2004). Another marked characteristic of this amplification is its muscle length dependency, where PIC development has been demonstrated to be greater when muscles receive synaptic input at longer lengths (Hyngstrom et al., 2007). Thus, the presence of a sustained muscular force after vibration cessation and its length-dependent characteristics can be taken as evidence for PIC development in humans.

When performed in isolation the TVR typically recruits only low-threshold motor units resulting in small force outputs (Gorassini, Bennett, & Yang, 1998; Kamen, Sullivan, Rubinstein, & Christie, 2006; Kiehn & Eken, 1997). Recently, however, the imposition of high-frequency tendon vibration during electrically induced muscular contractions has elicited forces as high as 50% of maximal voluntary contractions, possibly providing evidence of higher-threshold units being recruited in response to the additional input from electrical stimulation (Magalhães & Kohn, 2010; Magalhães, de

Toledo, & Kohn, 2013). This stimulation-vibration technique can provide insights into the presence of PICs not only in low-threshold motor units but also in higher-threshold units, which contribute more to maximal force production according to the size principle of motor unit recruitment (Henneman, 1985). Thus, the utilisation of electrical stimulation superimposed onto high-frequency tendon vibration provides a unique opportunity to investigate the development of PICs in humans.

Given that muscle stretching results in an acute central drive depression, a reduction in stretch-dependent afferent feedback after stretch might speculatively impact PIC development, and thus central (spinal) drive. The main aim of the present study was to examine the effect of muscle stretching on PIC development. The first specific aim, therefore, was to determine whether muscular force and electromyographic responses to simultaneous Achilles tendon vibration and muscle electrical stimulation would exhibit muscle length dependency, consistent with PIC-like properties in the human plantar flexors. The second purpose of the present study was to determine whether an acute bout of passive plantar flexor muscle stretching impairs the force and electromyographic responses to simultaneous tendon vibration and muscle electrical stimulation. It was hypothesised that: 1) vibration-induced contractions would be more pronounced at longer muscle lengths; and 2) passive stretch would decrease the reflexive plantar flexor contraction force and triceps surae muscle activity elicited by Achilles tendon vibration.

4.2 Methods

4.2.1 Subjects

Eleven healthy subjects (9 men and 2 women; mean ± SD: age, 28.9 ± 4.7 y; height, 1.77 ± 0.9 m; body mass, 74.8 ± 8.6 kg) without neuromuscular impairment volunteered for the study. The subjects reported not being engaged in flexibility training for at least 6 months prior to the study and refrained from such training during the data collection period. The subjects also refrained from vigorous exercise and alcohol consumption for 24 h, and stimulant (e.g. caffeine) use for 12 hours, prior to testing. The procedures performed during this research were approved by the Edith Cowan University Human Research Ethics Committee and were in agreement to the Declaration of Helsinki. All participants read and signed an informed consent document.

4.2.2 Study design and overview

All data collection was performed in a single session lasting approximately 1 hour and 30 min, during which the subjects performed two experiments. Prior to Experiment 1 the subjects were seated upright in the chair of an isokinetic dynamometer (Biodex System 3 Pro, Biodex Medical System, Shirley, New York, USA) with the knee fully extended (0º) and ankle at neutral (0º) position. They were then instructed to practice four voluntary submaximal isometric plantar flexion contractions (two contractions at 60% and two at 80% of perceived maximal effort) in order to become familiar with the contractions and to pre-condition the tendon for subsequent strain (Maganaris, Narici, & Maffulli, 2008). After practice, two maximal voluntary contractions were performed

with a 1-min passive rest interval and the contraction with the maximum torque recorded for subsequent analysis. The muscle electromyogram (EMG) was also recorded simultaneously from soleus (Sol), medial gastrocnemius (MG) and lateral gastrocnemius (LG).

4.2.2.1 Experiment 1

Experiment 1 was designed to determine whether the torque produced by the electrical stimulation superimposed on tendon vibration (vib+stim) would exhibit muscle length dependence, which is typically found in animal models and suggested to be a marked PIC characteristic. Thus, the knee remained fully extended throughout Experiment 1 and the isometric torque and EMG amplitude elicited by vib+stim were evaluated with the ankle in three different joint positions: neutral (0º), plantar flexion (+10º) and dorsiflexion (-10º).

4.2.2.2 Experiment 2

Experiment 2 was designed to investigate the effect of acute passive stretching on the reflexive torque and muscle activity elicited by vib+stim. Subjects were assessed with the ankle in a dorsiflexed position (-10º) at 5 min (control 1) and 1 min (control 2; pre-stretch) before the stretching as well as immediately, 5 min, 10 min and 15 min after.

4.2.3 Tendon vibration and superimposed muscle stimulation (Experiments 1 and 2)

A constant current electrical stimulator (DS7, Digitimer Ltd, Welwyn Garden City, UK) was used to deliver an electrical square-wave stimulus (1-ms pulse width) to the plantar flexor muscle belly through two self-adhesive electrodes (9 × 5 cm, Dura-Stick® II, Chattanooga Group, Hixon, USA). The cathode was placed distal to the popliteal crease and the anode over the distal myotendinous junction of soleus. For all electrical stimulations, the intensity necessary to reach 20% of MVC with a 0.5-s 20 Hz tetanic stimulation was used.

The Achilles tendon was mechanically vibrated at 70 Hz (based on pilot data) and 1 mm of amplitude by a vibrator (LymphoGenics, Medelect, Perth, Australia). The tip of the vibrator was firmly attached to the tendon with a clip to maintain steady pressure at a fixed position on the tendon and the vibration was applied continuously for of 33 s. Ten seconds after vibration onset five 2-s bursts of 20-Hz electrical stimulation separated by 2-s intervals were also applied (Figure 4.1).

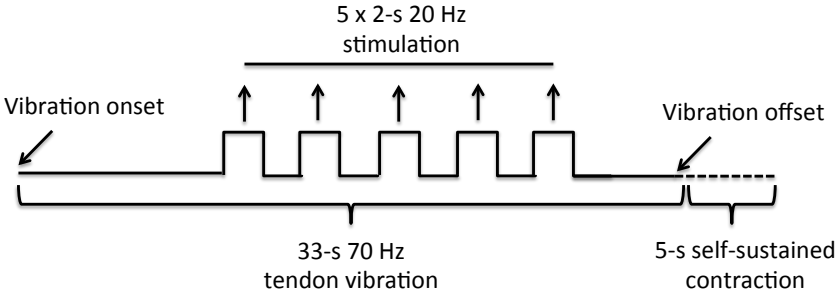

Figure 4.1. Schematic representation of the tendon vibration and superimposed muscle stimulation protocol used to elicit reflexive muscular contractions.

4.2.4 Voluntary and evoked torque measurements (Experiments 1 and 2)

The peak isometric plantar flexor torque, assessed during MVCs (described previously), was used to normalise the torque and EMG (see below) elicited by the stim+vib protocol. 'Reflexive torque' was measured as the mean torque in a 1-s window at two time points: (1) during vibration immediately after the 5th (last) burst of electrical stimulation (Torque vibration; T_{vib}), and (2) 3 s after vibration ceased (Torque sustained; T_{sust}) (Figure 4.2). After the torque returned to baseline levels at each time point an extra 20 Hz tetanic stimulation (using the same parameters described above) was delivered. As stimulation applied to resting muscle usually does not involve reflexive pathways the peak torque ($T_{stim,rest}$) was used to determine if the stretch

protocol affected the muscle's contractile potential, i.e. the ability to produce torque without central command. As plantar flexor muscles impose a small passive torque even when the muscle is relaxed, all the torque values were normalised and presented as changes from the baseline (resting) value.

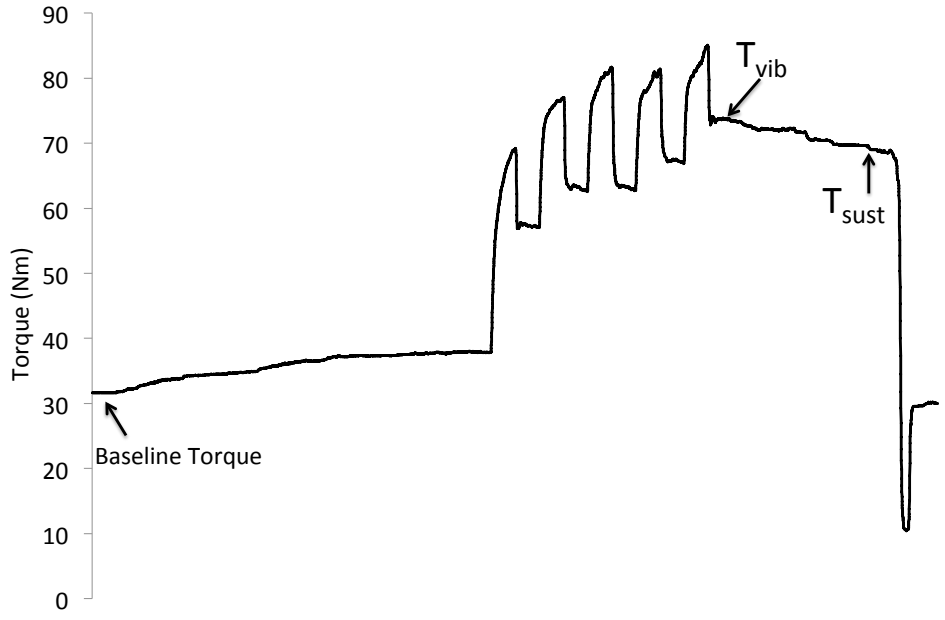

Figure 4.2. Example of reflexive torque elicited by the stimulation protocol and the respective time points at which torque was recorded over 1-s windows. T_{vib}, torque measured after the 5th (last) bout of electrical stimulation; T_{sust}, torque measured 3 s after vibration cessation (self-sustained torque).

4.2.5 Measurement of muscle activity (EMG)

Surface EMG was recorded from soleus (Sol), lateral gastrocnemius (LG) and medial gastrocnemius (MG) using a bipolar electrode configuration at a 4000 Hz analogue-digital conversion rate (bandwidth 10 to 500 Hz) using the Bagnoli-8 Main Unit EMG

system (DelSys, Inc., MA, USA). Electrodes were positioned according to SENIAM's recommendations (Hermens et al., 1999). The inter-electrode distance was 1 cm and a reference electrode was placed on the lateral malleolus. The skin under the electrodes was shaved, abraded and cleaned with alcohol to reduce the inter-electrode resistance below 5 kΩ. EMG data were also recorded during the stretching manoeuvres to ensure that muscle activation remained below 5% of the maximal value; a small muscle activity response is often seen even when the subjects are required to remain completely relaxed (Blazevich, et al., 2012). Muscle activity was expressed as the root mean square EMG amplitude (1-s averaging window) measured for each muscle (Sol, EMG_{sol}; LG, EMG_{LG}; and MG, EMG_{MG}) over the same time period as torque measurements (T_{vib} and T_{sust}). Ankle joint torque, joint angle and EMG data were simultaneously recorded using LabChart v.6.1.3 Software (PowerLab System, ADInstruments, NSW, Australia).

4.2.6 Muscle stretching protocol

The stretch procedures were performed on an isokinetic dynamometer with the muscles relaxed. The plantar flexors were stretched five times separated by 10-s non-stretch intervals by rotating the ankle into dorsiflexion at $5^{o} \cdot s^{-1}$ until a maximal tolerable stretch was attained and then held at stretched position for 1 min. This 5-min duration stretch protocol was chosen because previous studies showed that a similar 5-min intermittent stretch can reduce maximal voluntary torque and neural drive to the muscle and (Study 2; Chapter 3).

4.2.7 Statistical analysis

Separate one-way repeated measures ANOVAs were performed to compare changes in all variables (T_{vib}, T_{sust}, EMG_{SOL}, EMG_{LG}, EMG_{MG}) at different joint angles (Experiment 1; neutral, plantar flexion, and dorsiflexion) and over time (Experiment 2; before and immediately, 5, 10, and 15 min after stretch). Pairwise comparisons were performed as follow-up tests. Statistical significance was set at an α level of 0.05. Intra-class correlations (ICC) were computed to evaluate reliability of T_{vib} and T_{sust} torque measurement between control 1 and control 2 time points.

4.3 Results

4.3.1 Experiment 1

4.3.1.1 Torque

There was significant effect ($p<0.05$) of joint angle on both T_{vib} and T_{sust}. Post-hoc analyses revealed that T_{vib} and T_{sust} were higher (71 and 69%, respectively) when ankle joint was in dorsiflexion compared to plantar flexion and the neutral position (67 and 60%) (Figure 4.3).

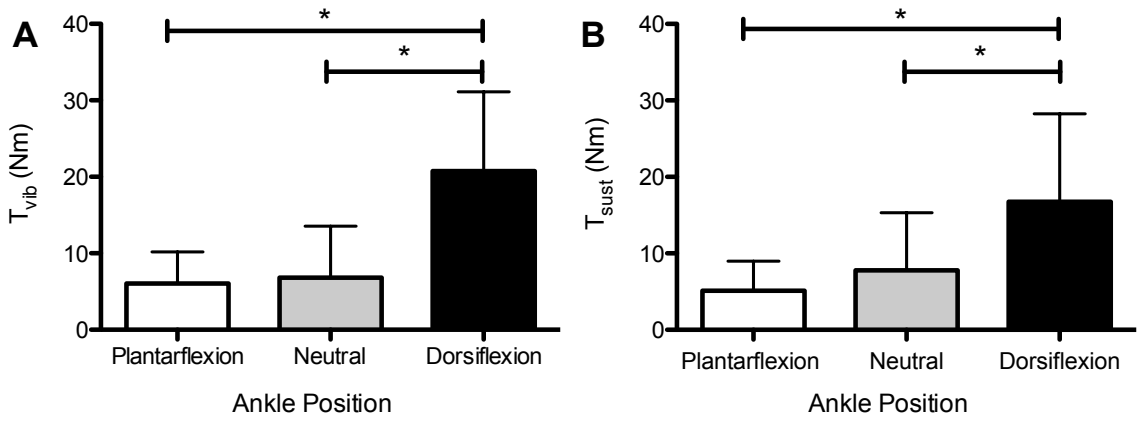

Figure 4.3. Panel A: Greater torque was developed after the 5th (last) stimulation during vibration (T_{vib}) when the ankle joint was held in dorsiflexion. Panel B: The same response was observed for self-sustained torque (T_{sust}). * $p<0.05$.

4.3.1.2 Muscle activity

There was a significant effect ($p<0.05$) of joint angle on EMG_{Sol} amplitude when measured during T_{vib} and T_{sust} (Figure 4.4). Post-hoc analyses showed that EMG_{Sol} amplitude was 32% greater when measured during T_{vib} and 28% greater when measured during T_{sust} when the ankle was held in dorsiflexion compared to plantar flexion ($p<0.05$). Similarly, EMG_{LG} was 27% greater when measured during T_{vib} when the muscle was held in dorsiflexion compared to plantar flexion.

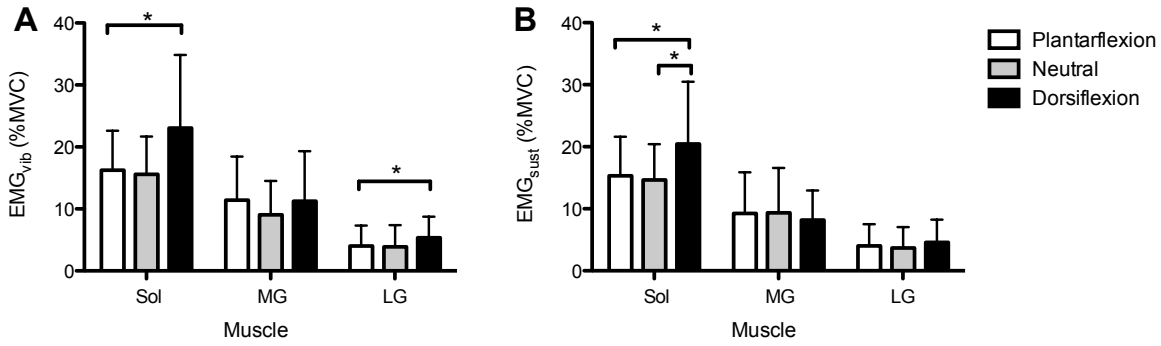

Figure 4.4. Panel A: Soleus (Sol), medial gastrocnemius (MG) and lateral gastrocnemius (LG) EMG amplitudes (root mean squared) measured after the 5th (last) stimulation during vibration (EMG_{vib}) with the ankle held in three different positions. Panel B: The same response was observed for self-sustained torque ($EMG_{sust.}$) * $p<0.05$

4.3.2 Experiment 2

4.3.2.1 Reflexive torque

ICC values describing the reliability of T_{vib} and T_{sust} between control 1 and control 2 (i.e. before muscle stretch) were 0.95 and 0.96, respectively, suggesting that the measures were reliable. There was a significant time effect ($p<0.05$) for both torque measures, with post-hoc analyses indicating that T_{vib} was reduced by 60% immediately after stretch and remained depressed by 32% at 5 min after stretch ($p<0.05$; see figure 4.5). Torque remained elevated after vibration cessation, however T_{sust} magnitude was also reduced by 65% immediately after stretch ($P<0.05$) and recovered at 5 min.

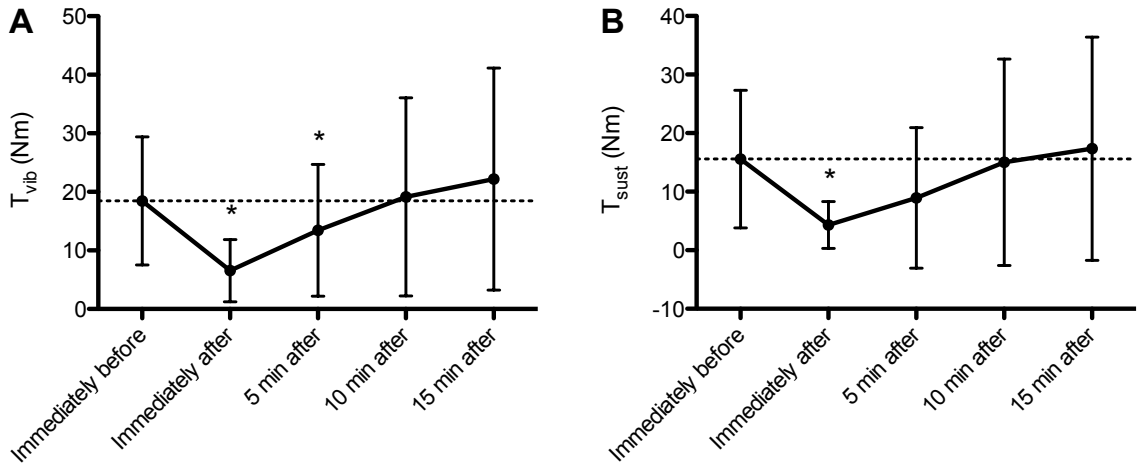

Figure 4.5. Panel A: The time course of torque changes measured after the 5th (last) stimulation during vibration (T_{vib}) immediately before and immediately and 5, 10, and 15 min after muscle stretching. Panel B: The same response was observed for self-sustained torque (T_{sust}). * p<0.05 when compared to immediately before stretch.

4.3.2.2 Muscle contractile capacity ($T_{stim,rest}$)

There was no significant time effect for $T_{stim,rest,}$ suggesting that the muscle's contractile torque was not affected by the stretch protocol.

4.3.2.3 Muscle activity

A significant time effect was found for EMG_{MG} amplitude when measured during T_{vib} and for EMG_{Sol} when measured during T_{sust}. Post-hoc analyses revealed that EMG_{MG} amplitude during T_{vib} was reduced by 41% immediately after stretch ($p<0.05$) and EMG_{Sol} amplitude measured during T_{sust} was reduced by 44% immediately after stretch ($p<0.05$). However, they were both recovered by 5 min after stretch and were increased by 16% and 10%, respectively, by 15 min ($p<0.05$) (Figure 4.6).

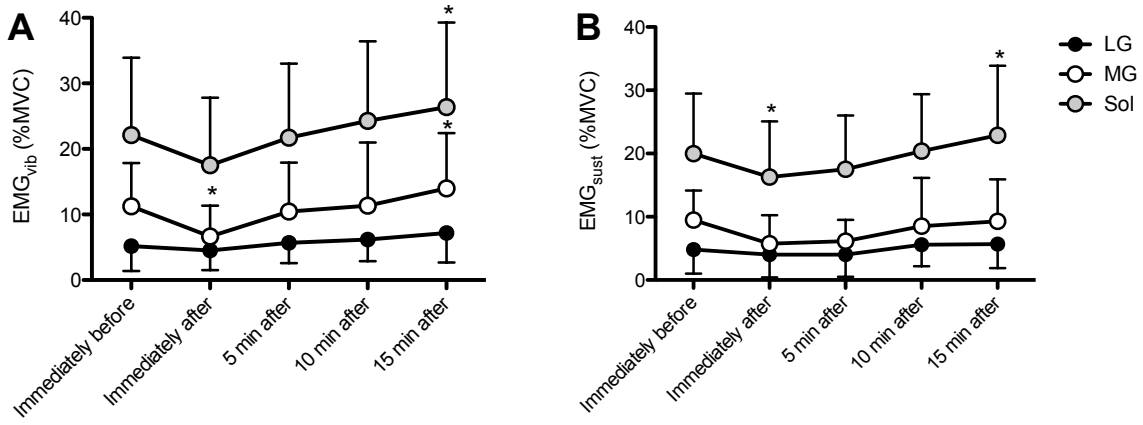

Figure 4.6. Panel A: Soleus (Sol), medial gastrocnemius (MG) and lateral gastrocnemius (LG) EMG amplitude measured after the 5th (last) stimulation during vibration (EMG_{vib}) immediately before and immediately, 5, 10, and 15 min after muscle stretching. Panel B: The same response was observed for self-sustained torque (EMG_{sust}). * $p<0.05$ when compared to immediately before stretch.

4.4 Discussion

Little is known about the effect of acute passive muscle stretching on motor neurone facilitatory pathways. The novel findings of this study were that: 1) the vib+stim protocol showed muscle length-dependence of torque and muscle activity and self-sustained firing was present after stimulation cessation, consistent with PIC-like behaviour; 2) passive muscle stretching decreased both the torque and muscle activity elicited by the vib+stim protocol; 3) the post-stretch inhibition lasted up to 5 min and was fully recovered by 10 min. These findings support the hypothesis that passive stretching may inhibit reflex-induced PIC development in the human plantar flexors.

One important aim of the present study was to test whether the stimulation protocol could elicit contractions consistent with PIC development. To test this hypothesis a

combined vibration-electrical stimulation (vib+stim) protocol was applied with the ankle joint in three different positions (plantar flexion, neutral and dorsiflexion). Experiments in decerebrate cats consistently reveal a muscle length-dependent modulation of PICs when a steady synaptic input is imposed by tendon vibration (Frigon et al., 2011; Hyngstrom et al., 2007). This length-dependent modulation seems to be caused by an increase in disynaptic Ia reciprocal inhibition that occurs when the antagonist muscle (i.e. tibialis anterior) is held in a long muscle position exciting muscle spindle primary (Ia) afferents and increasing agonist inhibition (Hyngstrom et al., 2007). In the present study the ankle joint angle-modulated reflexive torque and muscle activity were consistent with the expectation according to the results of studies in animal models (i.e. greater amplification when the muscle is held at a longer length). The increase in reflex-induced torque and muscle activity when the ankle was dorsiflexed, together with the apparent self-sustained firing after vibration cessation can be taken as indirect, yet strong, evidence of the development of PICs using the present protocol. Previous studies have used tendon vibration to estimate the contribution of PICs in human motor neurones, especially in patients with motor impairment (McPherson et al., 2008; Suresh et al., 2011), and self-sustained firing behaviour has been already reported in the literature after low-intensity contractions elicited by tendon vibration (Gorassini et al., 1998; Kamen et al., 2006; Kiehn & Eken, 1997). However, this appear to be the first study to demonstrate length-dependent modulation of reflexive contractions evoked by tendon vibration in humans, increasing the body of evidence supporting the possibility that contractions elicited by tendon vibration are mediated by PICs. Also, the significantly greater EMG_{Sol} amplitudes observed during T_{vib} and T_{sust} with the ankle in dorsiflexion suggest that sustained motor unit firing was present during and after vibration cessation. PIC amplification more typically produces self-

sustained firing in slow twitch type motor neurones (Lee & Heckman, 1998), and the finding that soleus EMG, but not EMG_{LG} and EMG_{MG}, amplitude were greater in the dorsiflexed position during T_{sust}, is consistent with this given that soleus is known to consist predominantly of slow-twitch fibres (Gollnick, Sjödin, Karlsson, Jansson, & Saltin, 1974). Thus, the utilisation of this protocol as an indirect and relative measure of PIC development in human studies seems to be justified.

The present study was the first to present evidence for the inhibitory effect of passive muscle stretching on spinal motor neurone facilitatory systems. In fact, the possibility that passive stretching could decrease Ia afferent efficiency has been demonstrated before by measuring H-reflexes concomitant with a decrease in force after prolonged (1 hour) repetitive fast muscle stretches (Avela et al., 1999). However, the H-reflex is a specific measurement (especially when measured in relaxed muscle) that cannot provide information regarding motor neurone facilitation and, more importantly, its resulting force modulation (Aagaard et al., 2002b; Knikou, 2008; Pierrot-Deseilligny, 1997; Pierrot-Deseilligny & Burke, 2005). The present data expand previous findings (Avela et al., 1999) by showing that moderate-duration (5 min) static muscle stretching impairs the ability to develop PICs in the plantar flexors. It is well known that motor neurones rely on a PIC-mediated facilitatory system that increases the gain of synaptic input in order to achieve maximal discharge frequency and thus to produce maximal levels of muscular force (Heckman et al., 2004; Hultborn et al., 2003). PICs have marked characteristics such as self-sustained firing and greater amplification when the agonist muscle is held at longer lengths, and both characteristics were demonstrated in the protocol used in the present study to elicit reflexive torque. Reductions in reflexive torque (T_{vib}) production during vibration and especially reductions in the ability to

sustain the torque without synaptic input (self sustained torque; T_{sust}) can be interpreted as an impairment in PIC development (see Figure 4.7). Importantly, T_{sust} and T_{vib} were statistically recovered by 5 and 10 min post-stretch, respectively, suggesting that the inhibitory effects of passive stretch did not last for longer than 10 min. This temporal profile is consistent with previous data showing that the reduced neural drive associated with force loss in response to muscle stretch should be recovered by at least 15 min post-stretch using a similar muscle stretching protocol (Studies 1 and 2). Unfortunately, force production was not measured at 5 and 10 min post-stretch in previous studies so a precise temporal comparison cannot be done. Additionally, the clear lack of changes in T_{rest} shows that muscle's ability to produce force through direct electrical stimulation was not affected, suggesting that any changes in reflexive torque production must have been caused by central rather than peripheral (i.e. muscle based) mechanisms. Moreover, post-stretch reductions in EMG_{MG} amplitude during $T_{vib,}$ as well as EMG_{Sol} amplitude during T_{sust} provide strong evidence for PIC-related reductions in motor unit activity after stretch. It is also interesting to note that an increase in EMG_{Sol} amplitude during T_{vib} and T_{sust} was found 15 min after stretch, suggesting the possibility of a facilitatory effect after the initial inhibitory effect. However, increases in muscle torque production subsequent to the post-stretch torque loss have not been previously reported so the functional significance of this finding is unclear.

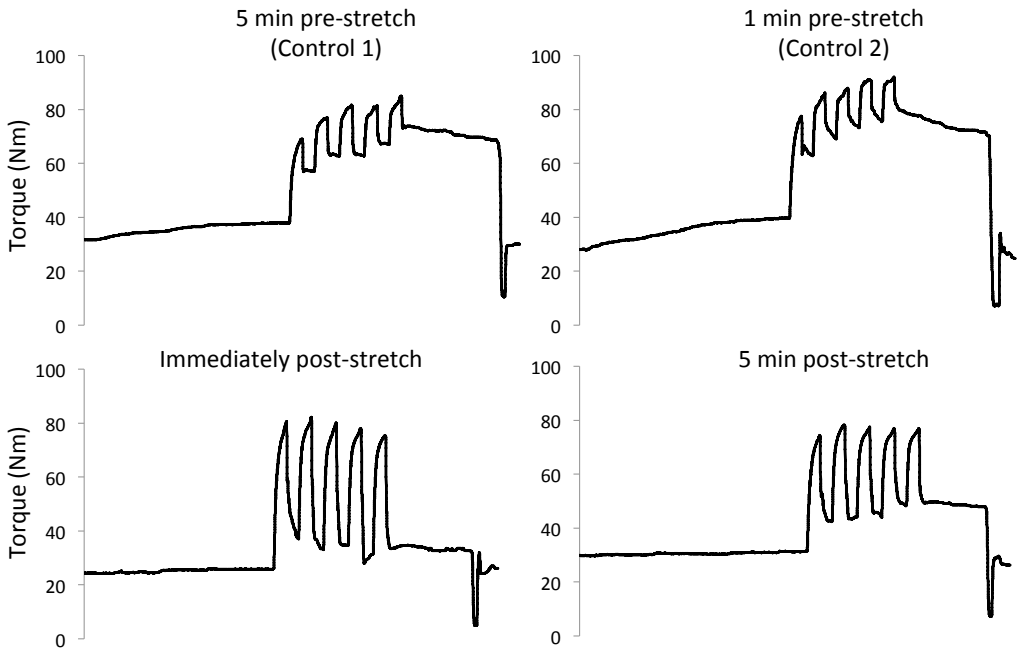

Figure 4.7. Example of torque data obtained during vib+stim protocol at 5 min (control 1) and 1 min (control 2; pre-stretch) before the stretching as well as immediately and 5 min after stretching.

With respect to the present study, some limitations should be highlighted. From the present data it was not possible to determine whether pre- and/or post-synaptic mechanisms inhibited PIC development after stretch. Pre-synaptic mechanisms could result in a reduced efficiency of the Ia pathway (Avela et al., 1999), including muscle-spindle desensitisation (Edin & Vallbo, 1988), increases in Ia afferent thresholds (Hayward, Nielsen, Heckman, & Hutton, 1986), prolonged pre-synaptic inhibition (Hultborn, Meunier, Morin, & Pierrot-Deseilligny, 1987; Hultborn, Meunier, Pierrot-Deseilligny, & Shindo, 1987; Meunier & Morin, 1989) and even neurotransmitter depletion at Ia synapses (Curtis & Eccles, 1960). Alternatively, post-synaptic mechanisms might involve a prolonged activation of inhibitory inter-neurones (Fetz et

al., 1979; Schieppati et al., 1990). Regarding inter-neuronal inhibition, another possible mechanism could be the activation of other proprioceptive structures during muscle stretching. For instance, it has been clearly shown in a series of experiments that stretch-sensitive free nerve endings are responsible for the homonymous-inhibitory clasp-knife reflex in response to large amplitude stretch of the extensor muscles in decerebrate cats, with the inhibitory effects persisting after stretch cessation (Cleland, Hayward, & Rymer, 1990; Cleland & Rymer, 1990, 1993; Cleland, Rymer, & Edwards, 1982). Thus, it is reasonable to speculate that prolonged stretch might also activate free nerve endings in healthy humans, inducing a similar inhibitory mechanism within the spinal circuitry. Also, the contribution of supra-spinal mechanisms cannot be ruled out. Human experiments have consistently demonstrated the possible involvement of cortical structures in response to stimulation of stretch-sensitive afferents (Cohen et al., 1985; Coxon et al., 2005; Marconi et al., 2008; Starr et al., 1981). Therefore, to better understand the precise mechanisms underpinning this prolonged inhibition further studies should examine the adaptation of spinal circuitry in animal models after passive stretching as well as determine the possible contribution of supra-spinal mechanisms to this phenomenon.

In summary, the present data indicate that motoneuronal facilitation, mediated by PICs, is affected for up to 5 min after prolonged (5 min) passive stretching. This conclusion was based on the significant reduction in the torque elicited by tendon vibration as well as self-sustained torque with a concomitant reduction in soleus and medial gastrocnemius EMG amplitudes immediately after muscle stretch. The stretch protocol used in this study did not affect the muscle's ability to produce contractile torque, so torque changes were not likely of peripheral origin. Future studies may focus on

strategies to up-regulate PIC activity (e.g. increasing monoaminergic drive) in order to mitigate the acute force-reducing effects of passive muscle stretching.

CHAPTER 5

Overall Discussion and Conclusion

The main aim of the present PhD thesis was to complete a detailed examination of the neuromuscular factors potentially influencing the immediate force loss that follows passive muscle stretching. The thesis was divided into three studies; the first two studies were designed to broadly determine the location of neuromuscular changes that occur after acute passive muscle stretching (e.g. central nervous system vs. muscular) and, based on the results of these two studies, a third study was completed to identify specific changes in neuromuscular function.

5.1 The contribution of central vs. peripheral mechanisms to stretch-induced force loss

The first study of the present thesis (Chapter 2) examined the contribution of central vs. peripheral factors to the stretch-induced torque loss in the human plantar flexors. The main finding was that changes in central (neural) drive after 5 min of continuous stretch were strongly related to both the torque reduction after stretch (Figure 2.3) and the torque recovery within 15 min of stretching. These findings indicate that central drive modifications influenced both the loss and the recovery of muscle force. Because three measures of central drive (EMG:M, %VA and V-wave) were obtained simultaneously, this study provides the clearest evidence of a reduced central drive influencing force production after stretch. This reduction may theoretically result from changes at cortical or sub-cortical (e.g. spinal) levels, however the present data provide no clear evidence

as to the precise location of modification in central drive. In addition to central drive changes, there was also a small reduction in electrical-elicited tetanic torque, indicating that the stretch had a small but detectable influence on muscle contractile capacity. However, the changes in tetanic torque were not correlated with the loss or recovery in voluntary torque, indicating that this minor peripheral change contributed little to the torque loss under the present experimental conditions. Given that these results cannot indicate the site/s of origin of the central drive limitation, further examination is required to examine whether adjustments in supra-spinal and/or spinal drive occur after passive muscle stretching.

5.2 The effect of intermittent passive muscle stretching on the force loss pattern and neuromuscular adjustments

One factor potentially influencing the magnitude, and possible location, of acute neuromuscular change in response to acute stretch is the use of continuous vs. intermittent (i.e. repeated) stretch. Theoretically, intermittent stretch might cause cycles of ischaemia and blood reperfusion and this would potentially increase the likelihood of contractile failure and increase the influence of peripheral changes on muscle force loss. Therefore, Chapter 3 was designed to determine whether intermittent stretch could cause a greater torque loss when compared to continuous stretch, and whether it had a different effect on peripheral mechanisms. The main findings of Chapter 3 were that: 1) continuous stretch improved range of motion more than intermittent stretch; 2) continuous stretch lowered muscle oxygenation levels more than intermittent stretch; 3) the torque loss that occurred after both conditions was associated with reductions in central drive; 4) intermittent stretch had a greater effect on torque loss magnitude and

duration than continuous stretch; and 5) the prolonged (30 min) force loss of ~5% found after intermittent stretching could not be explained by changes in central drive or E-C coupling efficiency and could thus be explicable by peripheral factors other than those measured in Chapter 3. This was the first study to directly compare between intensity-matched (i.e. equalised passive torque during stretch) intermittent and continuous stretches and further studies are therefore necessary to expand these findings. The hypothesis that intermittent stretch would cause a greater and prolonged force loss was confirmed, however this did not appear to be caused by impairments in E-C coupling efficiency as no changes in the 20:80 Hz electrical stimulation ratio was observed. The intermittent protocol reduced central drive (EMG:M and %VA) more after stretch than the continuous protocol; although these measures recovered by 15 min post-stretch and could not explain the prolonged (30 min) small (~5%) torque loss. Thus, intermittent stretching reduced torque production more than continuous stretching, yet central drive failure again appeared to explain much of the torque loss. Nonetheless, the mechanisms underpinning the prolonged torque loss caused by intermittent stretch are still not clear and the involvement of peripheral mechanisms other than those examined in this study requires further investigation.

5.3 The inhibitory effect of passive stretch on motor neurone facilitatory system

It is clear from Chapters 2 and 3 that central drive failure is a primary candidate mechanism underpinning the loss of muscle force shortly after acute passive muscle stretching. This central drive reduction may involve spinal and/or supra-spinal mechanisms. More specifically, at the spinal level it is reasonable to speculate that passive muscle stretching could affect the motor neurone afferent-mediated facilitatory

system. Chapter 4 of the present thesis was therefore implemented to examine the possible inhibitory effect of passive plantar flexor muscle stretching on the motor neurone facilitatory system. First, it was necessary to confirm whether a vibration-stimulation (vib+stim) protocol would elicit reflexive muscular contraction consistent with PIC-like characteristics. This was confirmed in the first set of experiments performed in the study. The vib+stim protocol elicited greater vibration-induced and self-sustained torque as well as greater muscle activity (EMG_{sol}) when the ankle joint was held in a plantar flexed position. These results were in line with previous findings in animal models reporting greater PIC development at longer muscle lengths as well as self sustained motor unit firing (Frigon et al., 2011; Hyngstrom et al., 2007), and they confirmed the validity of the technique for estimating PIC development. In the subsequent experiment it was found that the PIC-like characteristics elicited by the vib+stim protocol were depressed for 5 min after passive muscle stretch, but recovered completely within 10 min. These results suggest that passive muscle stretching negatively affects the ability of the plantar flexor motor units to develop PICs. This reduced PIC behaviour would likely reduce maximal muscle activity and could, at least partly, explain the depression in central drive observed in Chapters 2 and 3. Thus, a reduction in the ability to develop PICs after muscle stretch may be an important factor influencing the loss of muscle torque. Interventions that can minimise the loss of this facilitation, potentially including stimulant ingestion (e.g. caffeine) (Udina, D'Amico, Bergquist, & Gorassini, 2010; Walton, Kalmar, & Cafarelli, 2002), may help to minimise the loss of torque. Importantly, the methods used in Study 3 cannot delineate the specific mechanism affecting PIC development and futures studies are required to investigate the involvement of pre- and post-synaptic mechanisms contributing to this inhibitory effect.

5.4 Conclusion

In summary, the results of the present research indicate that a reduction in central (neural) drive to the muscle is the major factor affecting the stretch-induced force loss. This reduction in central drive was even more pronounced when an intermittent stretch protocol was utilised, however intermittent stretch also caused a small (~5%) but prolonged force loss that could not be explained by central factors and may be of peripheral origin. The immediate reduction in central drive and subsequent torque loss are likely to be influenced by an inhibition of the motor neurone facilitatory system (Chapter 4). It was demonstrated that the temporal profile of this inhibitory effect matches the time course of central drive reduction observed in Studies 1 and 2.

Neural control of skeletal muscle is clearly affected by passive stretching. The present study has provided novel information regarding the effect of passive muscle stretching on maximal muscle force production. Future studies are required to develop strategies to mitigate the effects of passive stretching on central drive reduction and the subsequent torque loss. The inhibitory effect of passive stretching on motor neurone facilitation described in this thesis has given a better understanding of the neural adjustments elicited by passive stretch and should be considered when designing training and rehabilitation routines.

REFERENCES

Aagaard, P. (2003). Training-induced changes in neural function. *Exerc Sport Sci Review, 31*(2), 61-67.

Aagaard, P., Simonsen, E. B., Andersen, J. L., Magnusson, P., & Dyhre-Poulsen, P. (2002a). Increased rate of force development and neural drive of human skeletal muscle following resistance training. *J Appl Physiol, 93*(4), 1318-1326.

Aagaard, P., Simonsen, E. B., Andersen, J. L., Magnusson, P., & Dyhre-Poulsen, P. (2002b). Neural adaptation to resistance training: changes in evoked V-wave and H-reflex responses. *J Appl Physiol, 92*(6), 2309-2318.

Abbate, F., Bruton, J. D., De Haan, A., & Westerblad, H. (2002). Prolonged force increase following a high-frequency burst is not due to a sustained elevation of [Ca2+]i. *Am J Physiol Cell Physiol, 283*(1), C42-47

Allen, D. G. (2004). Skeletal muscle function: role of ionic changes in fatigue, damage and disease. *Clin Exp Pharmacol Physiol, 31*(8), 485-493

Amako, M., Oda, T., Masuoka, K., & Campisi, P. (2003) Effect of static stretching on prevention of injuries for military recruits. *Mil Med*, 168(6), 442-446

Arabadzhiev, T.I., Dimitrov, V.G., Dimitrova, N.A., & Dimitrov, G.V. (2010). Interpretation of EMG integral or RMS and estimates of "neuromuscular efficiency" can be misleading in fatiguing contraction. *J Electromyogr Kinesiol, 20*(2), 223-232.

Arampatzis, Adamantios, Mademli, Lida, De Monte, Gianpiero, & Walsh, Mark. (2007). Changes in fascicle length from rest to maximal voluntary contraction affect the assessment of voluntary activation. *J Biomech, 40*(14), 3193-3200.

Armstrong, R. B., Duan, C., Delp, M. D., Hayes, D. A., Glenn, G. M., & Allen, G. D. (1993). Elevations in rat soleus muscle [Ca2+] with passive stretch. *J Appl Physiol, 74*(6), 2990-2997.

Avela, J., Kyrolainen, H., & Komi, P. V. (1999). Altered reflex sensitivity after repeated and prolonged passive muscle stretching. *J Appl Physiol, 86*(4), 1283-1291.

Avendaño, Carlos, Isla, Alberto J, & Rausell, Estrella. (1992). Area 3a in the cat II. Projections to the motor cortex and their relations to other corticocortical connections. *J Comp Neurol, 321*(3), 373-386.

Balog, E. M. (2010). Excitation-contraction coupling and minor triadic proteins in low-frequency fatigue. *Exerc Sport Sci Rev, 38*(3), 135-142.

Behm, D. G., Button, D. C., & Butt, J. C. (2001). Factors affecting force loss with prolonged stretching. *Can J Appl Physiol, 26*(3), 261-272.

Bentley, L. F., & Lehman, S. L. (2005). Doublets and low-frequency fatigue in potentiated human muscle. *Acta Physiol Scand, 185*(1), 51-60.

Bergquist, A. J., Clair, J. M., & Collins, D. F. (2011). Motor unit recruitment when neuromuscular electrical stimulation is applied over a nerve trunk compared with a muscle belly: triceps surae. *J Appl Physiol, 110*(3), 627-637.

Binder-Macleod, S. A., & Russ, D. W. (1999). Effects of activation frequency and force on low-frequency fatigue in human skeletal muscle. *J Appl Physiol, 86*(4), 1337-1346.

Binder-Macleod, S., & Kesar, T. (2005). Catchlike property of skeletal muscle: recent findings and clinical implications. *Muscle Nerve, 31*(6), 681-693.

Bixler, B., & Jones, R. L. (1992). High-school football injuries: effects of a post-halftime warm-up and stretching routine. *Fam Pract Res J*, *12*(2), 131-139.

Blaisdell, F William. (2002). The pathophysiology of skeletal muscle ischemia and the reperfusion syndrome: a review. *Cardiovasc Surg, 10*(6), 620-630.

Blazevich, A.J., Cannavan, D., Waugh, C.M., Fath, F., Miller, S.C., & Kay, A.D. (2012). Neuromuscular factors influencing the maximum stretch limit of the human plantar flexors. *J Appl Physiol, 113(9), 1446-1455*.

Blazevich, A.J., Kay, A.D., Waugh, C., Fath, F., Miller, S., & Cannavan, D. (2012). Plantarflexor stretch training increases reciprocal inhibition measured during voluntary dorsiflexion. *J Neurophysiol, 107*(1), 250-256.

Bojsen-Møller, J., Schwartz, S., Kalliokoski, K.K., Finni, T., & Magnusson, S.P. (2010). Intermuscular force transmission between human plantarflexor muscles in vivo. *J Appl Physiol, 109*(6), 1608-1618.

Borg, Thomas K, & Caulfield, James B. (1980). Morphology of connective tissue in skeletal muscle. *Tissue Cell, 12*(1), 197-207.

Brown, Ian E, Cheng, Ernest J, & Loeb, Gerald E. (1999). Measured and modeled properties of mammalian skeletal muscle. II. The effectsof stimulus frequency on force-length and force-velocity relationships. *J Muscle Res Cell Motil, 20*(7), 627-643.

Bruton, Joseph D, Place, Nicolas, Yamada, Takashi, Silva, José P, Andrade, Francisco H, Dahlstedt, Anders J, Westerblad, Håkan. (2008). Reactive oxygen species and fatigue-induced prolonged low-frequency force depression in skeletal muscle fibres of rats, mice and SOD2 overexpressing mice. *J Physiol, 586*(1), 175-184.

Burke, R. E., Rudomin, P., & Zajac, F. E., 3rd. (1970). Catch property in single mammalian motor units. *Science, 168*(927), 122-124.

Button, D.C., & Behm, D.G. (2008). The effect of stimulus anticipation on the interpolated twitch technique. *J Sport Sci Med, 7*, 520-524.

Chambers, Melissa A, Moylan, Jennifer S, Smith, Jeffrey D, Goodyear, Laurie J, & Reid, Michael B. (2009). Stretch−stimulated glucose uptake in skeletal muscle is mediated by reactive oxygen species and p38 MAP−kinase. *J Physiol, 587*(13), 3363-3373.

Cheng, Arthur J, Place, Nicolas, Bruton, Joseph D, Holmberg, Hans−Christer, & Westerblad, Håkan. (2013). Doublet discharge stimulation increases sarcoplasmic reticulum Ca2+ release and improves performance during fatiguing contractions in mouse muscle fibres. *J Physiol 591(15), 3739-3748*.

Christie, Anita, Inglis, J Greig, Kamen, Gary, & Gabriel, David A. (2009). Relationships between surface EMG variables and motor unit firing rates. *Eur J Appl Physiol, 107*(2), 177-185.

Cleland, Corey L, Hayward, Linda, & Rymer, WZ. (1990). Neural mechanisms underlying the clasp-knife reflex in the cat. II. Stretch-sensitive muscular-free nerve endings. *J Neurophysiol, 64*(4), 1319-1330.

Cleland, Corey L, & Rymer, WZ. (1990). Neural mechanisms underlying the clasp-knife reflex in the cat. I. Characteristics of the reflex. *J Neurophysiol, 64*(4), 1303-1318.

Cleland, Corey L, & Rymer, WZ. (1993). Functional properties of spinal interneurons activated by muscular free nerve endings and their potential contributions to the clasp-knife reflex. *J Neurophysiol, 69*(4), 1181-1191.

Cleland, Corey L, Rymer, WZ, & Edwards, Frank R. (1982). Force-sensitive interneurons in the spinal cord of the cat. *Science, 217*(4560), 652-655.

Cohen, LG, Starr, Arnold, & Pratt, Hillel. (1985). Cerebral somatosensory potentials evoked by muscle stretch, cutaneous taps and electrical stimulation of peripheral nerves in the lower limbs in man. *Brain, 108*(1), 103-121.

Cornwell, A., Nelson, A. G., & Sidaway, B. (2002). Acute effects of stretching on the neuromechanical properties of the triceps surae muscle complex. *Eur J Appl Physiol, 86*(5), 428-434.

Coxon, James P, Stinear, James W, & Byblow, Winston D. (2005). Amplitude of muscle stretch modulates corticomotor gain during passive movement. *Brain res, 1031*(1), 109-117.

Cramer, J. T., Beck, T. W., Housh, T. J., Massey, L. L., Marek, S. M., Danglemeier, S., . . . Egan, A. D. (2007). Acute effects of static stretching on characteristics of the isokinetic angle - torque relationship, surface electromyography, and mechanomyography. *J Sports Sci, 25*(6), 687-698.

Cramer, J. T., Housh, T. J., Weir, J. P., Johnson, G. O., Coburn, J. W., & Beck, T. W. (2005). The acute effects of static stretching on peak torque, mean power output, electromyography, and mechanomyography. *Eur J Appl Physiol, 93*(5-6), 530-539.

Cresswell, Andrew G, Löscher, WN, & Thorstensson, Alf. (1995). Influence of gastrocnemius muscle length on triceps surae torque development and electromyographic activity in man. *Exp Brain Res, 105*(2), 283-290.

Curtis, DR, & Eccles, JC. (1960). Synaptic action during and after repetitive stimulation. *J Physiol, 150*(2), 374.

De Haan, A., Gerrits, KHL, & de Ruiter, CJ. (2009). Counterpoint: the interpolated twitch does not provide a valid measure of the voluntary activation of muscle. *J Appl Physiol, 107*(1), 355.

Dimitrova, N. A., & Dimitrov, G. V. (2003). Interpretation of EMG changes with fatigue: facts, pitfalls, and fallacies. *J Electromyogr Kinesiol, 13*(1), 13-36.

Duclay, J., & Martin, A. (2005). Evoked H-reflex and V-wave responses during maximal isometric, concentric, and eccentric muscle contraction. *J Neurophysiol, 94*(5), 3555-3562.

Edin, BB, & Vallbo, AB. (1988). Stretch sensitization of human muscle spindles. *J Physiol, 400*(1), 101-111.

Edwards, R. H., Hill, D. K., Jones, D. A., & Merton, P. A. (1977). Fatigue of long duration in human skeletal muscle after exercise. *J Physiol, 272*(3), 769-778.

Ekstrand, J., Gillquist, J., & Liljedahl, S. O. (1983). Prevention of soccer injuries Supervision by doctor and physiotherapist. *Am J Sports Med*, *11*(3), 116-120.

Farina, D., Arendt-Nielsen, L., & Graven-Nielsen, T. (2005). Experimental muscle pain reduces initial motor unit discharge rates during sustained submaximal contractions. *J Appl Physiol, 98*(3), 999-1005.

Farina, D., Arendt-Nielsen, L., Merletti, R., & Graven-Nielsen, T. (2004). Effect of experimental muscle pain on motor unit firing rate and conduction velocity. *J Neurophysiol, 91*(3), 1250-1259.

Farina, D., Merletti, R., & Enoka, R.M. (2004). The extraction of neural strategies from the surface EMG. *J Appl Physiol, 96*(4), 1486-1495.

Farina, Dario, Cescon, Corrado, Negro, Francesco, & Enoka, Roger M. (2008). Amplitude cancellation of motor-unit action potentials in the surface electromyogram can be estimated with spike-triggered averaging. *J Neurophysiol, 100*(1), 431.

Farina, Dario, Merletti, Roberto, Nazzaro, Marisa, & Caruso, Ignazio. (2001). Effect of joint angle on EMG variables in leg and thigh muscles. *IEEE Eng Med Biol Mag, 20(6), 62-71.*

Fetz, E.E., Jankowska, E., Johannisson, T., & Lipski, J. (1979). Autogenetic inhibition of motoneurones by impulses in group Ia muscle spindle afferents. *J Physiol, 293*(1), 173-195.

Fowles, J. R., Sale, D. G., & MacDougall, J. D. (2000). Reduced strength after passive stretch of the human plantarflexors. *J Appl Physiol, 89*(3), 1179-1188.

Frigon, Alain, Carroll, Timothy J, Jones, Kelvin E, Zehr, E Paul, & Collins, David F. (2007). Ankle position and voluntary contraction alter maximal M waves in soleus and tibialis anterior. *Muscle Nerve, 35*(6), 756-766.

Frigon, Alain, Thompson, Christopher K, Johnson, Michael D, Manuel, Marin, Hornby, T George, & Heckman, CJ. (2011). Extra forces evoked during electrical stimulation of the muscle or its nerve are generated and modulated by a length-dependent intrinsic property of muscle in humans and cats. *J Neurosci, 31*(15), 5579-5588.

Gandevia, S. C. (2001). Spinal and supraspinal factors in human muscle fatigue. *Physiol Rev, 81*(4), 1725-1789.

Gandevia, SC, Petersen, N, Butler, JE, & Taylor, JL. (1999). Impaired response of human motoneurones to corticospinal stimulation after voluntary exercise. *J Physiol, 521*(3), 749-759.

Gellhorn, E, & Hyde, J. (1953). Influence of proprioception on map of cortical responses. *J Physiol, 122*(2), 371-385.

Gollnick, Philip D, Sjödin, Bertil, Karlsson, Jan, Jansson, Eva, & Saltin, Bengt. (1974). Human soleus muscle: a comparison of fiber composition and enzyme activities with other leg muscles. *Pflügers Archiv, 348*(3), 247-255.

Gondin, J., Duclay, J., & Martin, A. (2006). Soleus- and gastrocnemii-evoked V-wave responses increase after neuromuscular electrical stimulation training. *J Neurophysiol, 95, 3328-3335*

Gorassini, Monica A, Bennett, David J, & Yang, Jaynie F. (1998). Self-sustained firing of human motor units. *Neuroscience letters, 247*(1), 13-16.

Granger, D.N. (1988). Role of xanthine oxidase and granulocytes in ischemia-reperfusion injury. *American Journal of Physiology-Heart and Circulatory Physiology, 255*(6), H1269.

Gute, Dean C, Ishida, Tetsuya, Yarimizu, Koji, & Korthius, Ronald J. (1998). Inflammatory responses to ischemia, and reperfusion in skeletal muscle. *Mol Cell Biochem, 179*(1-2), 169-187.

Hamaoka, Takafumi, McCully, Kevin K, Quaresima, Valentina, Yamamoto, Katsuyuki, & Chance, Britton. (2007). Near-infrared spectroscopy/imaging for monitoring muscle oxygenation and oxidative metabolism in healthy and diseased humans. *J Biomed Optics, 12*(6), 062105-062105-062116.

Hayward, Linda F, Nielsen, Richard P, Heckman, Charles J, & Hutton, Robert S. (1986). Tendon vibration-induced inhibition of human and cat triceps surae group I reflexes: evidence of selective Ib afferent fiber activation. *Exp Neurol, 94*(2), 333-347.

Heckman, CJ, & Binder, Marc D. (1988). Analysis of effective synaptic currents generated by homonymous Ia afferent fibers in motoneurons of the cat. *J Neurophysiol, 60*(6), 1946-1966.

Heckman, CJ, & Enoka, Roger M. (2012). Motor unit. *Compr Physiol*, 2, 2629-2682

Heckman, CJ, Gorassini, M.A., & Bennett, D.J. (2004). Persistent inward currents in motoneuron dendrites: implications for motor output. *Muscle Nerve, 31*(2), 135-156.

Heckmann, C. J., Gorassini, M. A., & Bennett, D. J. (2005). Persistent inward currents in motoneuron dendrites: implications for motor output. *Muscle Nerve, 31*(2), 135-156.

Henneman, Elwood. (1985). The size-principle: a deterministic output emerges from a set of probabilistic connections. *J Exp Biol, 115*(1), 105-112.

Herda, T. J., Costa, P. B., Walter, A. A., Ryan, E. D., Hoge, K. M., Kerksick, C. M., . . . Cramer, J. T. (2011). The Effects of Two Modes of Static Stretching On Muscle Strength and Stiffness. *Med Sci Sports Exerc*.

Hermens, Hermie J, Freriks, Bart, Merletti, Roberto, Stegeman, Dick, Blok, Joleen, Rau, Günter, . . . Hägg, Göran. (1999). *European recommendations for surface electromyography*: Roessingh Research and Development The Netherlands.

Hore, J, Preston, JB, & Cheney, PD. (1976). Responses of cortical neurons (areas 3a and 4) to ramp stretch of hindlimb muscles in the baboon. *J Neurophysiol, 39*(3), 484-500.

Huerta, MF, & Pons, TP. (1990). Primary motor cortex receives input from area 3a in macaques. *Brain Res, 537*(1), 367-371.

Huffman, Kelly J, & Krubitzer, Leah. (2001). Area 3a: topographic organization and cortical connections in marmoset monkeys. *Cereb Cortex, 11*(9), 849-867.

Hultborn, H, Meunier, S, Morin, C, & Pierrot-Deseilligny, E. (1987). Assessing changes in presynaptic inhibition of I a fibres: a study in man and the cat. *J Physiol, 389*(1), 729-756.

Hultborn, H, Meunier, S, Pierrot-Deseilligny, E, & Shindo, M. (1987). Changes in presynaptic inhibition of Ia fibres at the onset of voluntary contraction in man. *J Physiol, 389*(1), 757-772.

Hultborn, H., Denton, M. E., Wienecke, J., & Nielsen, J. B. (2003). Variable amplification of synaptic input to cat spinal motoneurones by dendritic persistent inward current. *J Physiol, 552*(Pt 3), 945-952.

Hyngstrom, A.S., Johnson, M.D., Miller, J.F., & Heckman, CJ. (2007). Intrinsic electrical properties of spinal motoneurons vary with joint angle. *Nat Neurosci, 10*(3), 363-369.

Jankowska, E., & Hammar, I. (2002). Spinal interneurones; how can studies in animals contribute to the understanding of spinal interneuronal systems in man? *Brain Res Rev, 40*(1), 19-28.

Jones, D. A. (1996). High-and low-frequency fatigue revisited. *Acta Physiol Scand, 156*(3), 265-270.

Kagaya, A., & Muraoka, Y. (2005). Muscle architecture and its relationship to muscle circulation. *Int J Sport Health Sci, 3*, 171-180.

Kamen, Gary, Sullivan, Ryan, Rubinstein, Scott, & Christie, Anita. (2006). Evidence of self-sustained motoneuron firing in young and older adults. *J Electromyogr Kinesiol, 16*(1), 25-31.

Kay, A. D., & Blazevich, A. J. (2009a). Isometric contractions reduce plantar flexor moment, Achilles tendon stiffness, and neuromuscular activity but remove the subsequent effects of stretch. *J Appl Physiol, 107*(4), 1181-1189.

Kay, A. D., & Blazevich, A. J. (2009b). Moderate-duration static stretch reduces active and passive plantar flexor moment but not Achilles tendon stiffness or active muscle length. *J Appl Physiol, 106*(4), 1249-1256.

Kay, A. D., & Blazevich, A. J. (2010). Concentric muscle contractions before static stretching minimize, but do not remove, stretch-induced force deficits. *J Appl Physiol, 108*(3), 637-645.

Kay, A. D., & Blazevich, A. J. (2012). Effect of acute static stretch on maximal muscle performance: a systematic review. *Med Sci Sports Exerc, 44*(1), 154-164.

Keenan, Kevin G, Farina, Dario, Maluf, Katrina S, Merletti, Roberto, & Enoka, Roger M. (2005). Influence of amplitude cancellation on the simulated surface electromyogram. *J Appl Physiol, 98*(1), 120-131.

Keenan, Kevin G, Farina, Dario, Merletti, Roberto, & Enoka, Roger M. (2006). Amplitude cancellation reduces the size of motor unit potentials averaged from the surface EMG. *J Appl Physiol, 100*(6), 1928-1937.

Keeton, R. B., & Binder-Macleod, S. A. (2006). Low-frequency fatigue. *Phys Ther, 86*(8), 1146-1150.

Kent-Braun, J. A. (2009). Skeletal muscle fatigue in old age: whose advantage? *Exerc Sport Sci Rev, 37*(1), 3-9.

Kiehn, Ole, & Eken, Torsten. (1997). Prolonged firing in motor units: evidence of plateau potentials in human motoneurons? *J Neurophysiol, 78*(6), 3061-3068.

Knikou, Maria. (2008). The H-reflex as a probe: Pathways and pitfalls. *J Neurosci Meth, 171*(1), 1-12.

Le Pera, D., Graven-Nielsen, T., Valeriani, M., Oliviero, A., Di Lazzaro, V., Tonali, P.A., & Arendt-Nielsen, L. (2001). Inhibition of motor system excitability at cortical and spinal level by tonic muscle pain. *Clinl Neurophysiol, 112*(9), 1633-1641.

Lee, RH, & Heckman, CJ. (1998). Bistability in spinal motoneurons in vivo: systematic variations in persistent inward currents. *J Neurophysiol, 80*(2), 583-593.

Lepers, Romuald, Maffiuletti, Nicola A, Rochette, Ludovic, Brugniaux, Julien, & Millet, Guillaume Y. (2002). Neuromuscular fatigue during a long-duration cycling exercise. *J Appl Phsyiol, 92*(4), 1487-1493.

Maas, H., Meijer, H.J.M., & Huijing, P.A. (2005). Intermuscular interaction between synergists in rat originates from both intermuscular and extramuscular myofascial force transmission. *Cells Tissues Organs, 181*(1), 38-50.

Maas, H., & Sandercock, T.G. (2010). Force transmission between synergistic skeletal muscles through connective tissue linkages. *J Biomed Biotech, 2010*.

Magalhaes, F. H., & Kohn, A. F. (2010). Vibration-induced extra torque during electrically-evoked contractions of the human calf muscles. *J Neuroeng Rehabil, 7*, 26.

Magalhães, Fernando Henrique, de Toledo, Diana Rezende, & Kohn, André Fabio. (2013). Plantar flexion force induced by amplitude-modulated tendon vibration and associated soleus V/F-waves as an evidence of a centrally-mediated mechanism contributing to extra torque generation in humans. *J Neuroeng Rehabil, 10*(1), 32.

Maganaris, Constantinos N, Narici, Marco V, & Maffulli, Nicola. (2008). Biomechanics of the Achilles tendon. *Dis Rehab, 30*(20-22), 1542-1547.

Magnusson, S. P. (1998). Passive properties of human skeletal muscle during stretch maneuvers. A review. *Scand J Med Sci Sports, 8*(2), 65-77.

Magnusson, S. P., Simonsen, E. B., Dyhre-Poulsen, P., Aagaard, P., Mohr, T., & Kjaer, M. (1996). Viscoelastic stress relaxation during static stretch in human skeletal muscle in the absence of EMG activity. *Scand J Med Sci Sports, 6*(6), 323-328.

Marconi, Barbara, Filippi, Guido M, Koch, Giacomo, Pecchioli, Cristiano, Salerno, Silvia, Don, Romildo, . . . Caltagirone, Carlo. (2008). Long-term effects on

motor cortical excitability induced by repeated muscle vibration during contraction in healthy subjects. *J Neurol Sci, 275*(1), 51-59.

Martin, V., Millet, G. Y., Martin, A., Deley, G., & Lattier, G. (2004). Assessment of low-frequency fatigue with two methods of electrical stimulation. *J Appl Physiol, 97*(5), 1923-1929.

Matthews, Peter BC. (1991). The human stretch reflex and the motor cortex. *Trends Neurosci, 14*(3), 87-91.

McCully, K. K. (2010). The influence of passive stretch on muscle oxygen saturation. *Adv Exp Med Biol, 662*, 317-322.

McHugh, M. P., & Cosgrave, C. H. (2010). To stretch or not to stretch: the role of stretching in injury prevention and performance. *Scand J Med Sci Sports, 20*(2), 169-181.

10.1111/j.1600-0838.2009.01058.x

McNair, P. J., Dombroski, E. W., Hewson, D. J., & Stanley, S. N. (2001). Stretching at the ankle joint: viscoelastic responses to holds and continuous passive motion. *Med Sci Sports Exerc, 33*(3), 354-358.

McPherson, Jacob G, Ellis, Michael D, Heckman, CJ, & Dewald, Julius PA. (2008). Evidence for increased activation of persistent inward currents in individuals with chronic hemiparetic stroke. *J Neurophysiol, 100*(6), 3236-3243.

Merton, P. A. (1954). Voluntary strength and fatigue. *J Physiol, 123*(3), 553-564.

Meunier, S, & Morin, C. (1989). Changes in presynaptic inhibition of Ia fibres to soleus motoneurones during voluntary dorsiflexion of the foot. *Exp Brain Res, 76*(3), 510-518.

Millet, G. Y., & Lepers, R. (2004). Alterations of neuromuscular function after prolonged running, cycling and skiing exercises. *Sports Med, 34*(2), 105-116.

Morse, Christopher I, Degens, Hans, Seynnes, Olivier Roger, Maganaris, Constantinos N, & Jones, David A. (2008). The acute effect of stretching on the passive stiffness of the human gastrocnemius muscle tendon unit. *J Physiol, 586*(1), 97-106.

Mottram, Carol J, Suresh, Nina L, Heckman, CJ, Gorassini, Monica A, & Rymer, William Z. (2009). Origins of abnormal excitability in biceps brachii motoneurons of spastic-paretic stroke survivors. *J Neurophysiol, 102*(4), 2026-2038.

Murray, Elisabeth A, & Coulter, Joe Dan. (1981). Organization of corticospinal neurons in the monkey. *J Comp Neurol, 195*(2), 339-365.

Murthy, G., Hargens, A.R., Lehman, S., & Rempel, D.M. (2001). Ischemia causes muscle fatigue. *J Orthop Res, 19*(3), 436-440.

Nelson, A. G., Guillory, I. K., Cornwell, C., & Kokkonen, J. (2001). Inhibition of maximal voluntary isokinetic torque production following stretching is velocity-specific. *J Strength Cond Res, 15*(2), 241-246.

Nielsen, Bjørn Gilbert. (2009). Calcium and the role of motoneuronal doublets in skeletal muscle control. *Eur Bioph J, 38*(2), 159-173.

Nordez, A, McNair, P, Casari, P, & Cornu, C. (2007). Acute changes in hamstrings musculo-articular dissipative properties induced by cyclic and static stretching. *Int J Sport Med, 29*(05), 414-418.

Otsuki, A., Fujita, E., Ikegawa, S., & Kuno-Mizumura, M. (2011). Muscle Oxygenation and Fascicle Length During Passive Muscle Stretching in Ballet-Trained Subjects. *Int J Sports Med*

Palomero, Jesus, Pye, Deborah, Kabayo, Tabitha, & Jackson, Malcolm J. (2012). Effect of passive stretch on intracellular nitric oxide and superoxide activities in single skeletal muscle fibres: Influence of ageing. *Free Rad Res, 46*(1), 30-40.

Pensini, M, & Martin, A. (2004). Effect of voluntary contraction intensity on the H-reflex and V-wave responses. *Neurosci lett, 367*(3), 369-374.

Phillips, CG, Powell, TPS, & Wiesendanger, M. (1971). Projection from low-threshold muscle afferents of hand and forearm to area 3a of baboon's cortex. *J Physiol, 217*(2), 419-446.

Pierrot-Deseilligny, E. (1997). Assessing changes in presynaptic inhibition of Ia afferents during movement in humans. *J Neurosci Methods, 74*(2), 189-199.

Pierrot-Deseilligny, Emmanuel, & Burke, David. (2005). *The circuitry of the human spinal cord: its role in motor control and movement disorders*: Cambridge University Press.

Pizza, Francis X, Koh, Timothy J, McGregor, Stephen J, & Brooks, Susan V. (2002). Muscle inflammatory cells after passive stretches, isometric contractions, and lengthening contractions. *J of Appl Physiol, 92*(5), 1873-1878.

Place, Nicolas, Yamada, Takashi, Bruton, Joseph D, & Westerblad, Håkan. (2008). Interpolated twitches in fatiguing single mouse muscle fibres: implications for the assessment of central fatigue. *J Physiol, 586*(11), 2799-2805.

Pope, R. P., Herbert, R. D., Kirwan, J. D., & Graham, B. J. (2000). A randomized trial of preexercise stretching for prevention of lower-limb injury. *Med Sci Sports Exerc, 32*(2), 271-277.

Power, K., Behm, D., Cahill, F., Carroll, M., & Young, W. (2004). An acute bout of static stretching: effects on force and jumping performance. *Med Sci Sports Exerc, 36*(8), 1389-1396.

Powers, S. K., & Jackson, M. J. (2008). Exercise-induced oxidative stress: cellular mechanisms and impact on muscle force production. *Physiol Rev, 88*(4), 1243-1276.

10.1152/physrev.00031.2007

Purslow, Peter P. (1989). Strain-induced reorientation of an intramuscular connective tissue network: implications for passive muscle elasticity. *J Biomech, 22*(1), 21-31.

Rack, Peter MH, & Westbury, DR. (1969). The effects of length and stimulus rate on tension in the isometric cat soleus muscle. *J Physiol, 204*(2), 443.

Rathelot, Jean-Alban, & Strick, Peter L. (2009). Subdivisions of primary motor cortex based on cortico-motoneuronal cells. *Proc Natl Acad Sci U S A, 106*(3), 918-923.

Ross, Emma Z, Middleton, Natalie, Shave, Rob, George, Keith, & Nowicky, Alex. (2007). Corticomotor excitability contributes to neuromuscular fatigue following marathon running in man. *Exp Physiol, 92*(2), 417-426.

Roszek, Boris, & Huijing, Peter A. (1997). Stimulation frequency history alters length-force characteristics of fully recruited rat muscle. *J of Electromyogr and Kinesiol, 7*(3), 161-177.

Rubini, E. C., Costa, A. L., & Gomes, P. S. (2007). The effects of stretching on strength performance. *Sports Med, 37*(3), 213-224

Ryan, E. D., Beck, T. W., Herda, T. J., Hull, H. R., Hartman, M. J., Stout, J. R., & Cramer, J. T. (2008). Do practical durations of stretching alter muscle strength? A dose-response study. *Med Sci Sports Exerc, 40*(8),

Schabrun, S.M., & Hodges, P.W. (2012). Muscle pain differentially modulates short interval intracortical inhibition and intracortical facilitation in primary motor cortex. *J Pain, 13(2) 187-194*

Schieppati, M., Romano, C., & Gritti, I. (1990). Convergence of Ia fibres from synergistic and antagonistic muscles onto interneurones inhibitory to soleus in humans. *J Physiol, 431*(1), 365-377.

Scott, Stephen H, Sergio, Lauren E, & Kalaska, John F. (1997). Reaching movements with similar hand paths but different arm orientations. II. Activity of individual cells in dorsal premotor cortex and parietal area 5. *J Neurophysiol, 78*(5), 2413-2426.

Shehab, R., Mirabelli, M., Gorenflo, D., & Fetters, M. D. (2006). Pre-exercise stretching and sports related injuries: knowledge, attitudes and practices. *Clin J Sport Med*, *16*(3), 228-231.

Shield, A., & Zhou, S. (2004). Assessing voluntary muscle activation with the twitch interpolation technique. *Sports Med, 34*(4), 253-267.

Shrier, I. (2004). Meta-analysis on preexercise stretching. *Med Sci Sports Exerc, 36*(10), 1832; author reply 1833.

Small, K., Mc Naughton, L., & Matthews, M. (2008). A systematic review into the efficacy of static stretching as part of a warm-up for the prevention of exercise-related injury. *Res Sports Med*, *16*(3), 213-231.

Smith, Craig A. (1994). The warm-up procedure: to stretch or not to stretch. A brief review. *Journal of Orthopaedic & Sports Physical Therapy, 19*(1), 12-17.

Søgaard, K., Gandevia, S.C., Todd, G., Petersen, N.T., & Taylor, J.L. (2006). The effect of sustained low-intensity contractions on supraspinal fatigue in human elbow flexor muscles. *J Physiol, 573*(2), 511-523.

Solstad, G. M., Fimland, M. S., Helgerud, J., Iversen, V. M., & Hoff, J. (2011). Test-Retest Reliability of V-Wave Responses in the Soleus and Gastrocnemius Medialis. *J Clin Neurophysiol*

Starr, Arnold, McKeon, Brian, Skuse, Nevell, & Burke, David. (1981). Cerebral potentials evoked by muscle stretch in man. *Brain, 104*(Pt 1), 149-166.

Suresh, Nina L, Wang, Inga, Heckman, CJ, & Rymer, W Zev. (2011). *Characterization of the tendon vibration reflex response in hemi-spastic stroke individuals.* Paper presented at the Engineering in Medicine and Biology Society, EMBC, 2011 Annual International Conference of the IEEE.

Taylor, J. L., & Gandevia, S. C. (2008). A comparison of central aspects of fatigue in submaximal and maximal voluntary contractions. *J Appl Physiol, 104*(2), 542-550.

Taylor, J.L. (2009a). Last Word on Point: Counterpoint: The interpolated twitch does/does not provide a valid measure of the voluntary activation of muscle. *J Appl Physiol, 107*(1), 367.

Taylor, J.L. (2009b). Point: Counterpoint: The interpolated twitch does/does not provide a valid measure of the voluntary activation of muscle. *J Appl Physiol, 107*(1), 354-355.

Thacker, S. B., Gilchrist, J., Stroup, D. F., & Kimsey, C. D., Jr. (2004). The impact of stretching on sports injury risk: a systematic review of the literature. *Med Sci Sports Exerc, 36*(3), 371-378.

Tidball, James G, Lavergne, Eliane, Lau, Kim S, Spencer, Melissa J, Stull, James T, & Wehling, Michelle. (1998). Mechanical loading regulates NOS expression and activity in developing and adult skeletal muscle. *Am J Physiol Cell Physiol, 275*(1), C260-C266.

Trajano, Gabriel S, Nosaka, Kazunori, Seitz, Laurent, & Blazevich, Anthony J. (2013). Intermittent Stretch Reduces Force and Central Drive more than Continuous Stretch. *Medi Sci Sport Exerc*. In press

Trajano, Gabriel S, Seitz, Laurent, Nosaka, Kazunori, & Blazevich, Anthony John. (2013). Contribution of central vs. peripheral factors to the force loss induced by passive stretch of the human plantar flexors. *J Appl Physiol, 115(2), 212-218*.

Udina, Esther, D'Amico, Jessica, Bergquist, Austin J, & Gorassini, Monica A. (2010). Amphetamine increases persistent inward currents in human motoneurons estimated from paired motor-unit activity. *J Neurophysiol, 103*(3), 1295.

Upton, A. R., McComas, A. J., & Sica, R. E. (1971). Potentiation of "late" responses evoked in muscles during effort. *J Neurol Neurosurg Psychiatry, 34*(6), 699-711.

Walton, Christine, Kalmar, Jayne M, & Cafarelli, E. (2002). Effect of caffeine on self-sustained firing in human motor units. *J Physiol, 545*(2), 671-679.

Yao, Wanxiang, Fuglevand, Rew J, & Enoka, Roger M. (2000). Motor-unit synchronization increases EMG amplitude and decreases force steadiness of simulated contractions. *J Neurophysiol, 83*(1), 441-452.

APPENDICES

Appendix 1: Ethical Approval

From: **Research Ethics** research.ethics@ecu.edu.au
Subject: 6965 TRAJANO ethics approval
Date: 30 November 2011 2:42 pm
To: Gabriel SIQUEIRA TRAJANO g.trajano@ecu.edu.au
Cc: Tony BLAZEVICH a.blazevich@ecu.edu.au, Ken NOSAKA k.nosaka@ecu.edu.au, Research Assessments researchassessments@ecu.edu.au

Dear Gabriel

Project 6965 TRAJANO
Neuromuscular factors affecting stretch induced force loss

Student Number: 10194461

The ECU Human Research Ethics Committee (HREC) has reviewed your application and has granted ethics approval for your research project. In granting approval, the HREC has determined that the research project meets the requirements of the *National Statement on Ethical Conduct in Human Research*.

The approval period is from 30 November 2011 to 31 December 2013.

The Research Assessments Team has been informed and they will issue formal notification of approval. Please note that the submission and approval of your research proposal is a separate process to obtaining ethics approval and that no recruitment of participants and/or data collection can commence until formal notification of both ethics approval and approval of your research proposal has been received.

All research projects are approved subject to general conditions of approval. Please see the attached document for details of these conditions, which include monitoring requirements, changes to the project and extension of ethics approval.

Please feel free to contact me if you require any further information.

Regards
Kim

Kim Gifkins
Research Ethics Officer
Edith Cowan University
270 Joondalup Drive
JOONDALUP WA 6027
Phone: (08) 6304 2170
Fax: (08) 6304 5044
Email: research.ethics@ecu.edu.au

Conditions of approval

1. Monitoring of Approved Research Projects

Monitoring is the process of verifying that the conduct of research conforms to the approved ethics application. Compliance with monitoring requirements is a condition of approval.

The *National Statement on Ethical Conduct in Human Research* indicates that institutions are responsible for ensuring that research is reliably monitored. Monitoring of approved projects is to establish that a research project is being, or has been, conducted in the manner approved by the Ethics Committee. Researchers also have a significant responsibility in monitoring, as they are in the best position to observe any adverse events or unexpected outcomes. They should report such events or outcomes promptly to the Ethics Committee and take prompt steps to

Appendix 2: Information Letter to Participants (Study 1)

Information Letter to Participants

Thank you for expressing your interest in this research. The purpose of this document is to explain the study that you may choose to participate in as a subject. Please read this document carefully, and do not hesitate to ask any questions.

Project Title

Neuromuscular Factors Affecting Stretch-induced Force Loss

Researchers

This research project is being undertaken as part of the requirements of a PhD candidature (Sport and Exercise Sciences) at Edith Cowan University (ECU).

PhD Candidate: Gabriel Trajano (g.trajano@ecu.edu.au) 6304 5819
Supervisor: A/Prof. Anthony Blazevich (a.blazevich@ecu.edu.au) 6304 5472
Co-supervisor: Prof. Ken Nosaka (k.nosaka@ecu.edu.au) 6304 5655

Further details of supervisors and the School of Exercise, Biomedical and Health Sciences are available at: http://www.sebhs.ecu.edu.au

Purpose of the study

The purpose of this study is to examine force production, low frequency fatigue, voluntary activation and the propensity to utilise muscle's catch-like properties after a continuous stretching protocols.

Research Outline

In order to participate in this study, you will be asked to complete a medical questionnaire and to refrain from performing sports or hard exercise training for one day prior to the experimental day. You are also required to abstain from taking any stimulants or depressants (including caffeine or alcohol) for at least 12 hours prior testing.

If you participate in this study, you will be asked to report to the Exercise Physiology Lab (Building 19, Room 19.150) on 3 days separated by one week (scheduling is flexible) at the same time of the day. Each day, before the

measurements start, you will be asked to do a 5-minute warm-up on a stationary bicycle. On the first day you will be acquainted with all testing procedures such as: muscle stretching, maximal voluntary contractions and electrical muscle stimulation techniques; muscle stretching will be performed at an intensity where you feel mild discomfort. Electrical stimulation procedures require a small electrical current to be applied to the calf muscle belly using self-adhesive surface electrodes. The stimulation will be started at very low intensities and progressively increased until your muscle is maximally activated or you feel discomfort; at maximal intensities the electrical stimulation might be uncomfortable.

On the second and third visits you will complete the experimental conditions (5 minutes stretching or 5 minutes resting) in a random order. Force output during voluntary muscle contractions, with and without electrical stimulation being applied, will be measured using different protocols before and 1, 10, 20 and 30 minutes after each experimental condition. Small self-adhesive skin-mounted electrodes will be used to record the small electrical signals emanating from your calf muscles during contractions (these sit passively on the skin and there is no discomfort) and near-infrared spectroscopy probes will be attached to your skin to record muscle tissue oxygenation. The skin under the electrodes will be gently abraded and cleaned with alcohol (the alcohol minimises the risk of skin infection). First day measurements will take about 1 hour and second and third days will take about 1.5 hours.

Eligibility

You will be eligible for this study if:

- you are between 18 and 35 years old
- you have no neuromuscular injuries
- you have not performed flexibility training for the ankle joint in the last three months
- you have not been engaged in strength or endurance training more than 3 times a week

Risks

- The stretching exercise will be performed to your maximum stretch tolerance, which can cause some discomfort.
- Electrical stimulation procedures can also be uncomfortable, but SHOULD NOT be painful; the researcher will ask for continuous feedback from you.

- The light skin abrasion performed immediately prior to the attachment of skin-based electromyogram electrodes can increase the chance of skin infections. To further reduce this small risk, alcohol wipes will be applied to the skin after abrasion as well as after removal of the electrodes.
- As with all tests of maximal muscle force production, there is the chance for muscle or tendon strain. This risk is low given that proper warm-up and familiarisation will be performed, the tests will be conducted by a researcher who is experienced in the procedures, and isometric muscle actions carry a relatively low risk of injury.

Benefits

- You will have a unique opportunity to learn about the neuromuscular system and see high-level data acquisition techniques.
- You will learn about research strategies and research design, and have the opportunity to ask questions about research or any aspect of sports science.
- You will get free ankle extension strength assessment.

Confidentiality of Information

Your anonymity is ensured as much as it is possible during the investigation by assigning number codes to your data by the investigator. All information provided by you will be treated with full confidentiality. Your contact information will only be accessible by the chief researcher during the period of the study and only the researcher and supervisors will have access to the raw information for this study. The information and data gathered from you during the study will be used to answer the research question of this study. Data will be stored in a password-protected computer and is only available to the researchers. Hard copy data will only be kept in the researcher's office and locked in a specific drawer/filling cabinet. All data will be stored according to ECU policy and regulations following the completion of the study.

Results of the Research Study

The results of this study are intended for completion of a PhD by research thesis and may be presented at conferences/seminars and published in peer-reviewed journals, as magazine articles, as an online article or part of a book section or report. Published results will not contain information that can be used to identify participants unless specific consent for this has been obtained. A copy of published results can be obtained from the investigator upon request.

Voluntary Participation

Your participation in this study is voluntary. No monetary reward will be provided. No explanation or justification is needed if you choose to not participate. Your decision if you not want to participate or continue to participate will not disadvantage you or involve any penalty.

Withdrawing Consent to Participate

You are free to withdraw your consent to further involvement in this project at any time. You also have the right to withdraw any personal information that has been collected during the research.

Questions and/or Further Information

If you have any questions or require any further information about the research project, please do not hesitate to contact:

Gabriel Trajano (PhD Student – Researcher)
Office 19.384
School of Exercise, Biomedical and Health Sciences, Edith Cowan University
270 Joondalup Drive, Joondalup, WA 6027, Australia
Ph: (+61 8) 6304 5819
E-mail: g.trajano@ecu.edu.au

If you have any concerns or complaints about the research project and wish to talk to an independent person, you may contact:

Research Ethics Officer
Edith Cowan University
270 Joondalup Drive
JOONDALUP WA 6027
Phone: (08) 6304 2170
Email: research.ethics@ecu.edu.au

This project has been approved by the ECU Human Research Ethics Committee.

Appendix 3: Information Letter to Participants (Study 2)

Information Letter to Participants

Thank you for expressing your interest in this research. The purpose of this document is to explain the study that you may choose to participate in as a subject. Please read this document carefully, and do not hesitate to ask any questions.

Project Title

Neuromuscular Factors Affecting Stretch-induced Force Loss

Researchers

This research project is being undertaken as part of the requirements of a PhD candidature (Sport and Exercise Sciences) at Edith Cowan University (ECU).

PhD Candidate: Gabriel Trajano (g.trajano@ecu.edu.au) 6304 5819
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Further details of supervisors and the School of Exercise, Biomedical and Health Sciences are available at: http://www.sebhs.ecu.edu.au

Purpose of the study

The purpose of this study is to examine force production, low frequency fatigue, voluntary activation and the propensity to utilise muscle's catch-like properties after continuous and intermittent stretching protocols.

Research Outline

In order to participate in this study, you will be asked to complete a medical questionnaire and to refrain from performing sports or hard exercise training for one day prior to the experimental day. You are also required to abstain from taking any stimulants or depressants (including caffeine or alcohol) for at least 12 hours prior testing.

If you participate in this study, you will be asked to report to the Exercise Physiology Lab (Building 19, Room 19.150) on 4 days separated by one week (scheduling is flexible) at the same time of the day. Each day, before the

measurements start, you will be asked to do a 5-minute warm-up on a stationary bicycle. On the first day you will be acquainted with all testing procedures such as: muscle stretching, maximal voluntary contractions and electrical muscle stimulation techniques; muscle stretching will be performed at an intensity where you feel mild discomfort. Electrical stimulation procedures require a small electrical current to be applied to the calf muscle belly using self-adhesive surface electrodes. The stimulation will be started at very low intensities and progressively increased until your muscle is maximally activated or you feel discomfort; at maximal intensities the electrical stimulation might be uncomfortable.

On the second, third and fourth visits you will complete the experimental conditions (5 sets of 1-minute stretching, 1 set of 5 minutes stretching or 5 minutes resting) in a random order. Force output during voluntary muscle contractions, with and without electrical stimulation being applied, will be measured using different protocols before and 1, 10, 20 and 30 minutes after each experimental condition. Small self-adhesive skin-mounted electrodes will be used to record the small electrical signals emanating from your calf muscles during contractions (these sit passively on the skin and there is no discomfort) and near-infrared spectroscopy probes will be attached to your skin to record muscle tissue oxygenation. The skin under the electrodes will be gently abraded and cleaned with alcohol (the alcohol minimises the risk of skin infection). First day measurements will take about 1 hour and second, third and fourth days will take about 1.5 hours.

Eligibility

You will be eligible for this study if:

- you are between 18 and 35 years old
- you have no neuromuscular injuries
- you have not performed flexibility training for the ankle joint in the last three months
- you have not been engaged in strength or endurance training more than 3 times a week

Risks

- The stretching exercise will be performed to your maximum stretch tolerance, which can cause some discomfort.

- Electrical stimulation procedures can also be uncomfortable, but SHOULD NOT be painful; the researcher will ask for continuous feedback from you.
- The light skin abrasion performed immediately prior to the attachment of skin-based electromyogram electrodes can increase the chance of skin infections. To further reduce this small risk, alcohol wipes will be applied to the skin after abrasion as well as after removal of the electrodes.
- As with all tests of maximal muscle force production, there is the chance for muscle or tendon strain. This risk is low given that proper warm-up and familiarisation will be performed, the tests will be conducted by a researcher who is experienced in the procedures, and isometric muscle actions carry a relatively low risk of injury.

Benefits

- You will have a unique opportunity to learn about the neuromuscular system and see high-level data acquisition techniques.
- You will learn about research strategies and research design, and have the opportunity to ask questions about research or any aspect of sports science.
- You will get free ankle extension strength assessment.

Confidentiality of Information

Your anonymity is ensured as much as it is possible during the investigation by assigning number codes to your data by the investigator. All information provided by you will be treated with full confidentiality. Your contact information will only be accessible by the chief researcher during the period of the study and only the researcher and supervisors will have access to the raw information for this study. The information and data gathered from you during the study will be used to answer the research question of this study. Data will be stored in a password-protected computer and is only available to the researchers. Hard copy data will only be kept in the researcher's office and locked in a specific drawer/filling cabinet. All data will be stored according to ECU policy and regulations following the completion of the study.

Results of the Research Study

The results of this study are intended for completion of a PhD by research thesis and may be presented at conferences/seminars and published in peer-reviewed journals, as magazine articles, as an online article or part of a book section or report. Published results will not contain information that can be

used to identify participants unless specific consent for this has been obtained. A copy of published results can be obtained from the investigator upon request.

Voluntary Participation

Your participation in this study is voluntary. No monetary reward will be provided. No explanation or justification is needed if you choose to not participate. Your decision if you not want to participate or continue to participate will not disadvantage you or involve any penalty.

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JOONDALUP WA 6027
Phone: (08) 6304 2170
Email: research.ethics@ecu.edu.au

This project has been approved by the ECU Human Research Ethics Committee.

Appendix 4: Information Letter to Participants (Study 3)

Information Letter to Participants

Thank you for expressing your interest in this research. The purpose of this document is to explain the study that you may choose to participate in as a subject. Please read this document carefully, and do not hesitate to ask any questions.

Project Title

Neuromuscular Factors Affecting Stretch-induced Force Loss

Researchers

This research project is being undertaken as part of the requirements of a PhD candidature (Sport and Exercise Sciences) at Edith Cowan University (ECU).

PhD Candidate: Gabriel Trajano (g.trajano@ecu.edu.au) 6304 5819
Supervisor: A/Prof. Anthony Blazevich (a.blazevich@ecu.edu.au) 6304 5472
Co-supervisor: Prof. Ken Nosaka (k.nosaka@ecu.edu.au) 6304 5655

Further details of supervisors and the School of Exercise, Biomedical and Health Sciences are available at: http://www.sebhs.ecu.edu.au

Purpose of the study

The purpose of this study is to examine the force produced by reflex pathways before and after an intermittent stretch protocol.

Research Outline

In order to participate in this study, you will be asked to complete a medical questionnaire and to refrain from performing sports or hard exercise training for one day prior to the experimental day. You are also required to abstain from taking any stimulants or depressants (including caffeine or alcohol) for at least 12 hours prior testing.

If you participate in this study, you will be asked to report to the Exercise Physiology Lab (Building 19, Room 19.150) on 2 days separated at least 2 days (scheduling is flexible) at the same time of the day. On the first day you will be acquainted with all testing procedures such as: muscle stretching, maximal

voluntary contractions, tendon vibration and electrical muscle stimulation techniques; muscle stretching will be performed at an intensity where you feel mild discomfort. Electrical stimulation procedures require a small electrical current to be applied to the calf muscle belly using self-adhesive surface electrodes. The stimulation will be started at very low intensities and progressively increase.

On the second day you will complete the experimental condition (5 sets of 1-minute stretching). Force output during both an electrical stimulation protocol and tendon vibration will be measured before and 1, 5 and 15 minutes after the experimental condition. Self-adhesive skin-mounted electrodes will be used to record the small electrical signals emanating from your calf muscles during contractions (these sit passively on the skin and there is no discomfort). The skin under the electrodes will be gently abraded and cleaned with alcohol (the alcohol minimises the risk of skin infection). An ultrasound probe will be attached in the junction between the muscle and tendon on your calf. First day measurements will take about 1 hour and second will take about 1.5 hours.

Eligibility

You will be eligible for this study if:

- you are between 18 and 35 years old
- you have no neuromuscular injuries
- you have not performed flexibility training for the ankle joint in the last three months

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- The light skin abrasion performed immediately prior to the attachment of skin-based electromyogram electrodes can increase the chance of skin infections. To further reduce this small risk, alcohol wipes will be applied to the skin after abrasion as well as after removal of the electrodes.
- As with all tests of maximal muscle force production, there is the chance for muscle or tendon strain. This risk is low given that proper warm-up and familiarisation will be performed, the tests will be conducted by a

researcher who is experienced in the procedures, and isometric muscle actions carry a relatively low risk of injury.

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- You will have a unique opportunity to learn about the neuromuscular system and see high-level data acquisition techniques.
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Your participation in this study is voluntary. No monetary reward will be provided. No explanation or justification is needed if you choose to not participate. Your decision if you not want to participate or continue to participate will not disadvantage you or involve any penalty.

Withdrawing Consent to Participate

You are free to withdraw your consent to further involvement in this project at any time. You also have the right to withdraw any personal information that has been collected during the research.

Questions and/or Further Information

If you have any questions or require any further information about the research project, please do not hesitate to contact:

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If you have any concerns or complaints about the research project and wish to talk to an independent person, you may contact:

Research Ethics Officer
Edith Cowan University
270 Joondalup Drive
JOONDALUP WA 6027
Phone: (08) 6304 2170
Email: research.ethics@ecu.edu.au

This project has been approved by the ECU Human Research Ethics Committee.

Appendix 5: Information Consent (Studies 1, 2 and 3)

DECLARATION

I [PRINT NAME] _____ have read the information provided and any questions I have asked have been answered to my satisfaction. I agree to participate in this activity, realising that I may withdraw at any time without reason without prejudice.

I understand that all information provided is treated as strictly confidential and will not be released by the investigator unless required to by law. I have been advised as to what data is being collected, what the purpose is, and what will be done with the data upon completion of the research. I agree that research data gathered for the study may be published provided my name or other identifying information is not used.

_____ _____

Signature Date

Appendix 6: Pre-exercise Medical Questionnaire

Pre-exercise Medical Questionnaire

The following questionnaire is designed to establish a background of your medical history, and identify any injury and/ or illness that may influence your testing and performance. If you are under 18 then a parent or guardian should complete the questionnaire on your behalf or check your answers and then sign in the appropriate section to verify that they are satisfied the answers to all questions are correct to the best of their knowledge.

Please answer all questions as accurately as possible, and if you are unsure about anything please ask for clarification. All information provided is strictly confidential.

Personal Details

Name:_____

Date of Birth (DD/MM/YYYY):_____ Gender: Female/ Male

PART A

1. Are you a male over 45 yr, or female over 55 yr or who has had a hysterectomy or are postmenopausal?

Yes No

If YES, please provide details

2. Are you a regular smoker or have you quit in the last 6 months?	Y	N		_____
3. Did a close family member have heart disease or surgery, or stroke before the age of 60 years?	Y	N	Unsure	_____
4. Do you have, or have you ever been told you have blood pressure above 140/90 mmHg, or do you current take blood pressure medication?	Y	N	Unsure	_____
5. Do you have, or have you ever been told you have, a total cholesterol level above 5.2 mmol/L (200 mg/dL)?	Y	N	Unsure	_____
6. Is your BMI (weight/height^2) greater than 30 kg/m^2?	Y	N	Unsure	_____

PART B

1. Have you ever had a serious asthma attack during exercise?	Y	N	_____
2. Do you have asthma that requires medication?	Y	N	_____
3. Have you had an epileptic seizure in the last 5 years?	Y	N	_____
4. Do you have any moderate or severe allergies?	Y	N	_____
5. Do you, or could you reasonably, have an infectious disease?	Y	N	_____
6. Do you, or could you reasonably, have an infection or disease that might be aggravated by exercise?	Y	N	_____
7. Are you, or could you reasonably be, pregnant?	Y	N	_____

PART C

1. Are you currently taking any prescribed or non-prescribed medications?

Y N _____

2. Have you had, or do you currently have, any of the following?

			If YES, please provide details
Rheumatic fever	Y	N	_____
Heart abnormalities	Y	N	_____
Diabetes	Y	N	_____
Epilepsy	Y	N	_____
Recurring back pain that would make exercise problematic, or where exercise may aggravate the pain	Y	N	_____

PART C cont'd

Recurring neck pain that would make exercise problematic, or where exercise may aggravate the pain	Y	N	_____
Any neurological disorders that would make exercise problematic, or where exercise may aggravate the condition	Y	N	_____
Any neuromuscular disorders that would make exercise problematic, or where exercise may aggravate the condition	Y	N	_____
Recurring muscle or joint injuries that would make exercise problematic, or where exercise may aggravate the condition	Y	N	_____
A burning or cramping sensation in your legs when walking short distances	Y	N	_____
Chest discomfort, unreasonable breathlessness, dizziness or fainting, or blackouts during exercise	Y	N	_____

PART D

Have you had flu in the last week?	Y	N	_____
Do you currently have an injury that might affect, or be affected by, exercise?	Y	N	_____

*Is there any other condition not previously mentioned that may affect your ability to participate in this study?

Y N _____

Have you ever been told by a medical practitioner or health care professional that you have a nerve or muscle disorder?[a]

Yes No

Do you have a heart pacemaker?[c]

Yes No

Do you have any metallic implants (e.g. bone pins)?[a]

Yes No

Declaration (to be signed in the presence of the researcher)

I acknowledge that the information provided on this form, is to the best of my knowledge, a true and accurate indication of my current state of health.

Participant

Name:_____Date (DD/MM/YYYY):_____

Signature:_____

Researcher:

Signature:_____

Date (DD/MM/YYYY):_____

Parent/ Guardian (only if applicable)

I, _____, as parent / guardian of Mr/ Miss _____, acknowledge that I have checked the answers provided to all questions in the medical questionnaire and verify that they are correct to the best of my knowledge.

Signature: _____

Date (DD/MM/YYYY): _____

Practitioner (only if applicable)

I, Dr _____ have read the medical questionnaire and information/ consent form provided to my patient Mr/Miss/ Ms_____, and clear him/ her medically for involvement in exercise testing.

Signature:_____

Date (DD/MM/YYYY):_____

Appendix 7: Study 1 (Publication)

J Appl Physiol 115: 212–218, 2013.
First published May 9, 2013; doi:10.1152/japplphysiol.00333.2013.

Contribution of central vs. peripheral factors to the force loss induced by passive stretch of the human plantar flexors

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Trajano GS, Seitz L, Nosaka K, Blazevich AJ. Contribution of central vs. peripheral factors to the force loss induced by passive stretch of the human plantar flexors. *J Appl Physiol* 115: 212–218, 2013. First published May 9, 2013; doi:10.1152/japplphysiol.00333.2013.—The purpose of the present research was to identify the contribution of central vs. peripheral factors to the force loss after passive muscle stretching. Thirteen men randomly performed both a 5-min constant-torque stretch of the plantar flexors on an isokinetic dynamometer and a resting condition on 2 separate days. The triceps surae electromyogram (EMG) was recorded simultaneously with plantar flexor isometric torque. Measures of central drive, including the EMG amplitude normalized to the muscle compound action potential amplitude (EMG/M), percent voluntary activation and first volitional wave amplitude, and measures of peripheral function, including the twitch peak torque, 20-to-80-Hz tetanic torque ratio and torque during 20-Hz stimulation preceded by a doublet, were taken before and immediately and 15 min after each condition. Peak torque (−15.7%), EMG/M (−8.2%), and both twitch (−9.4%) and 20-Hz peak torques (−11.5%) were reduced immediately after stretch but recovered by 15 min. There were strong correlations between the torque loss and the reductions in central drive parameters (r = 0.65–0.93). Torque recovery was also strongly correlated with the recovery in EMG/M and percent voluntary activation (r = 0.77–0.81). The moderate decreases in measures of peripheral function were not related to the torque loss or recovery. These results suggest that *1*) central factors were strongly related to the torque reduction immediately after stretch and during torque recovery; and *2*) the muscle's contractile capacity was moderately reduced, although these changes were not associated with the torque reduction, and changes in excitation-contraction coupling efficiency were not observed.

muscle stretch; muscle activity; excitation-contraction coupling

PROLONGED (≥60 S) PASSIVE muscle stretch reduces maximal force production in human muscles (25). However, the mechanisms underpinning this loss have not been fully elucidated and effective strategies for minimizing the force loss have not been developed. A poststretch decrease in central (efferent) drive to the muscles has been considered to affect force production, evidenced by the reductions in electromyogram (EMG) amplitudes that are often observed (18, 27). However, the EMG signal can be affected by peripheral factors, including changes in muscle fiber action potential amplitude and propagation velocity (3, 16), so factors other than central drive limitations could also explain these results.

To better quantify changes in central drive, other techniques could be used, including normalization of EMG amplitudes to the maximal muscle compound action potential (M_{max}) amplitude (EMG/M) (3), the use of the interpolated twitch technique to estimate "percent voluntary activation" (%VA) (33, 40), and the measurement of V-wave amplitudes during maximum voluntary contractions (MVCs) (41). On the other hand, each of these measures is also considered potentially imperfect in some way (1, 3, 16, 40), so strong evidence for a central drive limitation subsequent to muscle stretch might only be indicated when a depression is observed in several simultaneously obtained measures, and these depressions are related to (i.e., correlated with) the loss of force. As yet, such a detailed examination has not been completed, so it is not clear whether a reduction in central drive is a key mechanism underpinning the force loss.

In addition to central factors, peripheral factors might influence the loss of force after stretch. For example, research using animal models has shown that passive muscle stretch can increase intracellular calcium concentration via stretch-activated channel activation and disturb calcium homeostasis (4). Such a disturbance can negatively affect the synergistic interaction between the calcium-release ryanodine receptor and voltage-sensitive dihydropyridine receptors, impairing excitation-contraction (E-C) coupling (6). In humans, it is possible to estimate the efficiency of this process by comparing the torque produced during low- vs. high-frequency electrical motor nerve stimulation trains (23, 32). In fact, it is also reasonable to expect changes in a muscle's response to short-interval double-spike stimuli when they precede a train of constant-frequency stimuli (i.e., a "catch-inducing" train) (7, 11) if calcium homeostasis is disrupted, because this response is thought to be influenced by the Ca^{2+} binding sensitivity to troponin (2, 35). Thus decreases in force production might occur even if no significant changes in central drive are produced and no metabolic disturbances are elicited. To date, the effect of static muscle stretch on muscle contractile properties remains relatively unexplored, so it is not clear if these are potential targets for intervention.

Given the above, the purpose of the present study was to establish the relative contribution of central vs. peripheral factors to the stretch-induced force loss after a 5-min continuous passive plantar flexor muscle stretch. We tested the hypothesis that impairments would be detected at both central and peripheral levels, and that these changes would be similarly correlated with changes in muscle force production. Three different examinations of central drive were completed {EMG/M, %VA [interpolated twitch technique (ITT)], and V-wave amplitude} to more robustly quantify potential central changes, while muscle and nerve stimulation procedures were used to gain information with regards to peripheral changes.

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Appendix 8: Study 2 (Publication)

Medicine & Science IN Sports & Exercise
The Official Journal of the American College of Sports Medicine
www.acsm-msse.org

. . . Published ahead of Print

Intermittent Stretch Reduces Force and Central Drive more than Continuous Stretch

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