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Neuromuscular factors contributing to reductions in muscle force after repeated, high-intensity muscular efforts

Benjamin J. C. Kirk
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**Neuromuscular factors contributing to
reductions in muscle force after repeated,
high-intensity muscular efforts**

This thesis is presented in partial fulfilment of the degree of
Master of Science (Sports Science)

Benjamin Jacob Crew Kirk

Edith Cowan University, Australia
School of Medical and Health Sciences

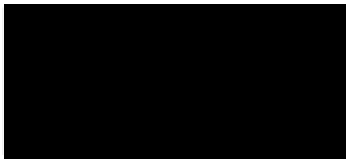
2018

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Abstract

Fatigue can accumulate sufficiently to limit muscular force production during repeated, forceful muscle contractions, including those that occur in the occupational, clinical and athletic settings. Fatigue during such efforts is likely to result from disturbances to multiple processes in the nervous system and muscle. However, previous research examining the mechanisms underpinning fatigue have typically required subjects to perform low-level constant-force contractions or to repeat maximal efforts in a single set format. Such tasks do not translate well to occupational, daily living or athletic situations where high-intensity, yet submaximal, repeated efforts may be performed in work bouts (or sets) with brief rest periods for recovery. Therefore, the overall aim of the present research was to investigate the neuromuscular mechanisms contributing to force loss after repeated, high-intensity muscular efforts with longer (90 s) periods of rest separating repetitions into sets of contractions.

In Experiment 1, 16 resistance trained men performed 6 sets of unilateral isometric plantar flexor contractions of the right leg (3 s contraction/2 s rest) reaching a target level of 85% maximal voluntary contraction (MVC). Sets were separated by a 90-s inter-set rest and completed to failure (i.e. <85% MVC) both with and without caffeine ingestion ($3 \text{ mg} \cdot \text{kg}^{-1}$) on two separate days. Caffeine was used to help identify potential physiological changes that might be responsible for the loss of force during high-intensity muscular efforts. Maximal isometric plantar flexor strength, efferent neural drive, corticospinal excitability (motor evoked potential amplitude normalised to M-wave amplitude; MEP/M) and intracortical inhibition (cortical silent period) were assessed before (PRE) and immediately (POST), 10 min (POST-10) and 20 min (POST-20) after the exercise using isokinetic dynamometry, muscle electromyography (EMG normalised to M-wave amplitude; EMG/M) and transcranial magnetic stimulation during both the non-caffeine and

caffeine conditions. In Experiment 2, tetanic stimulations (20 Hz, 80 Hz and variable frequency trains [VFT; 20-Hz trains commencing with 100-Hz ‘doublet’]) were imposed to quantify excitation-contraction coupling (E-C coupling) efficiency. Additionally, neuromuscular electrical stimulation was imposed during ongoing Achilles tendon vibration (VIB+STIM; 115 Hz vibration) in order to estimate persistent inward current (PIC)-mediated facilitation of the motor neurone (MN) pool before and after the same exercise intervention in 17 resistance trained men. During the VIB+STIM test, MN (PIC) facilitation was quantified as the mean torque at two time points throughout the test; during vibration (T_{vib}) and 500 ms after vibration was ceased (T_{sust}). Results are reported relative to baseline.

In Experiment 1, a significant reduction in maximum voluntary isometric plantar flexion torque (12.2%; $p < 0.001$) was observed post-exercise, which did not recover by POST-20. Significant reductions in triceps surae EMG/M (-6%; $p = 0.024$) and MEP/M amplitude (9%; $p = 0.01$) were found post-exercise but recovered by POST-10. Cortical silent period (an indicator of GABA_B-mediated intracortical inhibition) was reduced (-4%; $p = 0.016$) post-exercise and did not recover by POST-20. In Experiment 2, temporal changes in torque were similar to Experiment 1. Significant reductions in the evoked torque response from 20 Hz ($p < 0.001$), 80 Hz ($p < 0.001$) and VFT ($p < 0.001$) stimulations were observed at POST and did not recover by POST-20, however no changes in 20:80 and 20:VFT ratios were observed. Finally, significant reductions in both T_{vib} (-13%; $p = 0.035$) and T_{sust} (-25%; $p = 0.035$) were found post-exercise but recovered by POST-10. The ingestion of caffeine allowed for a greater overall torque production and neural drive (EMG/M) but the lack of condition \times time interaction effect indicated that it did not clearly affect the time course of fatigue or recovery. Further, no detectable effects were observed compared to the non-caffeine condition in corticospinal excitability, MN excitability or E-C coupling, as

shown by the negligible changes in MEP/M amplitude, PIC facilitation, and torque during 20-Hz, 80-Hz and VFT stimulations.

These data suggest that corticospinal tract efficiency and PIC-mediated facilitation of the MN pool can be compromised and are likely to account in part for the force loss immediately following an acute bout of repeated, high-intensity muscular efforts performed in sets (with 90 s rest). However, changes in E-C coupling efficiency (i.e. ‘peripheral fatigue’) are likely to explain the ongoing, prolonged loss of force, at least to 20 min post-exercise. Therefore, it is likely that both changes in the nervous system as well as the muscle contribute to the loss of force following repeated, high-intensity muscular efforts.

List of publications

Conference Presentations

Kirk, B., Pulverenti, T., Rowe, G., Trajano, G., Blazevich, A.J. (2017). *Neural mechanisms that regulate repeated, high-intensity muscular performance – new insights into fatigue?* Journal of Science and Medicine in Sport, Vol. 20, S41–S42.

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List of Abbreviations

| | |
|------------------------|---|
| ANOVA | Analysis of variance |
| ADP | Adenosine diphosphate |
| ATP | Adenosine triphosphate |
| Ca ²⁺ | Calcium ions |
| CI | Confidence interval |
| CMEP | Cervicomedullary motor evoked potential |
| CON | Control |
| cSP | Cortical silent period |
| E-C Coupling | Excitation-contraction coupling |
| EMG | Electromyography / electromyographic activity |
| GABA _B | Gamma-aminobutyric acid |
| H ⁺ | Hydrogen ions |
| HFF | High-frequency fatigue |
| K ⁺ | Potassium ions |
| LFF | Low-frequency fatigue |
| MANOVA | Multivariate analysis of variance |
| MEP | Motor evoked potential / measured during MVC |
| MEP _{REST} | Motor evoked potential measured during rest |
| MEP/M | Motor evoked potential measured during a maximal voluntary contraction normalised to maximal motor wave |
| MEP _{REST} /M | Motor evoked potential measured during rest normalised to maximal motor wave |
| MG | Medial gastrocnemius |
| MG/M | Medial gastrocnemius root mean square electromyography normalised to maximal motor wave |
| M _{max} | Maximal motor wave |
| Na ⁺ | Sodium ions |
| MN | Motor neurone |
| MSO | Maximal stimulator output |
| MVC | Maximum voluntary contraction |
| M-wave | Motor wave |
| P ⁺ | Inorganic phosphate |
| PIC | Persistent inward current |
| POST | After the intervention |
| POST-10 | 10 min after intervention |
| POST-20 | 20 min after intervention |
| PRE | Before the intervention |
| RMANOVA | Repeated measures analysis of variance |
| RMS | Root mean square |
| RyR | Ryanodine receptors |
| SD | Standard deviation |
| SE | Standard error of the mean |

| | |
|--------------------|--|
| SOL | Soleus |
| SOL/M | Soleus root mean square electromyography normalised to maximal motor wave |
| SOL _{PSU} | Soleus pseudo-monopolar electromyographic electrode configuration |
| SPSS | Statistical Package for the Social Sciences |
| TA | Tibialis anterior |
| TMEP | Thoracic motor evoked potential |
| TMS | Transcranial magnetic stimulation |
| T _{sust} | Sustained torque |
| T _{vib} | Torque during vibration |
| TS | Triceps surae |
| TS/M | Triceps surae root mean square electromyography normalised to maximal motor wave |
| VFT | Variable frequency train |
| VIB+STIM | Tendon vibration plus muscle stimulation |
| 20 Hz | Torque during electrical stimulation of muscle at 20 Hz |
| 5-HT | Serotonin |
| 80 Hz | Torque during electrical stimulation of muscle at 80 Hz |

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Introduction and Overview

Humans commonly perform repeated high-intensity, yet submaximal, muscular efforts. This is done in occupational settings where repetitive lifting (e.g. moving objects, shovelling, etc.) is regularly performed, clinical settings where individuals with functional limitations are required to work at a higher proportion of their maximum (compared to non-limited individuals) to complete activities of daily living, and athletic pursuits where repeated high-intensity efforts are required for successful performance. Such efforts are also common to strength training, which is traditionally used as a tool to improve strength, power and muscle mass (Stone, Stone, & Sands, 2007) in order to improve sporting/athletic performance or to prevent skeletal muscle loss, reduce morbidity and mortality, and improve physical function in older and clinical populations (Liu & Latham, 2009; Warburton, Nicol, & Bredin, 2006). However, the ability to perform repeated high-intensity muscular efforts is limited by the accumulation of fatigue, where the work output required to continue the activity can no longer be sustained. Thus, there is a need to understand the factors influencing, and mechanisms underpinning, fatigue during such tasks in order that strategies might be developed to mitigate against it and, in turn, improve exercise (physical work) performance.

Muscle fatigue can be defined as any exercise-induced reduction in the ability to produce force or power with a muscle or muscle group and can result from changes in function at multiple sites along the neuromuscular pathway (Bigland-Ritchie & Woods, 1984; Gandevia, 2001; Taylor & Gandevia, 2008). From a nomenclature perspective, fatigue resulting from processes at or distal to the neuromuscular junction has been commonly referred to as “peripheral fatigue”, whilst fatigue that manifests above the neuromuscular junction (i.e. brain, spinal cord and motor neurones) has often been referred to as “central fatigue” (Taylor & Gandevia, 2008). It is likely that both peripheral and central aspects of fatigue play a role in

the response to repeated high-intensity muscular efforts. However, previous research examining the mechanisms underpinning fatigue have typically focused on examinations of a relatively small number of changes within the neuromuscular pathway, which precludes the development of a clear picture of changes throughout the whole neuromuscular system. Furthermore, the majority of research investigating the effects of fatigue on muscular performance has typically imposed either sustained or repeated maximal efforts for time or predefined level of maximal force output, or sustained low-intensity submaximal voluntary contractions (Hoffman, Oya, Carroll, & Cresswell, 2009; Taylor, Butler, Allen, & Gandevia, 1996; Taylor & Gandevia, 2008). However, these types of tasks rarely apply in occupational, daily living or athletic situations, and it is not known how much understanding can be gained from the use of these paradigms. Furthermore, bouts of work are often performed with intervening rest periods in most occupational, daily living or athletic tasks, and is a hallmark of strength training practice (Bompa & Haff, 2009). However, these are rarely performed in research studies. Therefore, it is possible that the physiological changes leading to fatigue in the work bouts with different work:rest ratios used in previous studies are different, or at least of different relative importance, to those leading to fatigue in the repeated high-intensity efforts performed in many occupational, clinical and athletic environments. Finally, a significant part of our understanding comes from *ex vivo* work in single fibre preparations (Edman & Lou, 1990; Lamb, 2002). While such work has contributed significantly to our understanding of the fatigue process, at least at the peripheral level, consideration of the test protocols is needed in order to estimate the relevance to organism-level changes.

In the following review of literature, the potential for central and peripheral mechanisms to influence fatigue-induced force loss will be considered with a view to developing specific testable hypotheses for future research examining the fatigue responses to the repeated high-intensity muscular efforts that are performed as work bouts with short inter-

bout rest periods in many occupational, clinical and athletic environments, and especially in strength training.

1.1 Peripheral factors influencing fatigue

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

Peripheral changes contribute to the majority of fatigue induced by an acute bout maximal or high-intensity but submaximal exercise (Nordlund, Thorstensson, & Cresswell, 2004; Schillings, Hoefsloot, Stegeman, & Zwarts, 2003) and can be attributed to multiple mechanisms. Metabolic changes within the muscle are an important mechanism. These metabolic changes include reductions in ATP synthesis as well as accumulation of hydrogen ions (H^+), leading to a decrease in intracellular pH (i.e. acidosis), inorganic phosphate (P_i), which is hydrolysed from creatine phosphate in the resynthesis of ATP, and extracellular K^+ . These changes in ionic concentrations in the cell reduce sarcolemmal excitability and thus force development (McKenna, Bangsbo, & Renaud, 2008). Additionally, ADP accumulation adjacent to the contractile proteins is thought to negatively impact contractile function, although probably to a lesser extent other than metabolic changes (Allen, 2009; Allen, Lamb, & Westerblad, 2008; Debold, 2016).

At sub-physiological temperatures (10-15°C), elevated levels of P_i and H^+ to physiological fatiguing levels are known to decrease maximal isometric force by >50% (Debold, 2016; Nosek, Fender, & Godt, 1987). However, recent work has demonstrated that P_i and H^+ accumulation reduces isometric force by only 5-20% (Debold, Dave, & Fitts, 2004) and 10% (Knuth, Dave, Peters, & Fitts, 2006; Pate, Bhimani, Franks-Skiba, & Cooke, 1995), respectively, at near-physiological temperatures (30°C) for the same increase in P_i and H^+ seen during the low temperature conditions. However, when both metabolites are elevated

simultaneously, as is the case during exercise-related fatigue *in vivo*, their individual effects are additive, and have been shown to induce a greater level of fatigue (Debold, 2016; Nelson, Debold, & Fitts, 2014). Nonetheless, larger force losses are typically observed in single fibre studies when the same level of metabolite accumulation is evoked as in fatiguing exercise (Vedsted, Larsen, Madsen, & Sjøgaard, 2003). Therefore, it can be concluded that factors additional to the biochemical changes must contribute to the total force reduction in muscles during fatigue.

A second mechanism affecting fatigue at the peripheral level is a change in intracellular calcium handling, resulting from a reduction in sarcoplasmic reticulum calcium (Ca^{2+}) release as well as a reduced sensitivity of the contractile proteins to Ca^{2+} , which ultimately impairs the excitation-contraction (E-C) coupling process (Debold, 2016). Inside the muscle, increases in Ca^{2+} concentration mandate the binding of myosin to actin, and thus the development of muscular force, via the movement of tropomyosin which allows myosin to bind strongly to actin (Debold, 2016). Therefore, the efficiency of Ca^{2+} transport plays a key role in muscular contraction (Maffiuletti et al., 2016; Nielsen, 2009). It is believed that accumulation of metabolic by-products and compromised release of Ca^{2+} from the sarcoplasmic reticulum (SR) act synergistically to cause much of the loss in muscle function during fatigue (Debold, 2016).

In addition to reduced Ca^{2+} release, elevated levels of metabolic by-products have also been shown to decrease the sensitivity of the myofilaments (actin and myosin) to Ca^{2+} . The reduced Ca^{2+} sensitivity causes a rightward shift in the force-calcium relation that exacerbates the effects of reduced Ca^{2+} release such that the level of Ca^{2+} required to elicit 50% of the maximal isometric force is increased by 2-fold in the presence of a fatiguing level of P_i *in vitro*. Additionally, the level of Ca^{2+} needed to cause actomyosin interaction is even more pronounced near physiological temperatures. Therefore, elevated levels of metabolic by-products can cause

both a decrease in the release of Ca^{2+} as well as a reduction in the muscle's sensitivity to Ca^{2+} (Debold, 2016).

As changes in the E-C coupling process contribute to the loss of force during fatigue (Allen, Westerblad, Lee, & Lännergren, 1992; Pasquet, Carpentier, Duchateau, & Hainaut, 2000), further research is required in order to quantify the change in E-C coupling efficiency during repeated high-intensity muscular contractions. This might be assessed *in vivo* by comparing the peak torque produced during high- (e.g. 80 - 100 Hz) vs. low- (e.g. 20 Hz) frequency neuromuscular electrical stimulation, to quantify 'low-frequency fatigue' (LFF) (Edwards, Hill, Jones, & Merton, 1977; Jones, 1996; Martin, Millet, Martin, Deley, & Lattier, 2004). LFF is characterised by a proportionately greater loss of force in response to low- versus high-frequency muscle stimulation (Keeton & Binder-Macleod, 2006). It is understood that the greater loss of force in the low frequency stimulation is caused by an elevated Ca^{2+} concentration in the triadic junction (see Figure 1.1 below). This is attributable to impairment in the interaction between voltage-sensitive dihydropyridine receptors and calcium-release ryanodine receptors (RyR) (Balog, 2010; Lamb, 2000). One factor that might disrupt the interaction between receptors is an increased sarcoplasmic Ca^{2+} concentration causing a reduction in calcium release by the RyR during muscle contraction (Allen, 2004; Balog, 2010). In contrast, during the high frequency stimulation, force loss is typically less due to the supraphysiological stimulation rate which allows more Ca^{2+} to be released from the sarcoplasmic reticulum into the myoplasm (Edwards et al., 1977; Moss, Lynch, & Fitzsimons, 2017). However, if force loss does occur during high frequency stimulation, then it is possible that other processes such as Ca^{2+} sensitivity (partly caused by increased H^+ or P_i) or a loss of force transmission through the series elastic component of the muscle-tendon unit might be factors (Debold, 2016; Mademli & Arampatzis, 2005). In addition, LFF and increased sarcoplasmic calcium concentration are most notably observed during eccentric contractions

or isometric exercises at a long muscle lengths (Jones, 1996; Keeton & Binder-Macleod, 2006; Martin et al., 2004), which suggests that E-C coupling efficiency may be affected differently depending on the type of exercise being performed. Therefore, assessing reductions in this ratio (20:80 ratio) might provide valuable insight into the mechanisms underpinning E-C coupling efficiency (Jones, 1996; MacIntosh & Rassier, 2002; Martin et al., 2004), and the use of LFF tests might provide valuable insight into the mechanisms of fatigue during repeated high-intensity exercise.

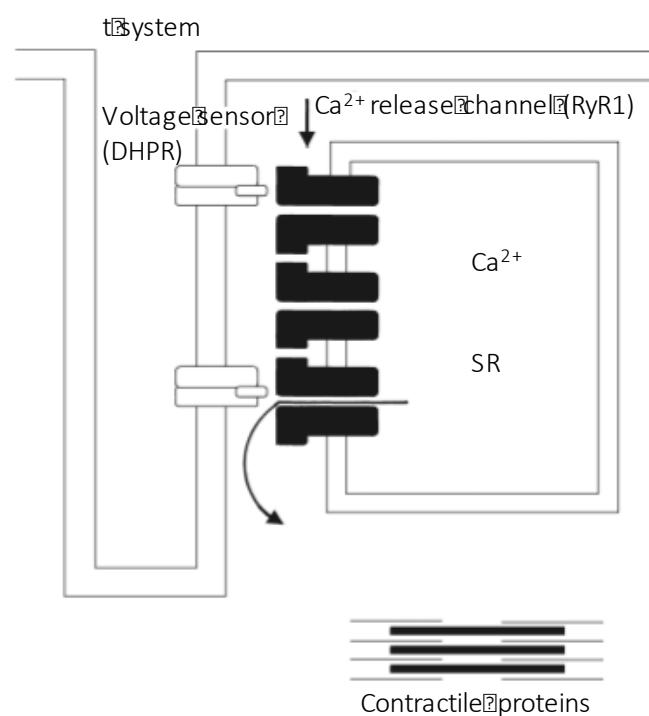


Figure 1.1. Schematic diagram of calcium release in skeletal muscle. The depolarisation of an action potential is detected by the voltage sensitive dihydropyridine receptors in the t system, which directly controls the opening and closure of the Ca^{2+} release channels (RyR) in the adjacent SR without the need for influx of extracellular Ca^{2+} (modified from Lamb, 2000). Increased sarcoplasmic Ca^{2+} concentration is believed to disrupt the interaction between receptors (Balog, 2010).

1.1.2 Muscle “catch-like” properties

The catch-like property of skeletal muscle refers to the force augmentation that occurs 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; Garland & Griffin, 1999). This high-frequency burst at the motor neurone (MN)

serves to enhance muscle force without the need to increase mean firing frequency/discharge rate (Binder-Macleod & Kesar, 2005). It is believed that the catch-like property can be evoked by the nervous system during volitional movements to compensate for the loss of muscle force that accompanies fatigue (Binder-Macleod & Kesar, 2005; Griffin, Garland, & Ivanova, 1998). Two primary mechanisms have been proposed to explain the force enhancing effects: 1) increased sarcoplasmic reticulum calcium release via the RyR receptor (Cheng, Place, Bruton, Holmberg, & Westerblad, 2013) and 2) increased stiffness of the series elastic elements of muscle (Binder-Macleod & Barrish, 1992; Burke, Rudomin, & Zajac, 1976; Parmiggiani & Stein, 1981). The latter mechanism is thought to work by the rapid shortening of contractile elements stretching the muscle's series elastic elements thus increasing the stiffness of the system and in turn force transmission velocity (Binder-Macleod & Kesar, 2005; Parmiggiani & Stein, 1981). Interestingly, the force enhancing effects of the high-frequency burst at stimulation onset are more pronounced during low-frequency fatigue (Bentley & Lehman, 2005). This is suggestive that the catch-like property might be an essential mechanism for counteracting the loss of force during fatigue by upregulating sarcoplasmic reticulum Ca^{2+} release (Bentley & Lehman, 2005; Binder-Macleod & Russ, 1999; Cheng et al., 2013) and, in turn, increasing the number of binding sites for cross-bridge formation (Bakker, Cully, Wingate, Barclay, & Launikonis, 2017; Cheng et al., 2013).

In order to gain a greater understanding of the catch-like effect on muscle contractile force and fatigue the use of muscle or nerve stimulation can be used by applying a brief, high-frequency burst of two to four electrical pulses followed by a constant low-frequency stimulation train to a muscle or nerve (i.e. a variable frequency train; VFT) (Binder-Macleod & Kesar, 2005; Burke et al., 1970). It is believed the initial high-frequency burst at the beginning of the train increases the myofibrillar affinity for Ca^{2+} , resulting in an increased rate of force development and peak contractile torque when compared to a constant-frequency train

of equivalent intensity and frequency (Bakker et al., 2017; Cheng et al., 2013). Therefore, the use of a VFT, along with measurements of the 20:80 Hz ratio, should provide valuable information with regard to calcium handling during repeated high-intensity exercise.

1.2 Central factors influencing fatigue

Whilst many of the changes influencing muscle function during fatiguing exercise occur peripherally, at least part of this fatigue occurs because the central nervous system loses its ability to fully activate the muscles (called ‘central drive’ failure) (Gandevia, 2001; Goodall, Charlton, Howatson, & Thomas, 2015). Determining the mechanisms that underpin this loss of central drive is essential in order to develop strategies to reduce fatigue and thus improve exercise performance.

In a voluntary contraction, an excitatory signal is initiated in the motor cortex that descends largely via the corticospinal tract to the MN. If the net excitatory synaptic input, from temporal and spatial summation of excitatory postsynaptic potentials, is of sufficient strength to depolarise the MN to its spiking threshold then an action potential is generated at the axon initial segment of the MN. The resulting MN action potential(s) then propagates along the axon to the neuromuscular junction and triggers postsynaptic action potentials in the membranes (sarcolemma) of muscle fibres (Gandevia, 2001; Heckman & Enoka, 2012a), resulting in calcium release from the sarcoplasmic reticulum and actomyosin interaction (i.e. E-C coupling) (Balog, 2010). The force produced in the contraction is dependent on both the number of motor units recruited as well as their discharge rate (Gandevia, 2001; Henneman, 1985). Therefore factors reducing the descending drive to the muscle should significantly and negatively affect E-C coupling and in turn muscular force production.

1.2.1 Supra-spinal mechanisms

Activity within the motor cortex determines the level descending drive (i.e. to the muscle), therefore alterations in supra-spinal drive can noticeably affect muscular force production (Gandevia, 2001). A reduction in cortical outflow has been shown to result in a loss of muscle force due to the inability of the descending supra-spinal drive to maximally activate the muscle's motor neurone pool (Gandevia, 2001). This could result from alterations in the net excitability of the motor cortex in response to peripheral sensory feedback from group III/IV nociceptive afferents, which could activate intracortical inhibitory circuits and reduce motor cortical output (Nijs et al., 2012; Taylor, Butler, & Gandevia, 2000; Taylor & Gandevia, 2008), and thus muscle force during fatigue. 'Supra-spinal fatigue' has been shown to contribute up to approximately one-quarter of the force loss during prolonged (2-min) maximal voluntary contractions (MVCs) (Taylor & Gandevia, 2008). Therefore force loss appears to be attributable in part to a reduction in motor cortical outflow (Gandevia, Allen, Butler, & Taylor, 1996; Gandevia, Petersen, Butler, & Taylor, 1999). However, it is not known whether such changes contribute substantially to muscle work during strength training, or occupational and daily tasks that require the performance of high intensity, yet submaximal, force production with intermittent periods of rest.

1.2.2 Spinal Mechanisms

The spinal circuitry is a complex network of sensory neurones, inter-neurones and MNs. In addition to supra-spinal fatigue, the responsiveness of spinal α -motor neurones (MNs) to descending input also declines profoundly with fatigue during maximal muscular contractions (McNeil, Giesebrecht, Gandevia, & Taylor, 2011; Taylor, Butler, et al., 2000). Whilst it is sometimes assumed that MNs passively convey cortical drive to the muscles, intrinsic MN properties and sensory feedback to the spinal cord can alter descending drive by

inhibiting or facilitating MNs (Nielsen, 2004). Thus, the reduction in neural drive and muscular force during fatigue could potentially result from MN inhibition and/or disfacilitation at the spinal level (Gruet et al., 2013; McNeil, Martin, Gandevia, & Taylor, 2009). In fact, at high discharge rates such as those seen in strong contractions, MN output may be more limited by the properties of the MN pool rather than the motor cortex (Martin, Gandevia, & Taylor, 2006; McNeil et al., 2009). For example, force augmentation resulting from both stimulation of the motor cortex (using transcranial stimulation) and the descending corticospinal pathways (using cervicomedullary stimulation) during maximal isometric contractions were simultaneously reduced with increasing levels of muscular contraction strength (Martin et al., 2006), indicating that motor cortical output was not a limiting factor for force production at high levels of contraction force and that effects below the level of the motor cortex (likely at the MN) were responsible for the reduction in evoked output (i.e. increased central/neural drive). Therefore, it would be of interest to determine whether changes in intrinsic MN properties and sensory feedback also play a role in the fatigue response during repeated high-intensity yet submaximal contractions.

Although strong evidence indicates that a portion of the fatigue resulting from intense muscular contractions such as those performed during strength training and other intense exercise activities results from a reduction in MN excitability (McNeil et al., 2009; Taylor, Butler, et al., 2000), no evidence exists for the specific site at which these changes occur. One potential factor influencing MN excitability is a change in somatosensory feedback, which might affect pre- or post-synaptic sites. Butler, Taylor, and Gandevia (2003) investigated whether group III and IV afferents contributed to the decline in discharge rate by performing elbow flexor MVCs during ischaemic muscle conditions. They observed a decrease in MN excitability, however cervicomedullary evoked potential (CMEP) amplitudes did not differ between ischaemic and control conditions, indicating that group III and IV afferent activity did

not notably influence the force decline. McNeil, Giesebrecht, Khan, Gandevia, and Taylor (2011) later examined the effect of the withdrawal of excitatory input to MNs from muscle spindles during prolonged (2 min) MVCs by using tendon vibration to mechanically increase muscle spindle discharge rates and increase Ia-excitatory input to the MN pool. They observed an increase in the muscle torque evoked by tendon vibration during sustained MVCs, however the increase in spindle activity did not affect the conditioned CMEP size (an indicator of MN excitability). The authors concluded that although muscle spindle afferents contributed to MN pool excitability, changes in the intrinsic MN properties caused by repetitive discharge were most probably responsible for the reduction in MN excitability during fatigue. There is compelling evidence that sensory afferents such as stretch-sensitive muscle spindles (Ia and II), tension-sensitive golgi tendon organs (Ib), pressure-sensitive joint receptors (II), and pain- and stretch-sensitive free-nerve endings (II, III and IV) are able to alter the synaptic input to the MN pool via inhibitory and excitatory inputs. In fact, sensory inputs, especially from group Ia afferents, can strongly affect MN facilitatory/amplification mechanisms, which could play a larger role during fatigue (Gandevia, 1998; Powers & Binder, 2001; Taylor, Amann, Duchateau, Meeusen, & Rice, 2016). Several lines of evidence support this conclusion (Gandevia, 1998; Khan, Giesebrecht, Gandevia, & Taylor, 2012) and point to two potential mechanisms underpinning the loss in MN excitability during fatigue. The first relates to changes in neuromodulatory effects at the somato-dendritic end of the MN (Perrier, Rasmussen, Christensen, & Petersen, 2013). It has been shown that motor unit discharge rates slow during fatiguing voluntary contractions as the contraction and relaxation of muscle fibres slow (Gandevia, 1998; Taylor, Butler, et al., 2000). Although the mechanism by which the slowing of MN discharge is unclear, a reduction in persistent inward current (PIC) formation could possibly be a factor that has not yet been tested (Binder, 2003; Heckman, Gorassini, & Bennett, 2005). A PIC is a depolarising current generated by voltage-sensitive channels in the MN that

tend to remain active as long as the membrane potential remains above activation threshold (Heckman et al., 2005). When activated, PICs amplify and prolong synaptic input (Hultborn, Denton, Wienecke, & Nielsen, 2003) and thus change the input-output relationship at the synapse to produce a sustained depolarisation, particularly in low-threshold MNs (Heckman, Johnson, Mottram, & Schuster, 2008; Lee & Heckman, 1996). This amplification allows the MN to fire more rapidly to produce maximal levels of muscular force (Hultborn et al., 2003).

PICs are primarily located at the dendrites of the MN and are commonly generated by two types of voltage-sensitive channels: L-type Ca^{2+} channels and persistent Na^{+} channels (Bennett, Hultborn, Fedirchuk, & Gorassini, 1998; Carlin, Jones, Jiang, Jordan, & Brownstone, 2000; Hounsgaard & Kiehn, 1993; Lee & Heckman, 1999). These channels are highly receptive to monoaminergic drive, in particular serotonin (5-HT) and noradrenaline in the spinal cord (Heckman & Enoka, 2012a; Heckman, Lee, & Brownstone, 2003; Perrier et al., 2013). Both of these neurotransmitters have potent effects on MNs by hyperpolarising the activation voltage for PICs (Heckman & Enoka, 2012a) and therefore increasing the likelihood that a MN will fire an action potential. Monoaminergic drive is essential for the generation of PICs, as shown using *in vivo* experiments on decerebrate cats where PIC generation was found to disappear after spinalisation and reappear after intravenous injection of a 5-HT precursor (5-hydroxytryptophan) (Crone, Hultborn, Kiehn, Mazieres, & Wigström, 1988; Hounsgaard, Hultborn, Jespersen, & Kiehn, 1988). The importance of this is such that PICs can be modulated across different behavioural states by changing the monoaminergic drive to the MN dendrites and thus changing the excitability of the MN (Heckman et al., 2003), which should allow the generation of very high forces despite modest input levels (Binder, 2003).

Sustained force generation is likely to depend on PICs (Heckman et al., 2003). In the absence of neuromodulatory input, ionotropic input alone (i.e. $\text{Na}^{+}/\text{K}^{+}$ flux) is not sufficient to drive high threshold (fast-twitch) motor units at their maximum force outputs (Lee, Kuo, Jiang,

& Heckman, 2003). In the absence of monoaminergic drive, and thus absence of PIC amplification (Perrier & Delgado-Lezama, 2005), force generation above 50% of voluntary maximum would not be possible (Heckman et al., 2005). Therefore, PIC behaviour in MNs is essential for force production in both maximal and submaximal contractions. During fatigue, however, PICs are likely to be affected in two ways: 1) at the somato-dendritic site due to a reduction in serotonergic and/or noradrenergic drive, which could be caused by a reduction in output from nerves arising in the brainstem (locus coeruleus or raphe nucleus output) (Heckman et al., 2003), or 2) a reduction in stimulatory afferent feedback, especially reductions in feedback from Ia afferents due to reductions in muscle spindle discharge rates (Macefield, Hagbarth, Gorman, Gandevia, & Burke, 1991). Regardless of the mechanism, a reduction in PIC amplitude is likely to negatively affect MN excitability and thus there would be a requirement for an upregulation of motor cortical output in order to maintain the same force output (McNeil et al., 2009), ultimately leading to a reduction in voluntary force and a higher level of perceived effort (Taylor, Butler, et al., 2000). Nonetheless, it is not known whether there is a reduction in PIC amplitude during repeated high-intensity, voluntary muscular contractions, such as those performed during strength training or other forms of high-intensity exercise. It is therefore also not known whether interventions targeting the activation of PICs can alleviate fatigue symptoms.

A second mechanism that might influence the loss of MN excitability during fatigue is a functional change at the initial segment of the axon hillock (Khan et al., 2012). This may result from the spillover of serotonin (5-HT) from the somato-dendritic compartment to inhibitory 5HT_{1A} receptor sites at the axon initial segment during prolonged or high magnitude muscle activation and in turn inhibit the generation of action potentials. (Cotel, Exley, Cragg, & Perrier, 2013; D'Amico et al., 2015). While there is limited direct evidence to support the

hypothesis that serotonin spillover contributes to fatigue, the possibility exists that this mechanism plays a role.

1.2.3 Limitations to previous research examining neural (central) drive after muscle fatigue

One of the limitations of previous research examining the effects of supra-spinal drive and MN excitability in fatigue is the lack of applicability to occupational, clinical and athletic settings. Previous studies have tended to utilise either continual submaximal or maximal efforts where little to no rest is afforded throughout the intervention (McNeil et al., 2009; Taylor et al., 1996; Yoon, Schlinder Delap, Griffith, & Hunter, 2007), or maximal intermittent efforts to a predefined percentage of maximum capacity (Kalmar, Del Balso, & Cafarelli, 2006). Whilst these strategies are effective for determining fatigue-induced changes in central drive, they do lack in their applicability to the tasks encountered in the occupational, clinical and athletic settings. For instance, in practice, work bouts typically occur at a high yet submaximal intensity relative to maximum capacity. In addition, these work bouts are usually accompanied by rest interspaced between efforts. An example of this might be strength training where intensities typically range between 60-90% maximum capacity and are accompanied by small periods of rest between repetitions and sets of an exercise. Therefore, an intervention that accounts for intensity, contraction duration and the recovery between efforts might help to better explain the effect of central and peripheral factors on fatigue during occupational, clinical and athletic settings.

1.3 Measuring central factors influencing fatigue

There are a variety of methods available to assess the neurophysiological function of the corticospinal-motoneuronal pathway and its effect on muscular force development. These methods include electrical or magnetic stimulation of the motor cortex, corticospinal tract and peripheral nerves (see Capaday (1997); McNeil, Butler, Taylor, and Gandevia (2013); Petersen, Pyndt, and Nielsen (2003); Taylor and Gandevia (2004) for reviews). The techniques used in the present thesis are explained below.

1.3.1 Corticospinal Excitability and Transcranial Magnetic Stimulation

In order to test whether a loss in corticospinal excitability occurs after performing repeated high-intensity muscle contractions, electromyography (EMG) and transcranial magnetic stimulation (TMS) can be used by applying a single short-lasting supra-threshold magnetic pulse over the motor cortex to produce a short-latency excitatory response in muscle known as a motor-evoked potential (MEP) (Di Lazzaro, Rothwell, & Capogna, 2017; Petersen et al., 2003). MEPs can be recorded from both upper and lower-limb muscles (Rothwell, Thompson, Day, Boyd, & Marsden, 1991) and are considered to be a reliable method for assessing the function of the corticospinal pathway. As the MEP is influenced by the excitability of the corticospinal-motoneuronal pathway, the size (i.e. amplitude or area) of the MEP is often used as an indicator of corticospinal excitability (Petersen et al., 2003; Rothwell, 1997).

A TMS pulse can be delivered at rest or during muscle contraction, both of which can be used to assess different aspects of the corticospinal-motoneuronal pathway (McNeil et al., 2013). At rest, MEPs evoked from TMS provide information regarding the balance between excitation and inhibition in the corticospinal-motoneuronal pathway (Chen, 2004). However,

by performing the TMS pulse during a voluntary contraction, the amplitude of the MEP is significantly increased when compared to at rest (Nielsen & Petersen, 1995). This increase in MEP amplitude is believed to result from a larger fraction of MNs being excited by the descending corticospinal volley because MN excitability is increased as the synaptic facilitation of MNs brings some MNs closer to their firing threshold (Di Lazzaro et al., 2001; Kaneko, Kawai, Fuchigami, Shiraishi, & Ito, 1996). Therefore, MEPs evoked during a voluntary contraction can provide additional information on facilitatory pathways throughout the human motor system.

An added benefit of measuring MEPs during voluntary contractions is that a second evoked response can be elicited that provides information relating to specific aspects of intracortical inhibition. The evoked MEP from TMS during voluntary contraction elicits a brief period of near silence in the ongoing EMG, known as the cortical silent period (cSP) (Hallett, 2000; McNeil et al., 2013; Rösler, 2001; Rossi, Hallett, Rossini, Pascual-Leone, & Group, 2009; Taylor & Gandevia, 2001). The cSP can last up to 300 ms and is believed to be influenced by both spinal inhibitory (up to 150 ms of the cSP) (Yacyshyn, Woo, Price, & McNeil, 2016) and cortical mechanisms (which predominately contribute to the later period) (Chen, Lozano, & Ashby, 1999; McDonnell, Orekhov, & Ziemann, 2006), the latter of which is believed to result from activation of an intracortical inhibitory process mediated by long-lasting gamma-aminobutyric acid (GABA)_B receptors. In turn, the cSP is commonly used as an indicator of an intracortical inhibitory process that can influence voluntary neural drive to the muscle. Therefore, by imposing TMS pulses at rest and during voluntary contraction, subsequent MEP amplitude and cSP can be used to give insight into cortical and corticospinal mechanisms contributing to force loss after repeated bouts of high-intensity contractions, such as those performed during strength training and other forms of high-intensity exercise.

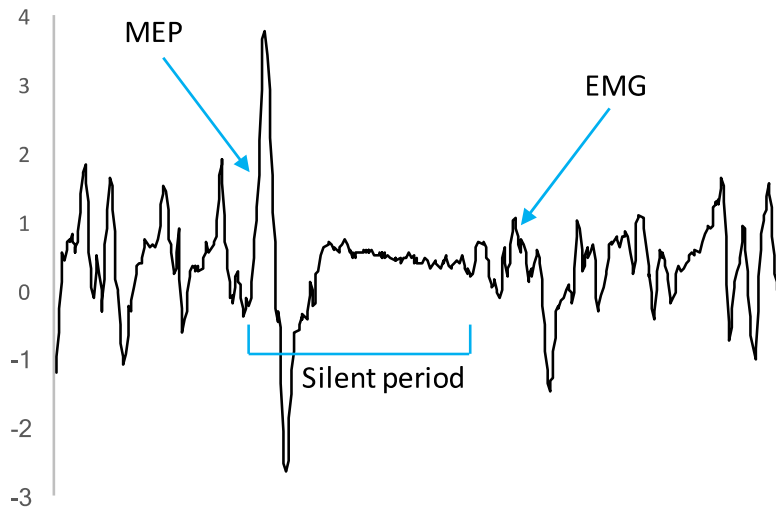


Figure 1.2. Sample recording of a TMS pulse during contraction. The MEP appears as a sharp spike in the EMG signal followed by a period of near silence (cSP). EMG then returns as the contraction recommences.

1.3.2 Motor neurone excitability and tendon vibration

In addition to TMS, tendon vibration methods can be used to assess MN excitability (Desmedt & Godaux, 1978; McNeil, Giesebrecht, Khan, et al., 2011; McPherson, Ellis, Heckman, & Dewald, 2008; Mottram, Suresh, Heckman, Gorassini, & Rymer, 2009; Suresh, Wang, Heckman, & Rymer, 2011; Trajano, Seitz, Nosaka, & Blazevich, 2014). At high frequencies (e.g. > 70 Hz), tendon vibration recruits motor units through the activation of Ia afferents arising from muscle spindles that induce progressive excitation to the homonymous MNs, and facilitates PICs in those MNs (Frigon et al., 2011; Heckman & Binder, 1988). The presence of this PIC can be seen as a slow progressive increase in isometric force during the tendon vibration sequence, as well as a visibly sustained force that persists beyond the cessation of vibration (Heckman et al., 2005). When performed alone, tendon vibration has been shown to induce small force outputs resulting from the recruitment of low-threshold motor units (Gorassini, Bennett, & Yang, 1998; Kamen, Sullivan, Rubinstein, & Christie, 2006; Kiehn & Eken, 1997). However, higher-force outputs may be elicited by imposing bursts of electrical stimulation simultaneously with tendon vibration to recruit higher-threshold motor units. With

this technique, forces of up to 50% of maximal contraction have been achieved, indicating the presence of fatigue-resistant as well as fast-fatigable motor unit recruitment (Magalhães, de Toledo, & Kohn, 2013; Magalhães & Kohn, 2010). This technique thus provides a unique opportunity to investigate PIC behaviour in humans (Trajano et al., 2014) and potentially its modulation with fatigue.

1.3.3 Caffeine and its modulation of corticospinal excitability

Although the techniques described above should provide a reasonable indication of motor neuronal tract efficiency and PIC amplitudes after an acute bout of maximal or near-maximal muscular contractions, they are not able to determine the motor neuronal site primarily responsible for the changes in motor unit discharge rate. Caffeine is often used to improve performance in fatiguing contractions, including those in strength training, and it is known to elicit both supraspinal (Cappelletti, Daria, Sani, & Aromatario, 2015; Nehlig, Daval, & Debry, 1992; Walton, Kalmar, & Cafarelli, 2002) and spinal (i.e. motor neuronal) (Walton, Kalmar, & Cafarelli, 2003) effects. Based on previous evidence, caffeine upregulates PIC amplitudes, partly because it affects noradrenergic and serotonergic drive (Walton et al., 2002) which positively augments PIC amplitudes and raises the resting membrane potential towards threshold (Heckman et al., 2008), and partly because it competes with adenosine on the post-synaptic membrane to increase membrane conductance (Kalmar & Cafarelli, 2004a; Walton et al., 2002). Additionally, caffeine increases the likelihood of self-sustained firing of MNs, which might indicate the presence of PICs (Walton et al., 2002). Thus, caffeine is believed to improve MN excitability at the somato-dendritic end of the MN and might rescue MN excitability, and hence force production, during fatigue. However, one could speculate also that caffeine might activate serotonergic receptors at the axon hillock and thus contribute to the loss of excitability (Cotel et al., 2013). A reasonable hypothesis, therefore, is that caffeine's positive pre-somatic

effects must dominate if spinal excitability increases and the force decline during fatiguing contractions is attenuated, but that its potential post-somatic effects must dominate (or that it had no discernible pre-somatic effect) if fatigue is notable.

1.4 Summary

This review provides evidence that both peripheral and central mechanisms contribute to the loss of force during fatiguing exercise. Regarding peripheral mechanisms, reduced E-C coupling efficiency due to increased metabolite accumulation, as well as reduced sarcoplasmic reticulum Ca^{2+} release and sensitivity of the contractile proteins to Ca^{2+} are likely contributors to the force loss seen during fatigue. Regarding central mechanisms, force loss may be due to reduced supra-spinal drive, spinal excitability and/or impaired MN facilitation processes. However, a paucity of research has investigated these mechanisms during high-intensity yet submaximal activities such as strength training where fatigue is elicited. It is therefore necessary to identify whether these mechanisms contribute to force loss and hence influence fatigue. Such research should improve strategies undertaken by strength coaches and clinicians for influencing strength, power and hypertrophy.

1.5 Aims of the thesis

The major aims of the present thesis are as follows:

Question 1: What is the relative contribution of central vs. peripheral mechanisms to the force loss after an acute bout of repeated fatiguing high-intensity muscular contractions performed with short periods of rest between bouts of contractions?

Hypothesis 1: It was hypothesised that both central and peripheral mechanisms would account for the loss in force following repeated fatiguing high-intensity muscular contractions, but that peripheral mechanisms would take longer to recover than central mechanisms.

Question 2: Does corticospinal excitability and PIC-mediated facilitation of the MN pool decrease following an acute bout of repeated fatiguing, high-intensity muscular contractions decrease?

Hypothesis 2: It was hypothesised that corticospinal excitability and PIC amplitude would be reduced following fatiguing, high-intensity muscular contractions, but that these would recover relatively quickly following the exercise.

Question 3: What are the effects of caffeine ingestion on corticospinal and MN excitability and does the ingestion of caffeine attenuate the loss of force following repeated, high-intensity muscular contractions?

Hypothesis 3: It was hypothesised that the ingestion of caffeine would maintain corticospinal and MN excitability during repeated high-intensity muscular contractions and minimise the loss of maximum force generating capacity.

Methods

The study was conducted in two parts; Experiment 1 and Experiment 2.

2.1.1 Participants

Sixteen resistance trained men volunteered to participate in Experiment 1 (age (mean \pm SD), 25.8 ± 3.6 y; height, 1.71 ± 0.3 m; body mass, 92.3 ± 24.3 kg) whilst seventeen volunteered in Experiment 2 (age, 25.5 ± 3.7 y; height, 1.76 ± 0.06 m; body mass, 86.6 ± 15.8 kg). A total of eight participants volunteered to participate in both experiments. All participants completed a Physical Activity Readiness Questionnaire and a TMS Readiness Questionnaire and reported no lower-limb neuromuscular disorders or contraindications to the safe use of TMS (i.e. no neurological disorders, medical implants or use of medications that alter neuronal activity). The participants volunteered on the basis that they were involved in a lower-body resistance training program for muscular strength and/or power development for at least 12 mo, had no known neurological or cardiovascular disease, and reported a minimal (<200 mg/day) caffeine consumption. They were required to abstain from taking any stimulants or depressants, including caffeine for at least 12 h and alcohol for at least 24 h, prior to testing and to refrain from performing sports or hard exercise training for 24 h prior to each experimental session. Written informed consent was obtained from all participants and all procedures in the study were conducted in accordance with the Declaration of Helsinki and were approved by the Edith Cowan University Human Research Ethics Committee.

Experiment 1: The effect of fatigue and recovery in response to repeated high-intensity muscle contractions on cortico-motoneuronal pathway efficiency.

2.2.1 Study design and overview

Participants visited the laboratory on three separate occasions at the same time of the day separated by a minimum of 72 h. In Session 1, the participants were familiarised with isometric plantar flexion test protocol and electrical nerve and magnetic cortical stimulation procedures. The subsequent two visits (non-caffeine and caffeine conditions) were used to examine the effect of fatiguing, repeated high-intensity muscle contractions on corticospinal excitability and neural drive to the muscle (described below). The exact number and intensity of muscle contractions was performed in the caffeine condition as in the non-caffeine condition, however the participants ingested 3 mg·kg⁻¹ body mass of caffeine 1 h prior to the commencement of the trial in the caffeine condition. Participants' peak isometric strength and neuromuscular function were tested before and immediately, 10 and 20 min after completion of a series of high-intensity, intermittent muscular contractions of the plantar flexors of the right leg (i.e. the fatiguing exercise protocol; see Figure 2.1 below for details). Each experimental session took place at the same time of day.

2.2.2 Non-caffeine condition (Session 2)

Upon arrival, each participant performed a standardised warm-up on a Monark cycle ergometer consisting of 5 min stationary cycling at 60 rpm with a 1-kg resistance. The participant was then seated on an isokinetic dynamometer (Biodex System 3 Pro, Biodex Medical System, Shirley, New York, USA) with the knee fully extended (0°) and ankle in the neutral position (0°; plane of foot relative to tibia) with the sole of foot perpendicular to the

shank and the lateral malleolus of the fibula aligned with the dynamometer's centre of rotation. To minimise movement of the dynamometer system during MVCs, the method of Cannavan, Coleman, and Blazeovich (2012) was used. First, the seat was positioned so that the knee angle was $\sim 30^\circ$ with the leg relaxed, then the leg was straightened so that the chair and dynamometer head were pressed apart, the mechanical compliance of the system reduced, and the leg acted like a strut with the muscles relaxed. Torque values were then expressed relative to the new baseline torque. The participant's upper body, knee and ankle were firmly secured to the dynamometer with straps and the contralateral leg rested on stool to avoid unwanted movements. Visual feedback of torque data was provided by a television monitor placed ~ 2 m in front of participants. All data was recorded synchronously at 2,000 Hz on a personal computer running LabChart software (version 8.1.5, ADInstruments, NSW, Australia) using a 16-bit analogue-to-digital converter (PowerLab 16/35, ADInstruments). Once seated the participant performed five 3-s isometric plantar flexions ranging from 20-100% of perceived maximum effort with 30 s of rest between contractions as a warm-up.

2.2.3 Tibial Nerve Stimulation

Percutaneous tibial nerve stimulation was used to evoke M-waves to assess potential changes in peripheral (muscle) function as well as to normalise both motor-evoked potential (MEP) and electromyography (EMG) measurements, as described below. After the warm-up, the stimulation intensity required to evoke the maximal M-wave amplitude was determined by delivering single 0.2-ms square-wave pulses to the tibial nerve of the right leg using a constant-current stimulator (DS7H, Digitimer Ltd, Welwyn Garden City, UK). The cathode electrode (pick-up area 77 mm²; Unilect 4535M, Ag/AgCl, Unomedical Ltd, Redditch, UK) was positioned over the tibial nerve in the popliteal fossa and the anode electrode (5 × 9 cm; Dura-Stick Plus, DJO Global LLC., Vista, USA) was positioned proximal to the patella. An elastic

band was placed around the knee over the cathode to hold the cathode in a constant position and to apply constant pressure throughout testing sessions. The stimulation site that elicited the greatest soleus (SOL) M-wave response at a submaximal stimulation intensity was located by a hand-held cathode electrode pen (Compex Motor Point Pen, DJO Global LLC., Vista, USA). The stimulation intensity for SOL M_{\max} was determined by increasing stimulator intensity in 5-10 mA increments from a sub-motor threshold intensity, where no evoked response was observed, until the M-wave amplitude plateaued, with 10-s intervals between stimuli while the participants were at rest in the isokinetic dynamometer. The stimulus intensity eliciting resting SOL M_{\max} was then increased by 50% to ensure a supramaximal stimulus intensity (150%) was used and to account for any depression in MN responsiveness during fatigue. The supramaximal stimulus intensity for M_{\max} was held constant throughout the experiment.

2.2.4 Transcranial magnetic stimulation (TMS) procedure

Transcranial magnetic stimulation (TMS) hotspot and test intensities were then determined. Prior to the experiment, each participant was familiarised with the device and completed a TMS safety checklist to screen for any contraindications. Magnetic stimuli were delivered to the contralateral (i.e. left) primary motor cortex by a double-cone coil (110 mm diameter) attached to a Magstim 200² stimulator. The coil was orientated to induce a posterior-anterior current. To find the optimum stimulation site (hotspot), the coil was moved over the motor cortex area contralateral to the plantar flexors of the right leg in 1-cm increments laterally, anteriorly and posteriorly to the vertex using a pre-defined grid marked on a cap worn by participants which was set according to the intersection of lines between the inion and nasion and the left and right ear tragus. The position that elicited the greatest average of three SOL MEPs while evoking minimal tibialis anterior (TA) response (< 50% of SOL MEP) was marked on the cap to ensure that accurate positioning was maintained. A custom-made coil holder and

an experienced investigator maintained coil placement throughout the experiments. Using Parameter Estimation by Sequential Testing (Awiszus, 2003) procedures through the TMS Motor Threshold Assessment Tool software (MTAT 2.0; (Awiszus and Brockhardt, 2011; Silbert et al., 2013a) resting motor threshold (RMT) was determined as the lowest TMS intensity that yielded a peak-to-peak resting SOL MEP amplitude of at least 60 μ V [mean \pm SD; non-caffeine condition (NON-CAF): $55.7 \pm 10.5\%$ of maximal stimulator output (MSO); caffeine condition (CAF): $53.1 \pm 7.4\%$ MSO]. TMS intensity was then set at 120% of RMT (NON-CAF: $62.7 \pm 19.9\%$ MSO; CAF: $59.8 \pm 17.3\%$ MSO) and this intensity was kept constant throughout the sessions (Soto, Valls-Solé, Shanahan, & Rothwell, 2006).

2.2.5 Fatiguing exercise protocol and test procedures

The fatiguing exercise protocol consisted of 6 sets of isometric calf contractions (3 s contraction/2 s rest) reaching a target level of 85% MVC with a 90-s rest between sets. Each set of contractions was completed when the required torque level was not attained on two consecutive repetitions, i.e. each set contained a different number of contractions, with fewer repetitions performed in the latter sets of the protocol. The test protocol was completed 180 s before (PRE) and immediately (POST), 10 min (POST-10) and 20 min (POST-20) after completing the fatiguing exercise protocol. The test protocol consisted of eight resting TMS pulses each separated by 5 s, six 3-s MVCs with superimposed TMS pulses separated by 30 s, and three M_{\max} measurements. Participants were instructed to perform the MVCs with the greatest possible rate of force development, to hold for 3 s (indicated by the experimenter) and then to relax as quickly as possible. Participants received strong verbal encouragement and visual feedback of performance from each MVC completed. Maximal voluntary isometric plantar flexion torque from the six MVCs at each time point were averaged and used as a measure of voluntary force production.

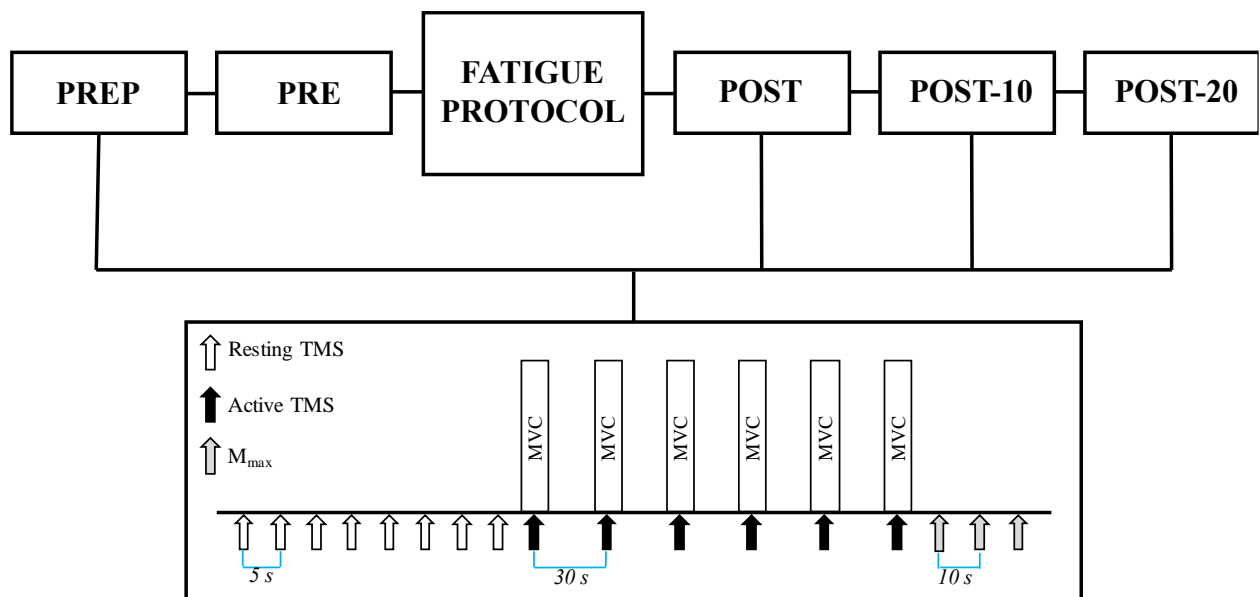


Figure 2.1. Experiment 1 design. Participants completed PREP before being tested before (PRE) and immediately (POST), 10 min (POST-10) and 20 min (POST-20) after the fatiguing contractions. The test protocol consisted of eight resting TMS pulses (white arrows), six active TMS pulses (black arrows) superimposed during MVC (active TMS) and 3 resting M_{max} stimuli (grey arrows).

2.2.6 Measurement of muscle activity (EMG)

Surface EMG was used to record muscle activity as well as evoked responses to TMS (i.e. MEP and cSP) and tibial nerve stimulation (M_{max}). Voluntary EMG was recorded from SOL, medial gastrocnemius (MG) and TA of the right leg using bipolar configurations of two Ag/AgCl self-adhesive electrodes (inter-electrode distance of 2 cm; Blue Sensor N-00-S, 28mm², Ambu, Ballerup, Denmark). SOL EMG location was ~3 cm below the distal head of MG. MG EMG was positioned over the most prominent area of MG when isometrically contracted and TA EMG was placed 1/3 of the distance between the lateral epicondyle of the tibia and the medial malleolus. To obtain clearer evoked responses (i.e. MEP, cSP, and M_{max} data) a pseudo-monopolar EMG configuration was also used over SOL (SOL_{PSU}). The active electrode was positioned just anterior to the SOL bipolar EMG electrodes and the dispersive electrode ~3 cm superior to the medial malleolus and over the Achilles tendon-soleus muscle-tendon junction (Blazevich, et al., 2012). The reference electrodes were positioned on the medial and lateral malleoli of the ankle. The skin beneath the electrodes was shaved, abraded

and cleaned with alcohol to reduce inter-electrode resistance below 5 k Ω . EMG was sampled at a 2000 Hz analogue-digital conversion rate using a Dual BioAmp EMG system (ADInstruments, NSW, Australia) and band-pass filtered (20-500 Hz) using LabChart Software (PowerLab System, ADInstruments, v. 8.1.5, NSW, Australia). SOL, MG and TA muscle activities during plantar flexion were expressed as root mean square EMG amplitudes (500-ms averaging window) and SOL and MG normalised to M_{\max} amplitude (EMG/M) (Kalmar & Cafarelli, 2004b). In addition, both SOL/M and MG/M were averaged to obtain a global measure of triceps surae EMG (TS/M) (Trajano, Seitz, Nosaka, & Blazevich, 2013). To measure MEP and M_{\max} amplitudes, EMG data remained unfiltered and the peak-to-peak amplitude was used. The TMS-induced EMG cortical silent period (cSP) duration was determined by visual inspection of the SOL_{PSU} EMG records and measured from the onset of the MEP to the return of voluntary EMG activity after the silent period (Damron, Dearth, Hoffman, & Clark, 2008; Säisänen et al., 2008). Changes in MEP/M ratio and cSP were used to assess corticospinal excitability and intracortical inhibition, respectively.

2.2.7 Caffeine condition (Session 3)

To ensure full recovery of force production in the plantar flexors after Session 2, Session 3 took place >72 h after Session 2. This session was completed identically to Session 2 with the exception that participants ingested 3 mg·kg⁻¹ (Walton et al., 2003; Walton et al., 2002) of U.S.P grade caffeine in tablet form (No-Doz, Key Pharmaceuticals Pty Ltd, Macquarie Park, NSW) 1 h prior to the commencement of the experiment (Graham, Rush, & Soeren, 1994). This allowed the testing of the effect of caffeine ingestion on fatigue-induced reductions in corticospinal and MN excitability and force production. During the exercise protocol, the intensity and number of repetitions attained in Session 2 were repeated exactly by providing a visual target of the plantar flexor torque level to be obtained on a repetition-by-repetition basis.

As caffeine has the potential to reduce the magnitude and rate of fatigue, and potentially increase the rate of recovery, the caffeine condition was always completed after the non-caffeine condition to ensure that participants were able to precisely repeat the required number and intensity of contractions. This ensured that direct comparisons of physiological changes associated with fatigue could be made between the non-caffeine and caffeine sessions. Blinding of condition was not considered to be necessary since the aim of the trial was not to assess the possible ergogenic effects of caffeine, but to use it as a pharmacological intervention to further probe potential physiological changes underpinning fatigue. Finally, chances of an order effect were minimised by allowing the participant to practice the plantar flexion task during the familiarisation session.

Experiment 2 – The effect of fatigue and recovery during repeated high-intensity muscle contractions on MN facilitation (PIC amplitude) and Ca^{2+} kinetics.

2.3.1 Study design and overview

Experiment 2 was conducted using the same experimental design as in Experiment 1. Participants were familiarised in Session 1, tested without caffeine in Session 2 and then tested after caffeine ingestion in Session 3, each at the same time of the day and separated by a minimum of 72 h.

2.3.2 Non-caffeine condition (Session 2)

Upon arrival, each participant performed a cycle warm-up as done in Experiment 1 before being seated on an isokinetic dynamometer. The stimulation intensity required to evoke the maximal M-wave amplitude was then determined (see above for M_{\max} set-up). Once seated the participant performed five 3-s isometric plantar flexions ranging from 20-100% of perceived maximum effort with 30 s of rest between contractions, followed by two additional MVCs. The best score from the three MVCs was recorded and used to attain the stimulation intensities for subsequent PIC and Ca^{2+} testing (see Figure 2.3 below for experimental design).

2.3.3 Muscle stimulation procedures (20:80 Hz torque ratio and torque response to constant vs. variable frequency 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 (5×9 cm; Dura-Stick Plus, DJO Global LLC., Vista, 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. Two elastic bands were placed around the calf, one over each electrode, which prevented the electrodes from falling off and applied a constant pressure throughout testing sessions.

For all tetanic stimulations, the intensity necessary to reach 50% of MVC with a 0.5-s 80-Hz tetanic stimulation was used (Martin et al., 2004). Three tetanic stimulations of the same duration (0.5 s) were delivered to test for excitation-contraction (E-C) coupling efficiency: 1) 20 Hz train; 2) variable frequency “catch inducing” train (i.e. 20 Hz train with the first two pulses at 100 Hz; VFT); and 3) 80 Hz train. The peak torque produced by the 20-Hz and 80-Hz stimulations were used to calculate the 20:80 Hz torque ratio, which was used as a measure of E-C coupling efficiency (Martin et al., 2004). The ratio of the torques evoked by the variable vs. constant frequency trains was used to estimate the muscle’s capacity to utilise a high-frequency double discharge at contraction onset, which is hypothesised to be strongly affected by Ca^{2+} sensitivity of the muscle (Binder-Macleod & Kesar, 2005; Burke et al., 1970).

2.2.4 Motor neurone facilitation (tendon vibration superimposed with neuromuscular electrical stimulation)

A combined tendon vibration plus muscle stimulation (VIB+STIM) technique was used to estimate PIC magnitude (Trajano et al., 2014). With the participant’s ankle in a slightly dorsiflexed position (-10°), the Achilles tendon of the right leg was mechanically vibrated at 115 Hz (1-mm deflections) by a hand-held vibrator (Vibrasens, Techno Concepts, Mane, France). The vibrator was held with steady pressure against a marked point in line with the medial malleolus on the Achilles tendon for 33 s. After the first 10 s of vibration, five 2-s bursts of neuromuscular electrical stimulation at a frequency of 20 Hz were superimposed on the ongoing tendon vibration at 4-s intervals (see Figure 2.2). The experimental setup for muscle

stimulation was the same as that used to measure E-C coupling efficiency (see Muscle stimulation procedures above). For all electrical stimulations, an intensity that elicited a torque response of 20% MVC with a 0.5-s 20 Hz tetanic stimulation was used. During the VIB+STIM protocol, participants were instructed to hold onto the shoulder straps and not voluntarily activate the plantar- or dorsi-flexor muscles. The left leg was positioned on a stool so that both legs were straight which prevented any extraneous movement throughout the test. To further reduce the chances of voluntary calf activation, participants were asked to count in two's from zero (i.e. 0, 2, 4, 6, etc.) at a self-selected pace throughout the VIB+STIM protocol.

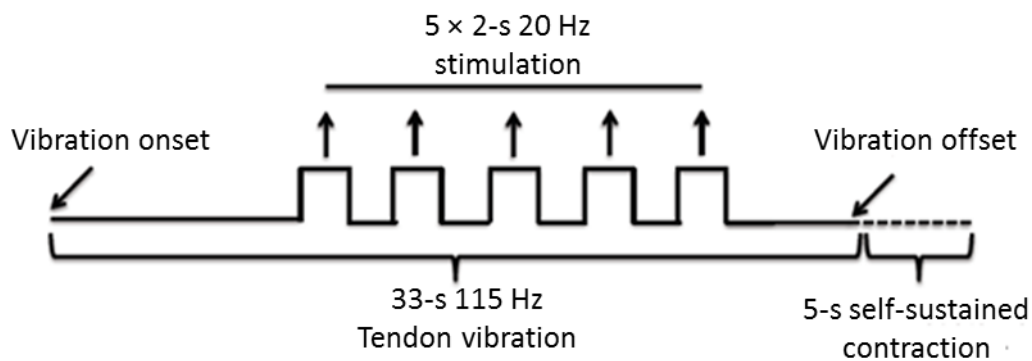


Figure 2.2. Schematic representation of the tendon vibration and superimposed tibial nerve stimulation protocol used to elicit reflexive muscular contractions.

2.2.5 Test procedures

Electrically evoked torque (20 Hz, VFT and 80 Hz) and VIB+STIM measurements were performed 7 min (within-day control; CON) and 2 min (PRE) prior to completion of the same fatiguing exercise protocol performed in Experiment 1 (see Fatiguing exercise protocol and test procedures above). Upon completion of the exercise protocol, electrically-evoked torque and VIB+STIM measurements were performed followed by one MVC and one M_{\max} measurement. These were again repeated 10 min (POST-10) and 20 min (POST-20) after the fatiguing exercise protocol.

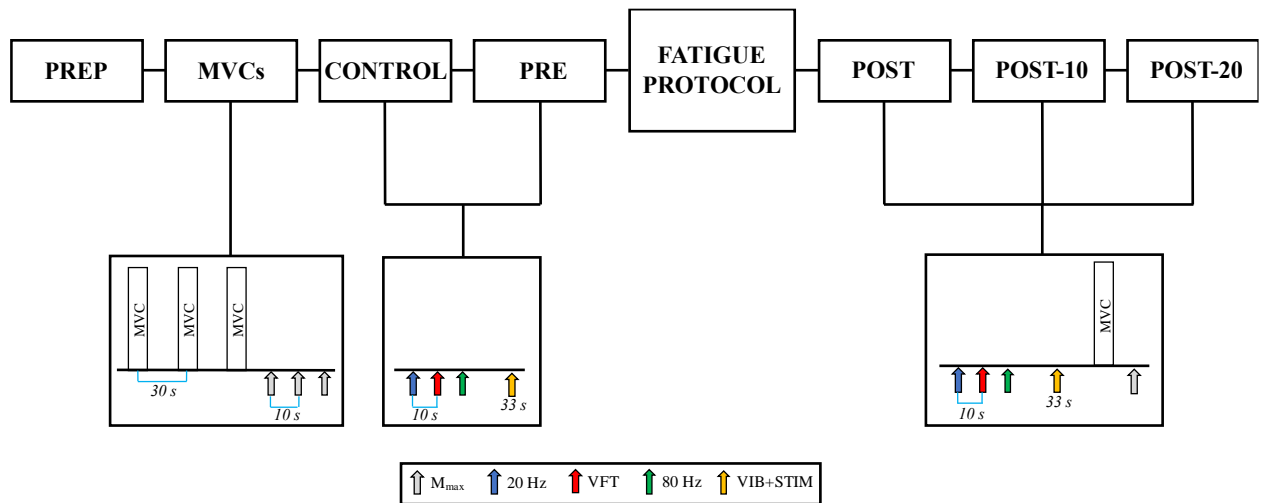


Figure 2.3. Experiment 2 design. Participants completed PREP, followed by three MVCs and M-waves, Control and PRE. Upon completion of the fatiguing exercise protocol, the participants were tested at POST, POST-10 and POST-20. The test protocol at Control and PRE consisted 20 Hz (blue arrow), VFT (red arrow), 80 Hz (green arrow) stimulations and VIB+STIM (yellow arrow). The POST test protocol consisted of 20 Hz, VFT, 80 Hz stimulations, VIB+STIM, one MVC and one M_{\max} measurement.

2.2.6 Voluntary and evoked torque measurements

Maximal voluntary isometric plantar flexion torque was used as a measure of voluntary force production. Evoked torque from the 20 Hz, 80 Hz and VFTs were analysed as the peak torque attained from each trial. Evoked torque from the VIB+STIM protocol (see Figure 2.4) was analysed as the mean torque in a 1-s window at two different time points during the protocol: (1) during vibration starting 500 ms after completion of the 5th (last) burst of tibial nerve stimulation (torque during vibration; T_{vib}), and (2) 500 ms after the vibration was ceased (sustained torque; T_{sust}). As plantar flexor muscles impose a small passive torque even when the muscle is relaxed, T_{vib} and T_{sust} torque values were normalised and presented as changes from the baseline (resting) value.

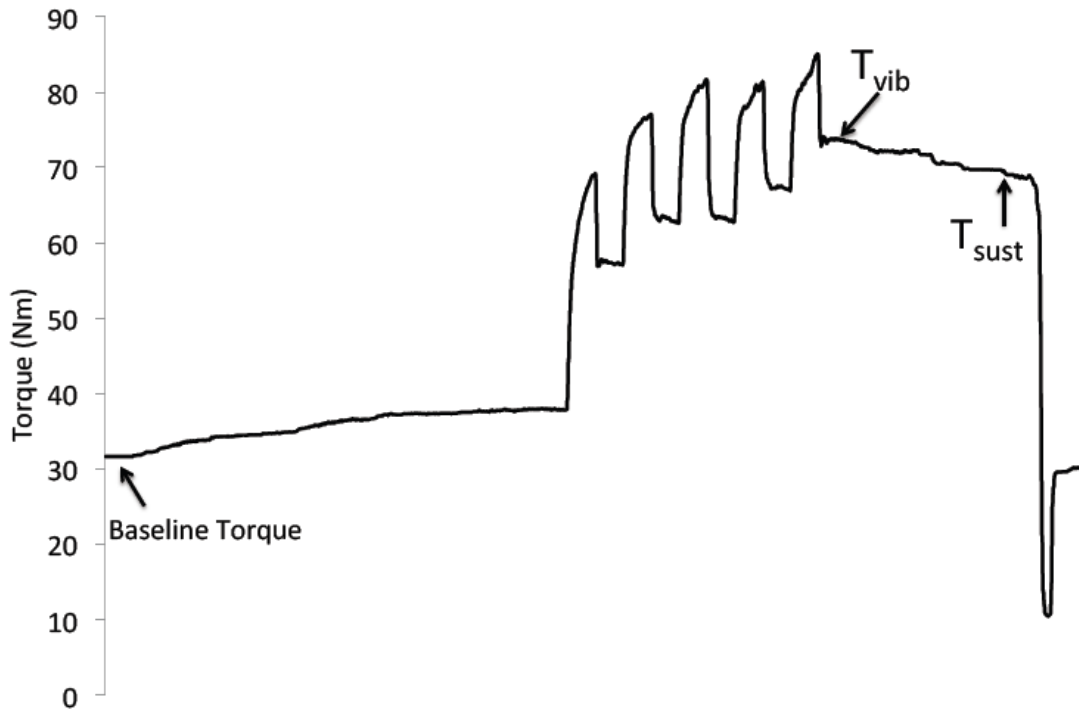


Figure 2.4. Example of the torque elicited during the combined tendon vibration plus tibial nerve stimulation protocol and time points at which torque is recorded. T_{vib} , torque is measured after the 5th (last) bout of electrical stimulation; T_{sust} , torque is measured 500 ms after vibration cessation (self-sustained torque).

2.2.7 Measurement of muscle activity (EMG)

EMG setup, recording and analysis was the same as Experiment 1 (see above for details).

2.2.8 Caffeine condition (Session 3)

Session 3 took place >72 h after Session 2. This session was completed identically to Session 2 with the exception that participants ingested $3 \text{ mg} \cdot \text{kg}^{-1}$ (see Experiment 1; Session 3 above for details).

2.2.9 Statistical analysis

Prior to analysis, the data were assessed for normality of distribution, homogeneity of variance (Levene's test) and sphericity (Mauchly's test). In Experiment 1, a two-way analysis

of variance (ANOVA) with repeated measures was used to compare changes in voluntary torque, MEP, MEP/M ratio cSP duration M_{\max} and TA variables between non-caffeine (NON-CAF) and caffeine (CAF) conditions and over time (PRE, POST, POST-10 and POST-20). A two-way repeated measures multivariate ANOVA (MANOVA) was performed to compare changes in SOL/M, MG/M, and TS/M between conditions and over time. Coefficient of variation (CV) was calculated to determine the variability in voluntary torque production over time and between conditions.

In Experiment 2, a two-way ANOVA with repeated measures was used to compare changes in voluntary torque, evoked torque from 20 Hz, 80 Hz and VFTs, 20:80 Hz and 20:VFT ratios, T_{vib} , T_{sust} , M_{\max} and TA variables between conditions (NON-CAF vs. CAF) and over time (PRE, POST, POST-10 and POST-20). A two-way repeated measures MANOVA was performed to compare changes in SOL/M, MG/M, and TS/M between conditions and over time. In cases where Mauchly's Test of Sphericity was violated, Greenhouse-Geisser corrections were used. When statistical differences were observed, post-hoc tests were used as appropriate (see Results) to determine the location of the differences. Pearson's product-moment correlations were calculated to determine relationships between changes in torque, EMG, MEPs and cSP immediately and 10 min after exercise in Experiment 1, and changes in torque, EMG, evoked torque and VIB+STIM measurements in Experiment 2. Statistical analysis was performed using SPSS version 24.0 (SPSS Inc., Chicago IL, USA). All data are reported as mean \pm SD in text (and mean \pm SE in graphs), confidence intervals (95%) and Hedges' g effect sizes. Statistical significance was accepted at an α level of 0.05.

Results

Experiment 1: The effect of fatigue and recovery in response to repeated high-intensity muscle contractions on cortico-motoneuronal pathway efficiency.

Example data traces for one participant in both the non-caffeine and caffeine conditions are presented in Figures 3.1 and 3.2, respectively.

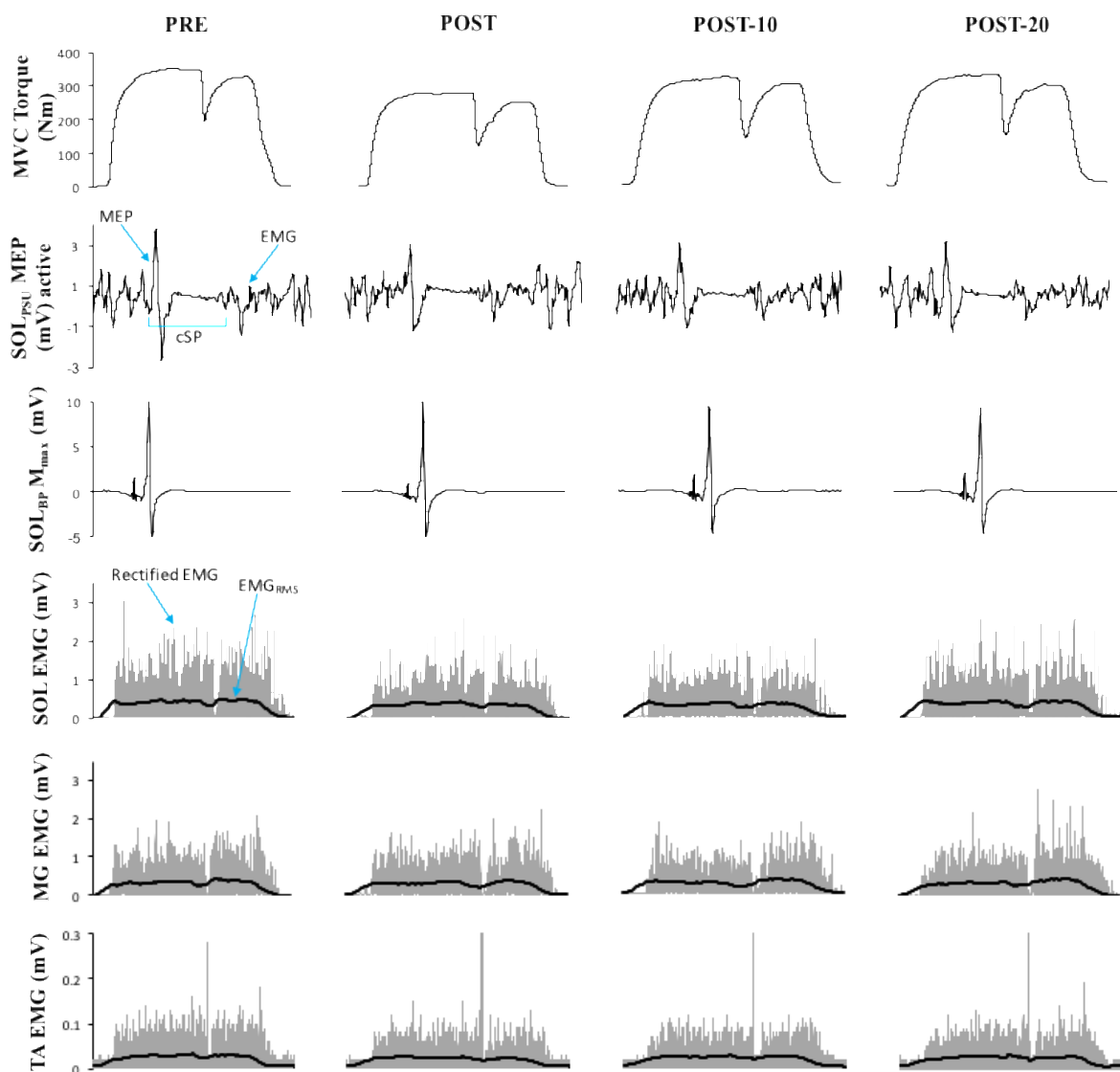


Figure 3.1. Traces of raw data from Experiment 1. Example data obtained from a single participant in the non-caffeine session immediately before (PRE) and immediately (POST), 10 min (POST-10) and 20 min (POST-20) after the exercise protocol. Decreases in MVC torque (first row), MEP amplitude (second row) and SOL_{RMS} are noticeable immediately after the exercise protocol.

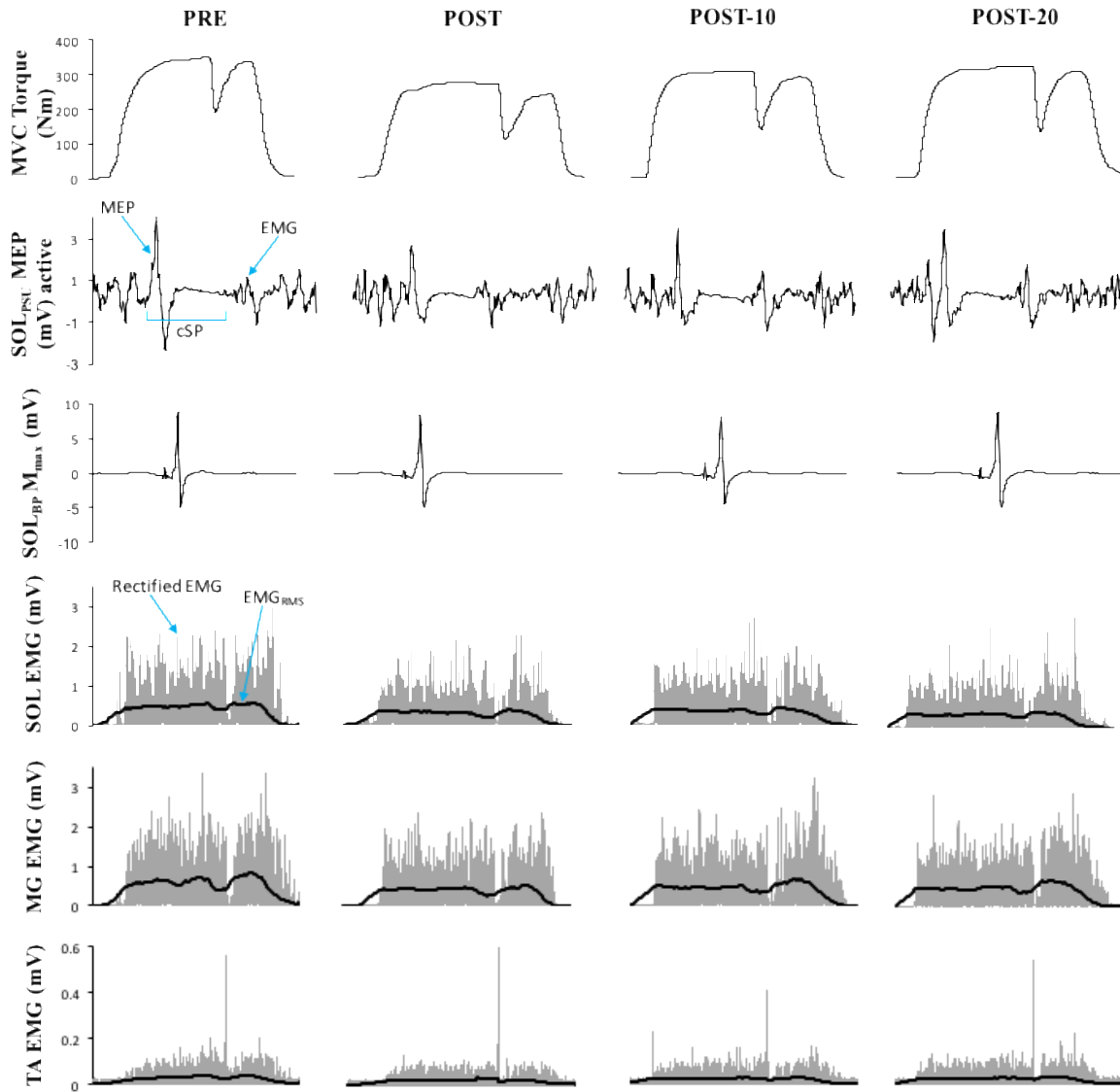


Figure 3.2. Traces of raw data from Experiment 1. Example data obtained from a single participant in the caffeine session immediately before (PRE) and immediately (POST), 10 min (POST-10) and 20 min (POST-20) after the exercise protocol. Decreases in MVC torque (first row), MEP amplitude (second row) and SOL_{RMS} are noticeable immediately after the exercise protocol.

3.1.1 Torque

Significant main effects of time ($F_{(3,15)} = 34.391$, $p = 0.000$; see Figure 3.3) and condition ($F_{(1,15)} = 10.843$, $p = 0.005$; see Figure 3.4) were observed for maximal plantar flexion torque, however there was no condition-by-time interaction effect ($F_{(2.655,15)} = 1.489$, $p = 0.235$). With respect to the time effect, pooled data (pooled between caffeine and non-caffeine conditions) for torque decreased immediately POST ($-12.2 \pm 6.5\%$ (CI = -22, -3%), $p < 0.001$,

$g = 0.642$) and did not recover by POST-10 ($-6.3 \pm 6.1\%$ (-16, 4%), $p < 0.001$, $g = 0.315$) or POST-20 ($-4.8 \pm 6.5\%$ (-15, 5%), $p = 0.002$, $g = 0.239$) relative to before exercise (PRE). Follow-up repeated measures ANOVAs (RM ANOVAs) examining the changes in each condition (caffeine and non-caffeine conditions separate) revealed a decrease in torque at POST in both NON-CAF ($-12.7 \pm 4.6\%$ (-26, 0%), $p < 0.001$, $g = 0.697$) and CAF ($-11.7 \pm 7.9\%$ (-26, 2%), $p < 0.001$, $g = 0.585$) conditions compared with PRE. At POST-10, torque was still reduced in both NON-CAF ($-7.8 \pm 4.9\%$ (-22, 6%), $p < 0.001$, $g = 0.394$) and CAF ($-4.8 \pm 6.8\%$ (-19, 10%), $p = 0.013$, $g = 0.238$). Torque did not return to baseline within the 20-min recovery period in either NON-CAF ($-6.2 \pm 6.7\%$, (-20, 8%), $p = 0.002$, $g = 0.298$) or CAF ($-3.3 \pm 6.1\%$ (-18, 11%), $p = 0.044$, $g = 0.179$).

With respect to the condition effect, Post-hoc analyses indicated that the between-condition effect (with a greater torque production observed in CAF than NON-CAF) resulted from significant differences at POST-10 ($6.9 \pm 7.4\%$ greater in CAF compared to NON-CAF (-9, 22%), $p = 0.003$, $g = 0.309$) and POST-20 ($6.8 \pm 7.6\%$ greater in CAF compared to NON-CAF (-9, 21%), $p = 0.004$, $g = 0.287$) with no statistical difference being observed at PRE ($3.4 \pm 6.2\%$ greater in CAF compared to NON-CAF (-11, 18%), $p = 0.6$, $g = 0.171$) or POST ($4.7 \pm 9.5\%$ greater in CAF compared to NON-CAF (-10, 20%), $p = 0.092$, $g = 0.287$).

Finally, CV for torque did not vary over time ($F_{(1.686,15)} = 0.825$, $p = 0.431$) or between conditions ($F_{(1,15)} = 0.752$, $p = 0.545$). The number of repetitions performed throughout the exercise protocol did not statistically differ between conditions ($p = 0.908$). Of the 16 participants, 9 replicated exactly the number of reps performed in the non-caffeine session, 4 missed by one rep, 2 missed by two reps and 1 missed by six reps.

3.1.2 Muscle activity

SOL/M, MG/M, TS/M and TA EMG measured at PRE, POST, POST-10 and POST-20 are presented in Table 3.1. In SOL/M, a significant main effect of time ($F_{(3,15)} = 6.233$, $p = 0.001$) was detected but no condition ($F_{(1,15)} = 2.670$, $p = 0.123$) or condition-by-time interaction effects ($F_{(3,15)} = 0.088$, $p = 0.966$) were observed. With respect to the time effect, SOL/M decreased immediately POST ($-7.6 \pm 14.9\%$ (-38, 22%), $p = 0.003$, $g = 0.186$) but recovered by 10 min ($-3.1 \pm 18.6\%$ (-25, 32%), $p = 0.599$, $g = -0.049$).

In MG/M, significant main effects of time ($F_{(3,15)} = 8.086$, $p < 0.001$) and condition ($F_{(1,15)} = 7.581$, $p = 0.015$) were observed, however there was no condition-by-time interaction effect ($F_{(3,15)} = 1.628$, $p = 0.196$). With respect to the time effect, a non-significant decrease of $-3.9 \pm 14.3\%$ (-25, 14%, $p = 0.076$, $g = 0.205$) was observed which recovered by 10 min ($4.4 \pm 13.6\%$ (-17, 22%), $p = 0.223$, $g = -0.135$). Follow-up RM ANOVAs examining the changes in each condition revealed no effect of time on MG/M in NON-CAF ($F_{(3,15)} = 1.615$, $p = 0.199$), however MG/M decreased in CAF at POST ($-6.6 \pm 9.3\%$ (-27, 9%), $p = 0.013$, $g = 0.326$) but recovered by POST-10 ($4.1 \pm 11.3\%$ (-15, 24%), $p = 0.237$, $g = -0.129$).

With respect to the condition effect, Post-hoc analyses indicated that the between-condition effect (greater MG/M observed in CAF than NON-CAF) resulted from significant differences at PRE ($22.0 \pm 33.8\%$ greater in CAF compared to NON-CAF (-3, 36%), $p = 0.02$, $g = 0.607$), POST-10 ($22.1 \pm 32.9\%$ greater in CAF compared to NON-CAF (-3, 34%), $p = 0.024$, $g = 0.559$) and POST-20 ($26.3 \pm 29.9\%$ greater in CAF compared to NON-CAF (-3, 40%), $p = 0.002$, $g = 0.821$), with no statistical difference being observed at POST ($17.1 \pm 33.0\%$ greater in CAF compared to NON-CAF (-9, 31%), $p = 0.111$, $g = 0.415$).

In TS/M, significant main effects of time ($F_{(3,15)} = 9.102$, $p < 0.001$; Figure 3.3) and condition ($F_{(1,15)} = 15.761$, $p = 0.001$; Figure 3.4) were observed, however there was no condition-by-time interaction effect ($F_{(3,15)} = 1.628$, $p = 0.196$). With respect to the time effect,

TS/M decreased immediately POST ($-5.8 \pm 12.6\%$ (-30, 15%), $p = 0.024$, $g = 0.243$) but recovered by 10 min ($-3.6 \pm 13.2\%$ (-16, 22%), $p = 0.242$, $g = -0.108$). Follow-up RM ANOVAs examining the changes in each condition revealed no effect of time on TS/M in NON-CAF ($F_{(1.907,15)} = 2.832$ $p = 0.078$), however TS/M decreased in CAF at POST ($-7.4 \pm 12.1\%$ (-25, 8%), $p = 0.033$, $g = 0.339$) and then recovered by POST-10 ($3.0 \pm 11.1\%$ (-15, 19%), $p = 0.428$, $g = 0.092$). No correlations were observed between changes in EMG/M variables and changes in torque.

With respect to the condition effect, post-hoc analyses indicated that the between-condition effect (greater TS/M observed in CAF than NON-CAF) resulted from significant differences at PRE ($15.1 \pm 20.2\%$ greater in CAF compared to NON-CAF (-6, 33%), $p = 0.011$, $g = 0.474$), POST ($11.3 \pm 20.5\%$ greater in CAF compared to NON-CAF (-12, 30%), $p = 0.048$, $g = 0.302$), POST-10 ($13.8 \pm 17.3\%$ greater in CAF compared to NON-CAF (-8, 30%), $p = 0.003$, $g = 0.435$) and POST-20 ($16.6 \pm 16.8\%$ greater in CAF compared to NON-CAF (-5, 34%), $p = 0.001$, $g = 0.548$).

In TA, no significant main effect of time ($F_{(1.746,15)} = 1.142$, $p = 0.328$), condition ($F_{(1,15)} = 0.033$, $p = 0.858$) or condition-by-time interaction effect ($F_{(1.630,15)} = 0.471$, $p = 0.591$) was observed.

Table 3.1. Plantar flexor torque, EMG, and TMS data measured in Experiment 1.

| | PRE | POST | POST-10 | POST-20 |
|---|----------------|------------------|------------------|------------------|
| Torque (Nm) | 218.3 ± 43.5 | 191.5 ± 38.9** | 204.6 ± 42.8** | 207.9 ± 43.0* |
| SOL/M | 0.031 ± 0.013 | 0.029 ± 0.013* | 0.032 ± 0.012 | 0.032 ± 0.012 |
| MG/M | 0.036 ± 0.010 | 0.034 ± 0.009 | 0.038 ± 0.010 | 0.038 ± 0.010* |
| TS/M | 0.068 ± 0.018 | 0.063 ± 0.017* | 0.070 ± 0.018 | 0.070 ± 0.018 |
| TA (mV) | 0.110 ± 0.041 | 0.101 ± 0.045 | 0.105 ± 0.045 | 0.095 ± 0.040 |
| MEP (mV) | 4.822 ± 1.553 | 4.272 ± 1.473* | 4.455 ± 1.445* | 4.300 ± 1.356* |
| MEP_{REST} (mV) | 1.081 ± 0.588 | 1.308 ± 0.775 | 1.177 ± 0.743 | 1.210 ± 0.796 |
| MEP/M | 0.233 ± 0.089 | 0.208 ± 0.084* | 0.224 ± 0.079 | 0.219 ± 0.080 |
| MEP_{REST}/M | 0.053 ± 0.029 | 0.066 ± 0.040 | 0.060 ± 0.036 | 0.063 ± 0.040 |
| cSP (ms) | 105.5 ± 16.4 | 100.4 ± 12.7* | 98.8 ± 14.0* | 98.7 ± 12.7* |
| SOL_{BP} M_{max} (mV) | 6.626 ± 3.011 | 6.206 ± 2.913* | 5.857 ± 2.782** | 5.885 ± 2.773** |
| MG M_{max} (mV) | 12.866 ± 3.392 | 11.883 ± 3.352** | 11.182 ± 3.324** | 11.098 ± 3.291** |
| SOL_{PSU} M_{max} (mV) | 21.541 ± 5.058 | 21.261 ± 4.817 | 20.388 ± 4.348* | 20.332 ± 4.495* |

Values are mean ± SD. Torque, mean torque during maximal voluntary isometric plantarflexion; SOL/M, MG/M, TS/M, soleus, medial gastrocnemius and triceps surae (SOL + MG) root-mean square EMG normalised to maximal M-wave amplitude; TA, tibialis anterior root-mean square EMG; MEP, motor-evoked potential amplitude during MVC; MEP_{REST}, motor-evoked potential amplitude during rest; MEP/M, motor-evoked potential amplitude during MVC normalised to M_{max}; MEP_{REST}/M, motor-evoked potential amplitude during rest normalised to M_{max}; SOL_{BP} M_{max}, soleus bipolar EMG; MG M_{max}, medial gastrocnemius; SOL_{PSU} M_{max}, soleus pseudo-monopolar EMG. *significantly different from PRE, p < 0.05, ** p < 0.001.

3.1.3 MEP amplitude

Active MEP amplitude (MEP) measured at PRE, POST, POST-10 and POST-20 are presented in Table 3.1. With respect to MEP, a significant main effect of time ($F_{(2,078,15)} = 4.257$, $p = 0.022$) was detected, but no condition ($F_{(1,15)} = 2.684$, $p = 0.122$) or condition-by-time interaction effects ($F_{(2,502,15)} = 0.223$, $p = 0.847$) were observed. With respect to the time effect, MEP decreased immediately POST ($-9.2 \pm 22\%$ ($-27, 4\%$), $p = 0.026$, $g = 0.360$) and did not recover by POST-10 ($-6.1 \pm 17\%$ ($-23, 8\%$), $p = 0.03$, $g = 0.242$) or POST-20 ($-9.3 \pm 16\%$ ($-26, 4\%$), $p = 0.003$, $g = 0.354$) relative to PRE.

3.1.4 MEP/M amplitude

MEP/M amplitudes measured at PRE, POST, POST-10 and POST-20 are presented in Table 3.1 and changes are shown in Figure 3.3. A significant main effect of time on MEP/M amplitude (MEP/M) ($F_{(3,15)} = 4.339$, $p = 0.009$) was detected, but no condition ($F_{(1,15)} = 0.563$, $p = 0.465$) or condition-by-time interaction effects ($F_{(3,15)} = 0.077$, $p = 0.972$) were observed. With respect to the time effect, MEP/M decreased immediately POST ($-8.8 \pm 20.8\%$ (-29, 8%), $p = 0.01$, $g = 0.281$) but recovered by 10 min ($-0.9 \pm 19.6\%$ (-22, 14%), $p = 0.184$, $g = 0.108$).

3.1.5 Resting MEP amplitude

Resting MEP (MEP_{rest}) and MEP_{rest}/M amplitudes measured at PRE, POST, POST-10, & POST-20 are presented in Table 3.1. In MEP_{rest} , no significant main effect of time ($F_{(1.881,15)} = 1.491$, $p = 0.232$), condition ($F_{(1,15)} = 0.32$, $p = 0.860$) or condition-by-time interaction effect ($F_{(1.938,15)} = 0.909$, $p = 0.413$) was detected. In MEP_{rest}/M , no significant main effect of time ($F_{(3,15)} = 1.807$, $p = 0.182$), condition ($F_{(1,15)} = 0.222$, $p = 0.645$), or condition-by-time interaction effect ($F_{(2.028,15)} = 1.356$, $p = 0.275$) was detected.

3.1.6 Cortical silent period

A significant main effect of time ($F_{(2.204,15)} = 7.189$, $p = 0.002$) was detected (Figure 3.3), but no condition ($F_{(1,15)} = 1.181$, $p = 0.294$) or condition-by-time interaction effects ($F_{(2.325,15)} = 1.930$, $p = 0.155$) were observed. With respect to the time effect, cortical silent period (cSP) decreased immediately POST ($-3.8 \pm 10.2\%$ (-11, 2%), $p = 0.016$, $g = 0.341$) and remained reduced at POST-10 ($-5.7 \pm 9.2\%$ (-13, 1%), $p = 0.004$, $g = 0.433$) and POST-20 ($-5.6 \pm 9.6\%$ (-13, 1%), $p = 0.004$, $g = 0.456$). No correlations were observed between changes in TMS variables and changes in torque.

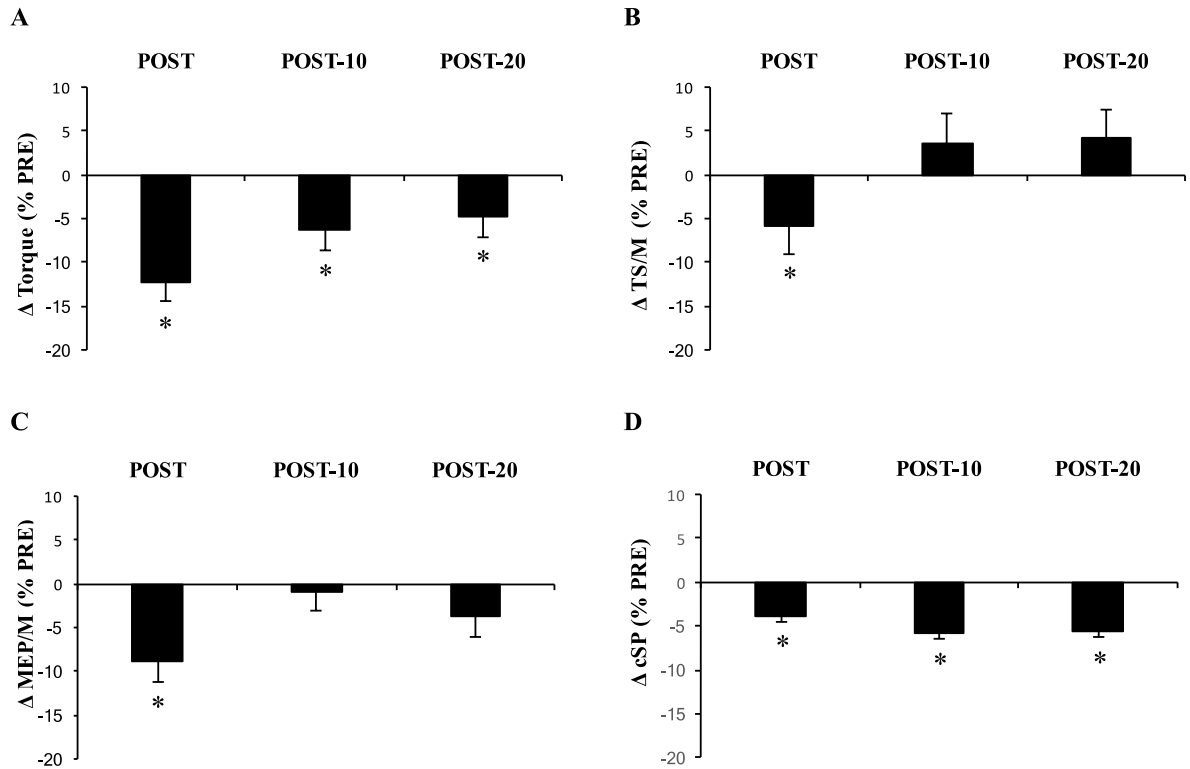


Figure 3.3. Changes in torque, EMG, MEP/M and cSP measured from PRE to immediately (POST), 10 min (POST-10) and 20 min (POST-20) after exercise. Changes in A) MVC torque, B) triceps surae EMG normalised to M-wave amplitude (EMG/M), C) motor-evoked potential amplitude normalised to M-wave amplitude (MEP/M), and D) cortical silent period (cSP). * significantly different from PRE, $p < 0.05$.

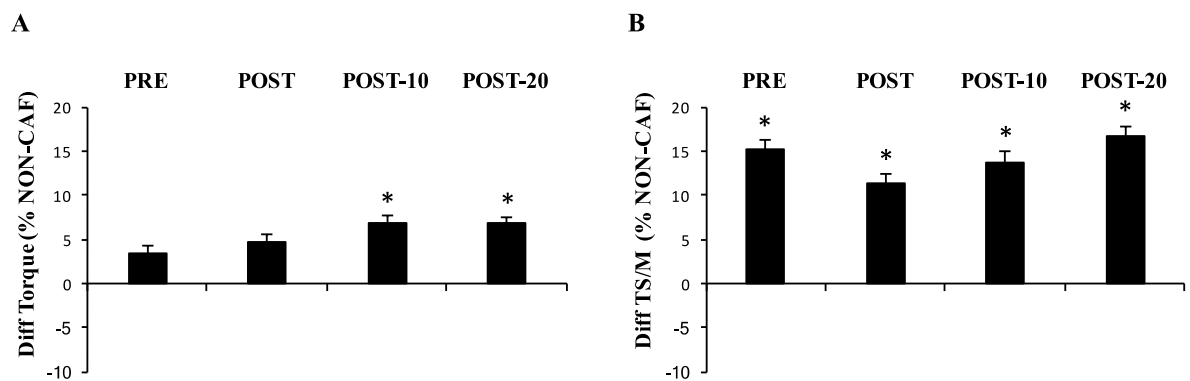


Figure 3.4. Differences between non-caffeine (NON-CAF) and caffeine (CAF) conditions for torque and EMG measured before exercise (PRE) and immediately (POST), 10 min (POST-10) and 20 min (POST-20) after exercise. Differences in A) MVC torque, and B) triceps surae EMG normalised to M-wave amplitude (TS/M). Torque production and EMG were greater in CAF compared to NON-CAF. No effect of caffeine was observed in MEP/M or cSP. * significantly different from NON-CAF, $p < 0.05$.

3.1.7 M-wave amplitude

Significant main effects of time ($F_{(3,15)} = 18.919$, $p < 0.001$) and condition ($F_{(1,15)} = 12.772$, $p = 0.003$) were observed in SOL M_{\max} , however there was no condition-by-time interaction effect ($F_{(3,15)} = 0.250$, $p = 0.861$). With respect to the time effect, SOL M_{\max} decreased immediately POST ($-6.7 \pm 9.6\%$ (-29, 16%), $p = 0.01$, $g = 0.140$) and remained depressed at 10 min ($-12.3 \pm 9.3\%$ (-33, 10%), $p < 0.001$, $g = 0.262$) and 20 min ($-12 \pm 8.3\%$ (-33, 11%), $p < 0.001$, $g = 0.253$). Follow-up RM ANOVAs examining the changes in each condition revealed a decrease in SOL M_{\max} at POST in NON-CAF ($-6.7 \pm 9.6\%$ (-38, 25%), $p = 0.014$, $g = 0.147$) that remained depressed at POST-10 ($-12.3 \pm 9.3\%$ (-43, 18%), $p < 0.001$, $g = 0.279$) and POST-20 ($-12.0 \pm 8.3\%$ (-42, 18%), $p < 0.001$, $g = 0.279$). In CAF, no statistical change in SOL M_{\max} was detected at POST ($-5.0 \pm 10.9\%$ (-38, 26%), $p = 0.063$, $g = 0.147$), however SOL M_{\max} was depressed at POST-10 ($-10.4 \pm 10.0\%$ (-43, 21%), $p = 0.001$, $g = 0.243$) and POST-20 ($-9.6 \pm 8.5\%$ (-42, 22%), $p = 0.001$, $g = 0.224$).

With respect to the condition effect, post-hoc analyses indicated that the between-condition effect (smaller SOL M_{\max} observed in CAF than NON-CAF) resulted from significant differences at PRE ($12.5 \pm 17.0\%$ less in CAF compared to NON-CAF (-44, 16%), $p = 0.004$, $g = 0.322$), POST ($10.5 \pm 18.6\%$ less in CAF compared to NON-CAF (-45, 18%), $p = 0.025$, $g = 0.300$), POST-10 ($11.2 \pm 12.4\%$ less in CAF compared to NON-CAF (-44, 19%), $p = 0.003$, $g = 0.280$) and POST-20 ($11.3 \pm 9.7\%$ less in CAF compared to NON-CAF (-44, 20%), $p < 0.001$, $g = 0.265$).

In MG M_{\max} , a significant main effect of time ($F_{(1,698,15)} = 53.563$, $p < 0.001$) was detected, but no condition $F_{(1,15)} = 0.021$, $p = 0.887$) or condition-by-time interaction effects ($F_{(1,15)} = 4.562$, $p = 0.019$) were observed. With respect to the time effect, MG M_{\max} decreased immediately POST ($-8 \pm 4.7\%$ (-21, 5%), $p < 0.001$, $g = 0.288$) and remained depressed at

10 min ($-13.6 \pm 7.2\%$ (-26, 0%), $p < 0.001$, $g = 0.495$) and 20 min ($-14.4 \pm 7.5\%$ (-27, -1%), $p < 0.001$, $g = 0.523$) after exercise.

In SOL PSU M_{\max} , a significant main effect of time ($F_{(1.768,15)} = 6.809$, $p = 0.005$) was detected, but no condition $F_{(1,15)} = 0.402$, $p = 0.536$) or condition-by-time interaction effects ($F_{(1.469,15)} = 1.718$, $p = 0.206$) were observed. With respect to the time effect, SOL PSU M_{\max} decreased immediately POST ($-1.1 \pm 9.3\%$ (-13, 10%), $p = 0.01$, $g = 0.056$) and remained depressed at 10 min ($-4.6 \pm 7.8\%$ (-16, 6%), $p < 0.001$, $g = 0.242$) and 20 min ($-4.9 \pm 7.6\%$ (-17, 5%), $p < 0.001$, $g = 0.250$) after exercise.

Experiment 2 – The effect of fatigue and recovery during repeated high-intensity muscle contractions on MN facilitation (PIC amplitude) and Ca^{2+} kinetics.

Example data traces for one participant in both the non-caffeine and caffeine conditions are presented in Figures 3.5 and 3.6, respectively.

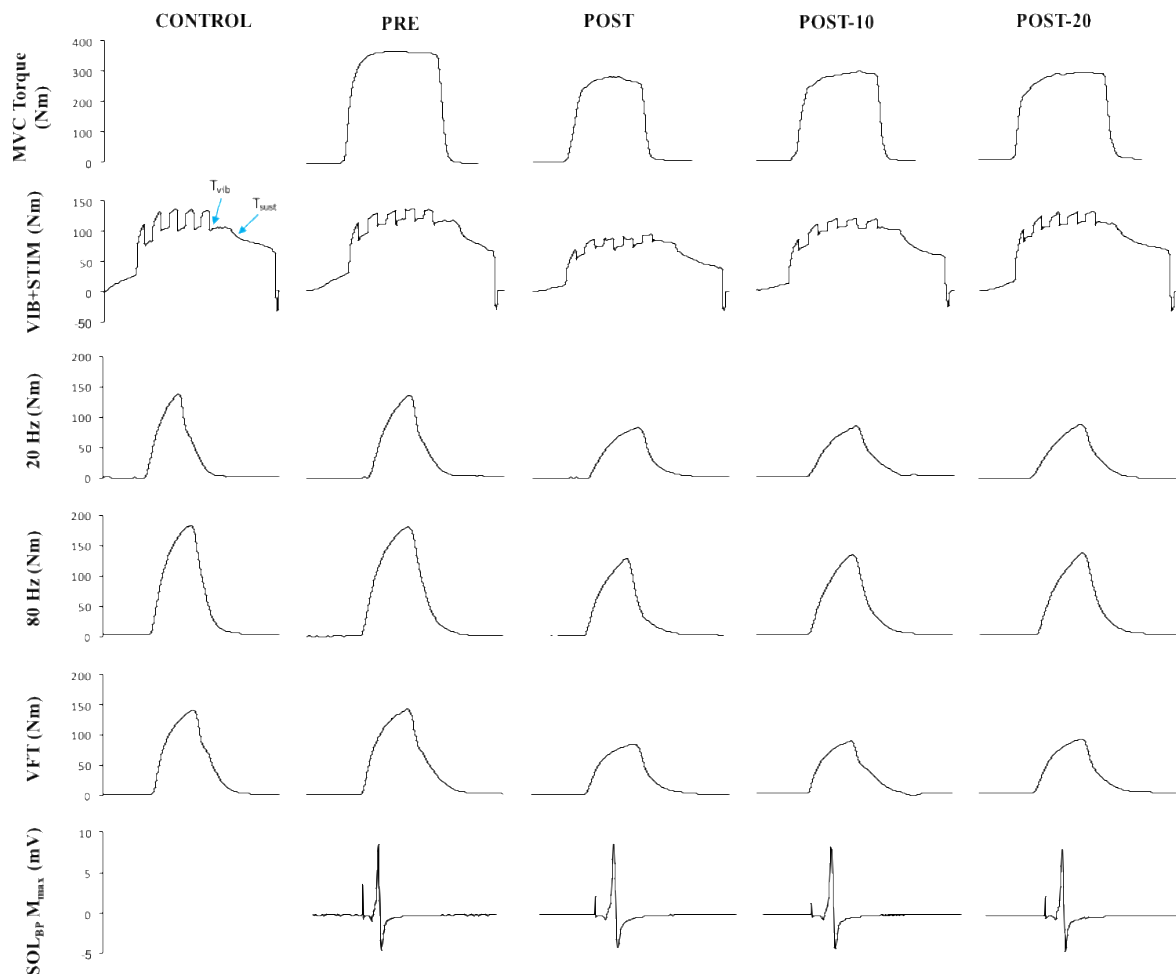


Figure 3.5. Traces of raw data from Experiment 2. Example data obtained from a single participant in the non-caffeine session immediately before (PRE) and immediately (POST), 10 min (POST-10) and 20 min (POST-20) after the exercise protocol. Decreases in MVC torque (first row), reflexive torque (second row) and tetanic stimulations are noticeable immediately after the exercise protocol.

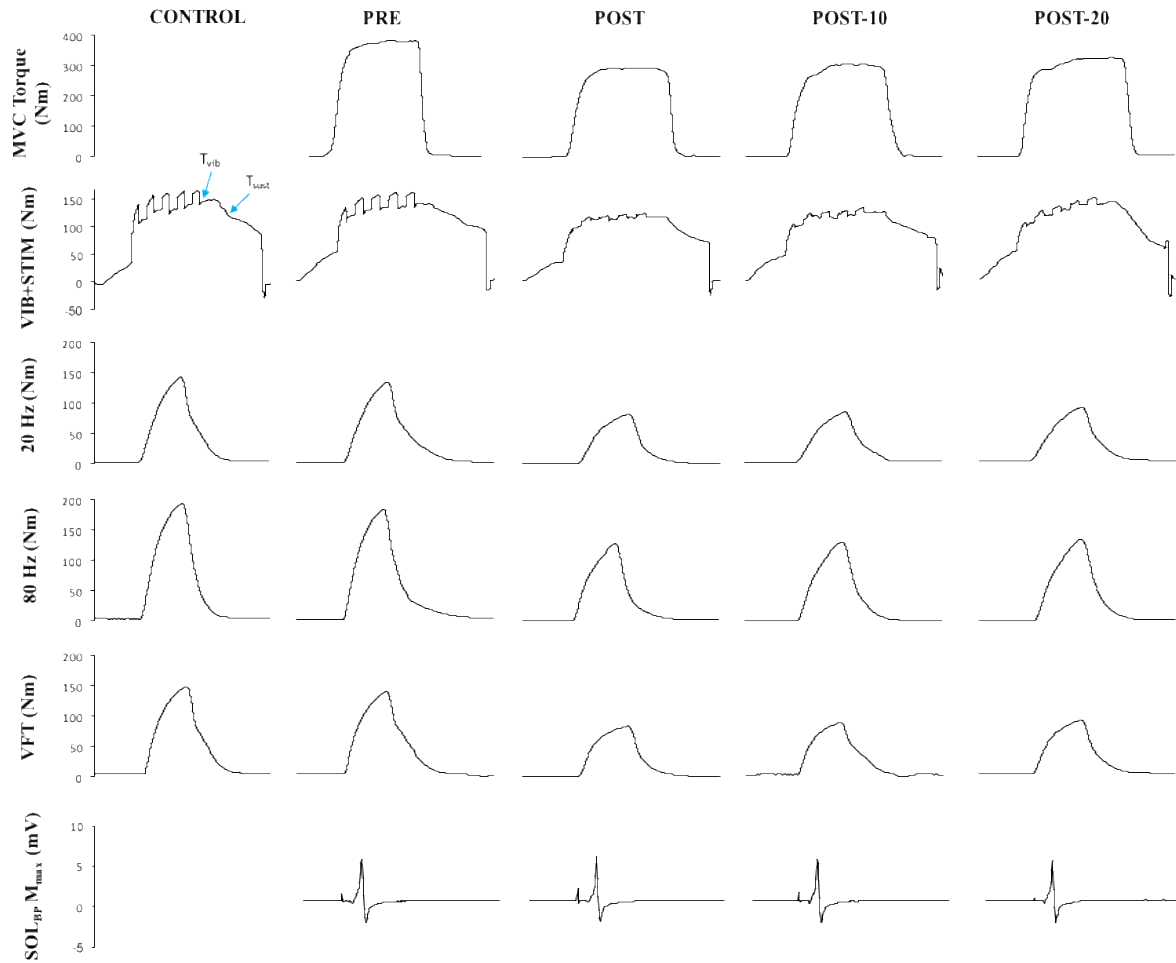


Figure 3.6. Traces of raw data from Experiment 2. Example data obtained from a single participant in the caffeine session immediately before (PRE) and immediately (POST), 10 min (POST-10) and 20 min (POST-20) after the exercise protocol. Decreases in MVC torque (first row), reflexive torque (second row) and tetanic stimulations are noticeable immediately after the exercise protocol.

3.2.1 Torque

Significant main effects of time ($F_{(1.596,16)} = 51.016$, $p < 0.000$; see Figure 3.7) and condition ($F_{(1,16)} = 8.529$, $p = 0.011$) were observed for maximal plantar flexion torque, however there was no condition-by-time interaction effect ($F_{(2.219,16)} = 2.985$, $p = 0.059$). With respect to the time effect, torque decreased immediately POST ($-16.9 \pm 7.3\%$ (-25, -8%), $p < 0.001$, $g = 0.924$) and did not recover by POST-10 ($-10.8 \pm 7.5\%$ (-20, -2%), $p < 0.001$, $g = 0.603$) or POST-20 ($-10.2 \pm 8\%$ (-19, -1%), $p < 0.001$, $g = 0.544$) relative to PRE. Follow-up RM ANOVAs examining the changes in each condition revealed a decrease in torque at POST

in both NON-CAF ($-16.5 \pm 7.9\%$ (-30, -4%), $p < 0.001$, $g = 0.899$) and CAF ($-17.4 \pm 6.6\%$ (-28, -4%), $p < 0.001$, $g = 0.930$) conditions compared with PRE. At POST-10, torque was still reduced in NON-CAF ($-9.9 \pm 8.4\%$ (-23, 4%), $p < 0.001$, $g = 0.520$) and CAF ($-11.7 \pm 6.3\%$ (-24, 0%), $p < 0.001$, $g = 0.682$), and did not return to baseline within the 20-min recovery period in either NON-CAF ($-12.2 \pm 9.2\%$ (-26, 2%), $p < 0.001$, $g = 0.615$) or CAF ($-8.3 \pm 5.8\%$ (-21, 4%), $p < 0.001$, $g = 0.470$).

With respect to the condition effect, post-hoc analyses indicated that the between-condition effect (greater torque production observed in CAF than NON-CAF), resulted from significant differences at PRE ($4.7 \pm 6.2\%$ greater in CAF compared to NON-CAF (-9, 18%), $p = 0.008$, $g = 0.219$) and POST-20 ($10.3 \pm 12.4\%$ greater in CAF compared to NON-CAF (-6, 24%), $p = 0.004$, $g = 0.410$) with no statistical difference being observed at POST ($4.6 \pm 9.5\%$ greater in CAF compared to NON-CAF (-10, 19%), $p = 0.097$, $g = 0.230$) or POST-10 ($2.9 \pm 7.2\%$ greater in CAF compared to NON-CAF (-12, 16%), $p = 0.212$, $g = 0.108$). The number of repetitions performed throughout the exercise protocol did not statistically differ between conditions ($p = 0.778$). Of the 17 participants, 10 replicated exactly the number of reps performed in the non-caffeine session, 5, 1 and 1 participants missed by 2, 3 and 5 repetitions respectively.

3.2.2 Muscle activity (electromyography)

SOL/M, MG/M, TS/M and TA measured at PRE, POST, POST-10 and POST-20 are presented in Table 3.2. In SOL/M, the trend towards a time effect did not reach statistical significance ($F_{(3,16)} = 2.385$, $p = 0.083$), and no condition ($F_{(1,16)} = 0.012$, $p = 0.914$) or condition-by-time interaction effects ($F_{(3,16)} = 1.977$, $p = 0.132$) were observed.

In MG/M, significant main effects of time ($F_{(1.815,15)} = 8.88$, $p < 0.002$) and condition ($F_{(1,16)} = 8.399$, $p = 0.012$) were observed, however there was no condition-by-time interaction effect ($F_{(2.373,16)} = 1.748$, $p = 0.185$). With respect to the time effect, MG/M decreased immediately POST ($-11.7 \pm 23.4\%$ (-37, 0%), $p = 0.009$, $g = 0.482$) but recovered by 10 min ($-0.2 \pm 23.3\%$ (-32, 5%), $p = 0.319$, $g = 0.36$). Follow-up RM ANOVAs examining the changes in each condition revealed a decrease in MG/M immediately POST in both NON-CAF ($-9.3 \pm 24.4\%$ (-36, 10%), $p = 0.039$, $g = 0.390$) and CAF ($-14.4 \pm 22.1\%$ (-40, 5%), $p = 0.01$, $g = 0.542$) compared to PRE, with a return to baseline at POST-10 in both NON-CAF ($4.2 \pm 27.7\%$ (-26, 23%), $p = 0.789$, $g = 0.041$) and CAF ($-4.9 \pm 16.1\%$ (-31, 19%), $p = 0.171$, $g = 0.166$).

The between-condition effect resulted from significant differences at PRE ($7.7 \pm 33.5\%$ greater in CAF compared to NON-CAF (-18, 36%), $p = 0.016$, $g = 0.255$) and POST-20 ($15.4 \pm 11.9\%$ greater in CAF compared to NON-CAF (-10, 39%), $p < 0.001$, $g = 0.394$), with no statistical difference being observed at POST ($10.1 \pm 30.6\%$ greater in CAF compared to NON-CAF (-21, 30%), $p = 0.473$, $g = 0.132$) or POST-10 ($5.6 \pm 20.7\%$ greater in CAF compared to NON-CAF (-18, 29%), $p = 0.363$, $g = 0.147$).

In TS/M_{max}, a significant main effect of time ($F_{(3,16)} = 6.656$, $p < 0.001$) was detected (see Figure 3.7) but no condition ($F_{(1,16)} = 0.478$, $p = 0.501$) or condition-by-time interaction effects ($F_{(3,16)} = 0.2.747$, $p = 0.055$) were observed. With respect to the time effect, TS/M decreased immediately POST ($-9.8 \pm 18\%$ (-24, 8%), $p = 0.001$, $g = 0.251$) but recovered by 10 min ($1.5 \pm 20.1\%$ (-13, 22%), $p = 0.854$, $g = -0.120$). No correlations were observed between changes in EMG/M variables and changes in torque.

In TA, no significant main effect of time ($F_{(3,16)} = 0.342$, $p = 0.795$), condition ($F_{(1,16)} = 0.104$, $p = 0.752$) or condition-by-time interaction effect ($F_{(3,16)} = 0.729$, $p = 0.540$) was observed.

Table 3.2. Plantar flexor torque, EMG, reflexive torque and electrically evoked torque data measured in Experiment 2.

| | CONTROL | PRE | POST | POST-10 | POST-20 |
|---|---------------|----------------|-----------------|-----------------|-----------------|
| Torque (Nm) | N/A | 249.4 ± 46.1 | 208.5 ± 41.2** | 222.4 ± 42.4** | 224.2 ± 45.3** |
| SOL/M | N/A | 0.041 ± 0.016 | 0.038 ± 0.017 | 0.043 ± 0.020 | 0.043 ± 0.024 |
| MG/M | N/A | 0.040 ± 0.015 | 0.034 ± 0.011* | 0.039 ± 0.013 | 0.041 ± 0.013 |
| TS/M | N/A | 0.079 ± 0.029 | 0.072 ± 0.022* | 0.082 ± 0.026 | 0.084 ± 0.030 |
| TA (mV) | N/A | 0.095 ± 0.086 | 0.079 ± 0.061 | 0.083 ± 0.061 | 0.081 ± 0.062 |
| T_{vib} (Nm) | 28.1 ± 28.0 | 30.7 ± 28.2 | 24.1 ± 25.3* | 31.1 ± 28.6 | 29.5 ± 28.1 |
| T_{sust} (Nm) | 27.9 ± 29.0 | 31.2 ± 28.5 | 22.6 ± 24.5* | 31.0 ± 27.9 | 29.1 ± 28.5 |
| 20 Hz (Nm) | 94.1 ± 17.2 | 92.2 ± 16.6 | 78.4 ± 20.8** | 78.3 ± 20.4** | 76.2 ± 21.3** |
| 80 Hz (Nm) | 124.1 ± 24.4 | 121.5 ± 24.1 | 103.6 ± 27.1** | 106.9 ± 28.4** | 105.4 ± 29.2** |
| VFT (Nm) | 95.8 ± 93.7* | 93.7 ± 17.4 | 80.0 ± 21.0** | 79.2 ± 21.1** | 77.4 ± 21.8** |
| 20:80 Hz ratio | 0.762 ± 0.052 | 0.763 ± 0.056 | 0.757 ± 0.057 | 0.735 ± 0.064 | 0.727 ± 0.063* |
| 20:VFT ratio | 0.984 ± 0.025 | 0.985 ± 0.025 | 0.979 ± 0.023 | 0.993 ± 0.051 | 0.986 ± 0.032 |
| SOL_{BP} M_{max} mV) | N/A | 5.916 ± 2.704 | 4.744 ± 2.300** | 4.577 ± 2.254* | 4.749 ± 2.575* |
| MG M_{max} (mV) | N/A | 13.263 ± 3.547 | 12.303 ± 2.850 | 11.449 ± 2.815* | 11.429 ± 2.666* |

Values are mean ± SD. Torque, peak torque during maximal voluntary isometric plantarflexion; SOL/M, MG/M, TS/M, soleus, medial gastrocnemius and triceps surae (SOL + MG) root-mean square EMG normalised to maximal M-wave amplitude; TA, tibialis anterior root-mean square EMG; T_{vib}, T_{sust}, mean torque after the 5th (last) stimulation during vibration and self-sustained torque 500 ms after vibration; 20 Hz, 80 Hz, VFT (variable frequency train) tetanic torque values during stimulation trains; 20:80 Hz, 20:VFT, stimulation train ratios; SOL_{BP} M_{max}, soleus bipolar EMG; MG M_{max}, medial gastrocnemius; SOL_{PSU} M_{max}, soleus pseudo-monopolar EMG. *significantly different from PRE, p < 0.05, ** p < 0.001.

3.2.3 Reflexive torque

In T_{vib}, a significant main effect of time ($F_{(4,16)} = 2.559$, $p = 0.048$) was detected (see Figure 3.7) but no condition ($F_{(1,16)} = 0.012$, $p = 0.916$) or condition-by-time interaction effects ($F_{(4,16)} = 2.460$, $p = 0.055$) were observed. With respect to the time effect, no statistical change was detected between CON and PRE ($-1.4 \pm 36.5\%$ ($-57, 39\%$), $p = 0.169$, $g = 0.090$). Following the exercise protocol, T_{vib} decreased immediately POST ($-13.2 \pm 60.5\%$ ($-70, 23\%$), $p = 0.035$, $g = 0.243$) but recovered by 10 min ($10.4 \pm 44.3\%$ ($-47, 51\%$), $p = 0.71$, $g = -0.016$).

In T_{sust} , a significant main effect of time ($F_{(4,16)} = 2.559$, $p = 0.048$) was detected (see Figure 3.7) but no condition ($F_{(1,16)} = 0.012$, $p = 0.916$) or condition-by-time interaction effects ($F_{(4,16)} = 2.460$, $p = 0.055$) were observed. With respect to the time effect, no statistical change was detected between CON and PRE ($-1.8 \pm 43.7\%$ (-55, 34%), $p = 0.169$, $g = 0.114$). Following the exercise protocol, T_{sust} decreased immediately POST ($-24.8 \pm 53.6\%$ (-69, 14%), $p = 0.035$, $g = 0.322$) but recovered by 10 min ($8.4 \pm 43.4\%$ (-44, 43%), $p = 0.71$, $g = 0.007$). No correlations were observed between changes in reflexive torque variables and changes in torque.

3.2.4 Tetanic evoked torque

Torque produced during 20 Hz, 80 Hz and variable-frequency (VFT) trains as well as the 20:80 Hz ratio and 20:VFT ratio measured at CON, PRE, POST, POST-10 and POST-20 are presented in Table 3.2. For torque in the 20-Hz train, a significant main effect of time ($F_{(1,487,16)} = 21.867$, $p < 0.001$) was detected (see Figure 3.7) but no condition ($F_{(1,16)} = 2.975$, $p = 0.104$) or condition-by-time interaction effects ($F_{(2,123,16)} = 0.175$, $p = 0.853$) were observed. With respect to the time effect, no statistical change was detected between CON and PRE ($2.3 \pm 7.3\%$ (-7, 11%), $p = 0.065$, $g = 0.112$). Following the exercise protocol, torque in the 20-Hz train decreased immediately POST ($-15.3 \pm 16.1\%$ (-24, -5%), $p < 0.001$, $g = 0.725$) and did not recover by POST-10 ($-15.1 \pm 17.8\%$ (-24, -5%), $p < 0.001$, $g = 0.734$) or POST-20 ($-17.8 \pm 16.5\%$ (-27, -7%), $p < 0.001$, $g = 0.826$) relative to PRE.

For torque in the 80-Hz train, a significant main effect of time ($F_{(1,653,16)} = 24.756$, $p < 0.001$) was detected (see Figure 3.7) but no condition ($F_{(1,16)} = 1.973$, $p = 0.179$) or condition-by-time interaction effects ($F_{(2,236,16)} = 0.079$, $p = 0.940$) were observed. With respect to the time effect, no statistical change was detected between CON and PRE ($2.4 \pm 6.8\%$ (-7, 12%), $p = 0.065$, $g = 0.108$). Following the exercise protocol, torque in the 80-Hz train decreased

immediately POST ($-15.3 \pm 12.9\%$ (-24, -4%), $p < 0.001$, $g = 0.689$) and did not recover by POST-10 ($-12.4 \pm 16.3\%$ (-22, -2%), $p < 0.001$, $g = 0.548$) or POST-20 ($-14 \pm 15.7\%$ (-23, -3%), $p < 0.001$, $g = 0.593$).

For torque in the VFT, a significant main effect of time ($F_{(1.524,16)} = 23.480$, $p < 0.001$) was detected (see Figure 3.7) but no condition ($F_{(1,16)} = 3.230$, $p = 0.091$) or condition-by-time interaction effects ($F_{(2.120,16)} = 0.125$, $p = 0.894$) were observed. With respect to the time effect, a small but significant change was detected between CON and PRE ($2.4 \pm 7.2\%$ (-7, 11%), $p = 0.046$, $g = 0.118$). Following exercise, VFT torque decreased at POST ($-14.9 \pm 15.6\%$ (-24, -5%), $p < 0.001$, $g = 0.701$) and did not recover by POST-10 ($-15.6 \pm 17.8\%$ (-25, -5%), $p < 0.001$, $g = 0.740$) or POST-20 ($-17.9 \pm 16.5\%$ (-27, -7%), $p < 0.001$, $g = 0.815$).

Regarding the 20:80 Hz ratio, a significant main effect of time ($F_{(2.242,16)} = 4.313$, $p = 0.018$) was detected but no condition ($F_{(1,16)} = 0.113$, $p = 0.741$) or condition-by-time interaction effects ($F_{(2.502,16)} = 0.377$, $p = 0.734$) were observed. With respect to the time effect, no statistical difference was detected between time points except for POST-20 ($-4.6 \pm 7.1\%$, $p < 0.001$, CI (-1, -2%), $g = -0.043$) relative to PRE.

For the 20:VFT ratio, no significant time ($F_{(2.315,16)} = 0.662$, $p = 0.543$), condition ($F_{(1,16)} = 0.1262$, $p = 0.278$) or condition-by-time interaction effects ($F_{(2.884,16)} = 0.554$, $p = 0.642$) were observed. No correlations were observed between changes in tetanic torque variables and changes in torque.

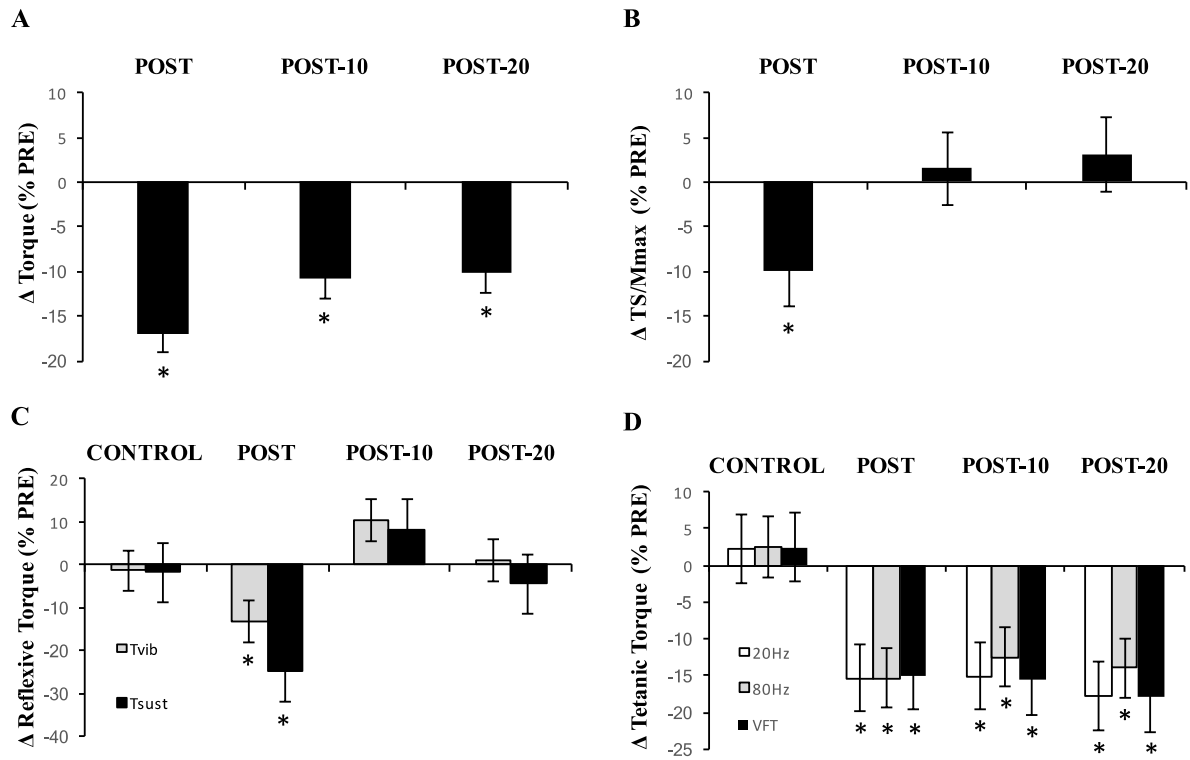


Figure 3.7. Changes in torque, EMG, reflexive torque and tetanic torque measured from before to immediately (POST), 10 min (POST-10) and 20 min (POST-20) post-exercise. A) MVC torque, B) triceps surae EMG normalised to M-wave (TS/M_{max}), C) mean torque after the 5th (last) stimulation during vibration (T_{vib}) and self-sustained torque (T_{sust}) measured 500 ms after vibration cessation, D) peak torque during 20-Hz, 80-Hz and VFT trains of electrical stimulation. *significantly different from PRE, $p < 0.05$.

3.2.5 M-wave amplitude

A significant main effect of time ($F_{(3,16)} = 8.975$, $p < 0.001$) was detected in SOL M_{max}, but no condition ($F_{(1,16)} = 0.121$, $p = 0.734$) or condition-by-time interaction effects ($F_{(3,16)} = 1.458$, $p = 0.240$) were observed. With respect to the time effect, SOL M_{max} decreased immediately POST ($-15.1 \pm 14.8\%$, $p < 0.001$, CI (-40, 1%), $g = 0.458$) and remained depressed at 10 min ($-18.5 \pm 16.1\%$, $p = 0.001$, CI (-43, -2%), $g = 0.528$) and 20 min ($-18.2 \pm 18.6\%$, $p = 0.003$, CI (-41, 2%), $g = 0.433$).

A significant main effect of time ($F_{(1.505,16)} = 10.379$, $p = 0.002$) was detected in MG M_{max} but no condition ($F_{(1,16)} = 1.657$, $p = 0.219$) or condition-by-time interaction effects ($F_{(2.139,16)} = 1.402$, $p = 0.256$) were observed. With respect to the time effect, no change in MG

M_{\max} was detected immediately POST ($-3.8 \pm 12.3\%$, $p = 0.211$, CI (-19, 5%), $g = 0.295$), however MG M_{\max} was depressed at 10 min ($-12 \pm 12.1\%$, $p = 0.004$, CI (-25, -2%), $g = 0.560$) and 20 min ($-11.9 \pm 11.8\%$, $p = 0.002$, CI (-25, -2%), $g = 0.578$) after exercise.

Discussion

The primary aim of the present research was to examine the neuromuscular factors influencing the force loss and then recovery following repeated bouts of high-intensity, yet submaximal, muscular efforts. The thesis was divided into two experiments. The first experiment aimed to investigate the potential contribution of changes in corticospinal excitability to the decreases in neural drive and voluntary force following repeated, high-intensity muscular efforts where fatigue was elicited in the human plantar flexors. The second experiment aimed to investigate whether inhibition of the MN facilitatory system or losses of muscle contractile capacity contributed to force decline. In both experiments, the isometric plantar flexion exercise induced a significant loss of peak isometric torque ($12.2 \pm 6.5\%$ in Experiment 1 and $16.9 \pm 7.3\%$ in Experiment 2). The small difference in torque loss ($p = 0.009$) between experiments can be explained by: (1) in Experiment 1, six MVCs were performed and the average used for analysis whilst in Experiment 2 a single MVC was performed, and (2) the electrical stimulation techniques used to identify MN facilitation and muscle contractile capacity in Experiment 2 might have induced some additional fatigue or attenuated the recovery between the last repetition of the exercise protocol and the MVC. Regardless, the reduction in torque is consistent with previous studies where repeated submaximal muscular efforts resulted in a loss of torque (e.g. $-43.3 \pm 3.6\%$ contracting at 50% MVC to failure in Neyroud et al. (2013), and $-20.3 \pm 12.0\%$ at 75% MVC in Rozand, Cattagni, Theurel, Martin, and Lepers (2015)).

The torque decreases were accompanied by reductions in the M-wave normalised EMG (EMG/M) immediately after exercise, which is indicative of a decrease in central/neural drive (Kalmar & Cafarelli, 2004b). However, the lack of significant correlation between the changes in these variables suggests that other factors play a larger role in the fatigue response. The simultaneous reduction in EMG/M and voluntary peak torque following high-intensity

muscular efforts are congruent with the findings of several other studies (Husmann et al., 2017; Kalmar et al., 2006; Place, Maffiuletti, Martin, & Lepers, 2007; Sogaard, Gandevia, Todd, Petersen, & Taylor, 2006) and suggests that impaired voluntary muscular force production after an acute bout of high-intensity exercise results in part from a reduction in neural drive.

The mechanisms underlying this decrease in neural drive after repeated bouts of high-intensity, yet submaximal, muscular contractions are unknown, although they are thought to include changes in descending input to the MNs (Taylor, Butler, et al., 2000). In the present study, MEP/M measured in the relaxed muscle was unchanged by the exercise protocol. When measured at rest, the MEP amplitude is believed to represent the balance between excitation and inhibition in the corticospinal-motoneuronal pathway, which can be modulated by sensory/afferent feedback (Chen, 2004). A reduction in MEP size following exercise might be explained by a reduction in cortical excitability, an increase in sensory inhibitory feedback to the MN (Taylor et al., 2016), and reductions in MN intrinsic properties (Heckman & Enoka, 2012b). Nonetheless, as no change in the resting MEP/M at rest was detected in the present study, it could suggest there were no detectable changes in each of these sites in the absence of facilitatory voluntary drive, or different sites may have responded differently, resulting in a lack of overall change in MEP.

MEP/M measured during MVC was found to decrease following high-intensity exercise and then recover by 10 min. This time course was consistent with the time course of change in EMG/M, which also recovered by 10 min, indicating a possible association between the two variables. This result is not consistent with previous findings in plantar flexor muscles, where MEP amplitude has been shown to increase (Hoffman et al., 2009) during submaximal sustained contractions or remain unchanged when assessed during a MVC after 45 intermittent maximal contractions (Iguchi & Shields, 2012). This finding is also observed in other muscle groups where MEPs has been shown to increase (Keller et al., 2011; Taylor, Allen, Butler, &

Gandevia, 2000) or remain unchanged (Fernandez-del-Olmo et al., 2013; Girard, Bishop, & Racinais, 2013; Goodall et al., 2015; Sidhu, Bentley, & Carroll, 2009; Yoon, Schlinder-Delap, Keller, & Hunter, 2011). The disparity between studies likely results from the high variability when utilising TMS techniques (Wassermann, 2002) as well as the testing and analysis methods used. It is interesting to note that it has been suggested that changes in MEP amplitude after fatiguing contractions could be dependent on the stimulation intensity used to evoke MEPs (Bachasson et al., 2016). In addition, differences in the fatiguing interventions (i.e. sustained or intermittent contractions, voluntary force level, contraction duration etc.) performed between studies is likely to influence the result. Finally, sample size is also likely to play a role in the statistical analysis of TMS variables, which was evident in Experiment 1 where significant changes in MEP amplitude and MEP/M were observed from PRE to POST when data were collapsed across conditions (CAF and NON-CAF) but not when each condition was examined separately (and sample size was thus reduced). Therefore, some caution is required when interpreting the results of the current study even though 16 participants were used. Regardless, the temporal alignment of changes in EMG/M and MEP/M amplitude provides reasonable evidence that a change in corticospinal excitability might be partly responsible for the loss in neural drive following high-intensity, submaximal exercise. It should be noted that the MEP amplitude itself was depressed throughout the recovery phase (i.e. to at least 20 min), however this coincided with an M-wave amplitude depression so MEP/M was observed to recover. This demonstrates the importance of accounting for possible peripheral changes at the muscle when assessing MEP changes; it certainly provides a better indicator of changes in corticospinal excitability following fatiguing exercise.

Whilst the single-pulse TMS methods used in this study were sufficient to detect changes in corticospinal excitability, this technique only provided an indication of global changes within the whole corticospinal pathway. In order to differentiate between cortical and

spinal sites the use of paired TMS and cervicomedullary or thoracic electrical stimulation techniques may be required. The thoracic motor evoked potential (TMEP) protocol was tested for use in the current study, however it proved difficult to obtain reliable TMEPs in the plantar flexors.

Of interest, the cortical silent period (cSP), considered to reflect changes in spinal and intracortical inhibitory mechanisms mediated by long-lasting gamma-aminobutyric acid (GABA)_B receptors (Chen et al., 1999; Gruet et al., 2013; Taylor et al., 1996; Werhahn, Kunesch, Noachtar, Benecke, & Classen, 1999), was reduced following the exercise protocol and did not recover over the 20-min post-exercise period. Contrary to our findings, previous studies have typically reported increases in cSP duration after performance of sustained or intermittent low-intensity contractions (Gruet et al., 2014; Yoon et al., 2011) and maximal contractions (Goodall, Howatson, & Thomas, 2017; Taylor et al., 1996; Vernillo, Temesi, Martin, & Millet, 2017). This increase in cSP duration is believed to arise from an increase in fatigue-related (e.g. group III/IV) afferents (Hilty et al., 2011; Sidhu et al., 2017). Nonetheless, others have observed either no change (Goodall et al., 2015; Jubeau et al., 2017) or decreases in cSP after fatiguing exercise (Girard et al., 2013). Possible explanations for the discrepant findings include differences in exercise intensity, duration and recovery, as well as methodological testing procedures used. Of particular relevance is that intermittent contractions, where a short rest period was afforded between repetitions, were performed in the present study. This may have resulted in less group III/IV inhibitory afferent activity compared to a sustained effort where no rest is afforded throughout the effort. It is believed that the reduction in intracortical inhibition results from repetitive activation of somatosensory input to the primary motor cortex, thus increasing neural drive to the MN pool (Kidgell, Bonanno, Frazer, Howatson, & Pearce, 2017). It might be the case that the cSP reduction in the present study attenuated some of the loss in corticospinal excitability (which could possibly be due to

a reduction in MN excitability), although this was insufficient to prevent the loss of neural drive, as estimated through the EMG/M ratio. However, whether changes in sensory input are able to modify the cSP response after a single exercise session remains to be elucidated. If this were the case, then it might be that whilst some afferent input to the primary motor cortex improves neural drive to the MN, an excess of input might inhibit drive, and that the type of training performed is an influential factor.

In addition to the loss in corticospinal excitability, MN disfacilitation is also a possible mechanism contributing to the loss of neural drive (Hultborn et al., 2003; Taylor, Butler, et al., 2000). Alpha-MNs are strongly dependent upon facilitatory inputs to achieve a maximal discharge frequency, and thus to produce high levels of muscular force (Hultborn et al., 2003). This facilitatory modulation occurs at the motor neurone dendrites and is controlled by the interaction between descending monoaminergic drive and spinal circuits, especially including the Ia afferents (Heckman et al., 2005). It is well known that MNs rely on a PIC-mediated facilitatory system that increases synaptic gain in order to achieve maximal discharge frequency and thus to produce maximal levels of muscular force (Heckman et al., 2005; Hultborn et al., 2003). To test whether a loss in PIC-mediated facilitation of the MN occurs following fatigue, a combined vibration-electrical stimulation (VIB+STIM) protocol was used. The VIB+STIM technique is believed to reflect PIC activation because the responses to it display many of the hallmarks of PIC-dependent change, such as muscle length dependence, where reflexive torque increases as the muscle is lengthened (i.e. the antagonist is shortened), there is a wind-up effect during repeated muscle stimulations, and a self-sustained torque phase is notable after vibration cessation (Trajano et al., 2014). Our results showed reductions in reflexive torque (T_{vib}) production during vibration as well as reductions in the ability to sustain the torque without synaptic input (self-sustained torque; T_{sust}) following the exercise. These results are considered to indicate an impairment in PIC activation. T_{vib} and T_{sust} were

statistically recovered by 10 min, suggesting that the effects of the fatiguing exercise were overcome relatively quickly. This temporal profile is consistent with the change in EMG/M observed in both experiments 1 and 2, indicating the possibility of an association between the changes. Although the correlations between changes in EMG/M and T_{vib} and T_{sust} were not significant, this may reflect the time taken to measure the PIC amplitude (33 s), during which some level of recovery might have occurred, and the effects of measurement variability on correlation magnitude. Regardless, the present study is the first to provide evidence for the inhibitory effect of fatiguing exercise on spinal MN facilitatory systems.

Both MEP amplitude and PIC amplification appeared to be depressed at POST but recovered by 10 min. This aligns temporally with the loss and recovery of EMG/M and strongly suggests that the current fatiguing exercise protocol was sufficient to reduce neural drive during an MVC completed immediately after the exercise, but that these changes recovered within 10 min. These changes therefore cannot completely explain the prolonged loss of force that was observed at 20 min post-exercise. Therefore, other factors must be considered.

Reductions in tetanic torque were evident in 20 Hz, 80 Hz and VFT tetanic stimulation conditions, suggesting that the muscle's contractile capacity was compromised. However, the loss of force was similar across stimulation conditions and we were therefore unable to ascertain a specific process within the muscle that was predominant in the force decline. Previous studies have identified significant reductions in the 20:80 Hz ratio following fatiguing exercise such as downhill running (Martin et al., 2004) and concentric leg extension/flexion tasks (Hill, Thompson, Ruell, Thom, & White, 2001) and this is believed to be caused by a reduced Ca^{2+} release or faster uptake of Ca^{2+} into the sarcoplasmic reticulum (Binder-Macleod & Kesar, 2005; MacIntosh & Rassier, 2002). However, the lack of relative differences in the torque change between the 20 Hz and 80 Hz trains in our experiment indicates a minimal effect of these mechanisms, which may have been due to the position of the ankle (0°) and the type

of muscle action (isometric) performed (Jones, 1996; Keeton & Binder-Macleod, 2006; Martin et al., 2004). The 20:VFT ratio is believed to represent changes in the muscle's sensitivity to Ca^{2+} , i.e. the ratio between the muscle's myoplasmic Ca^{2+} concentration and the muscle's force level. The lack of change in 20:VFT indicates that decreased Ca^{2+} sensitivity was not an overriding factor affecting force production following the fatiguing exercise. It is important to note, however, that because of the large compliance in the plantar flexors due to the long Achilles tendon the effect of the doublet stimulation on force production may be minimised when compared to other muscle groups such as the leg extensors (Binder-Macleod & Kesar, 2005) and this may have reduced our ability to detect small potential changes in the 20:VFT ratio. On the contrary, it might be the case that the doublet has a minimal effect in the plantar flexors and therefore a loss of the ability to use it, i.e. changes in Ca^{2+} sensitivity, may not be important. Alternatively, a loss of force per cross-bridge (Edman & Lou, 1990; MacIntosh & Rassier, 2002), reduced force transmission from the contractile elements to the bone, or the cumulative effect of small (difficult to detect) changes in multiple force generating processes may have underpinned the force decline. More research is needed to explicitly locate the mechanism/s, however the data strongly suggest that interventions that allow maintenance of muscular force production will help to reduce the force decline after high-intensity, repetitive muscular contractions, or at least increase the rate of recovery.

M-wave amplitudes were depressed following the fatigue exercise protocol and did not recover within the 20-min recovery period in both experiments; thus, M-wave depression was temporally associated with the loss of voluntary force. M-wave amplitude is considered to provide information relating to the ability of the sarcolemma to transmit excitatory potentials to the calcium release channels (Green, 2004; Rodriguez-Falces & Place, 2016), although interpretation of M-wave changes is complex due to the M-wave being highly sensitive to positional changes of the recording electrodes relative to the muscle-tendon complex

(Rodriguez-Falces & Place, 2014) as well as the changes in muscle geometry (pennation angle and fascicle length) that may occur during fatiguing exercise (Hodges, Pengel, Herbert, & Gandevia, 2003; Narici et al., 1996). Nonetheless, the repeatable findings (in Experiments 1 and 2) of M-wave depression indicates that a change in fibre membrane excitability may have occurred and could have contributed to the prolonged loss of force following the exercise. However, evidence against a physiological effect of the M-wave amplitude reduction in the current study comes from the lack of change in the 20:80 Hz ratio. If the 20:80 Hz ratio is sensitive enough to reflect changes in Ca^{2+} release from the Ca^{2+} release channels, then the lack of change in the current study suggests that possible reductions in sarcolemmal excitability were insufficient to meaningfully impede muscle Ca^{2+} release and thus muscle function. Future research might more explicitly examine the different phases of the M-wave, as each are believed to represent different characteristics in membrane excitability (Rodriguez-Falces & Place, 2017; Rodriguez-Falces & Place, 2016). This may provide further evidence as to the mechanisms influencing the M wave, specifically whether the changes in M-wave amplitude result from changes in membrane excitability (possibly due to the rise in extracellular K^+) or changes in muscle-tendon architectural features, as is more likely in the current study due to the lack of change in the 20:80 Hz ratio (Rodriguez-Falces & Place, 2016).

The second main purpose of the present study was to determine whether caffeine might reduce the fatiguing effect of the exercise. The results showed that the prior consumption of caffeine resulted in a robust increase in muscle force production and neural drive (EMG/M) measured during MVC across the points. (i.e. there was an effect of condition). This allowed for greater MVC force to be produced in the recovery from exercise, with statistical differences from the NON-CAF condition being observed at 10 and 20 min after the fatiguing bout in Experiment 1 as well as before and 20 min after the fatiguing bout in Experiment 2. Nonetheless, no condition-by-time interaction was observed, suggesting that caffeine did not

affect force production at any specific time point, i.e. before or in recovery from exercise. Therefore, caffeine was not observed to minimise the loss of force evoked by exercise. The greater force production in the caffeine condition was associated with an increase in neural drive, as estimated using the EMG/M ratio. This finding is consistent with evidence that caffeine exerts the majority of its effects on the nervous system (Fredholm, Bättig, Holmén, Nehlig, & Zvartau, 1999; Snyder, Katims, Annau, Bruns, & Daly, 1981; Tallis, James, Cox, & Duncan, 2012). The mechanisms of caffeine on the nervous system are multifaceted, and include adenosine antagonistic behaviour, which theoretically should increase MEP amplitude (Gandevia & Taylor, 2006; Kalmar & Cafarelli, 2004a), as well as the upregulation in noradrenergic and serotonergic drive, which could potentially increase PIC activation (Heckman et al., 2008) and thus improve excitability at the somato-dendritic end of the MN (Cotel et al., 2013). However, corticospinal excitability (MEP) and MN facilitation (sustained torque; T_{sust}) were not statistically affected by the ingestion of caffeine, thus it is likely that caffeine had no discernible effect on MN pre- or post-somatic sites and that caffeine exerted its effects on neural mechanisms not tested within the present study, or that our tests were not sensitive enough to detect differences in corticospinal excitability and MN facilitation between the non-caffeine and caffeine conditions.

Caffeine may also exert effects peripherally via alterations in intramuscular ion handling, such as increased Ca^{2+} mobilisation from the sarcoplasmic reticulum, and this may produce favourable effects on E-C coupling (Davis & Green, 2009; Magkos & Kavouras, 2005). However, no differences were detected in evoked torque (20 Hz, 80 Hz or VFTs) between the caffeine and non-caffeine conditions at any time point, which suggests that caffeine ingestion did not have a discernible effect on E-C coupling either before or after the high-intensity intermittent exercise. Therefore, it is likely that the ergogenic benefits of caffeine were confined to the nervous system.

It should be noted that an individual variability in the responsiveness to caffeine was detected between participants. On average, caffeine improved MVC torque production by >3% in approximately half (53%) of the participants across both experiments, whilst in 9% of participants the torque production was reduced; no effect was observed (the changes were $\pm 3\%$) in the remaining 38% of participants. Therefore, caffeine may have some effect on the neuromuscular function in some participants, suggesting that individual responses to caffeine need to be determined in order to decide whether an ergogenic effect is evoked before and/or after the performance of sets of high-intensity, fatiguing muscular efforts. It is also of note that the dosage of $3 \text{ mg} \cdot \text{kg}^{-1}$ body mass used in the present study is lower than that used in previous research (Behrens et al., 2015; Davis & Green, 2009; Walton et al., 2003; Walton et al., 2002), where doses of $6 \text{ mg} \cdot \text{kg}^{-1}$ body mass are used. The lower dose was chosen to better represent the dosages that might commonly be ingested through the consumption of energy drinks, coffee, pre-workout supplements, etc. Utilising a caffeine dosage greater than $3 \text{ mg} \cdot \text{kg}^{-1}$ might potentially produce results different to those seen in the present study, although their ecological validity might be reduced.

4.1 Conclusion

The results of the present research indicate that the performance of repeated high-intensity, but submaximal, muscular efforts results in a significant and prolonged (>20 min) loss of force which is likely to influence subsequent bouts of work. The loss of force was accompanied by a loss of neural drive measured during MVCs immediately after exercise, which were further accompanied by decreases in corticospinal excitability and MN facilitation. However, neural drive (and associated physiological changes) recovered quickly and cannot explain the ongoing loss of force, even if it contributed to the greater loss of force detected early in the recovery phase. Interestingly, cSP was shortened immediately after exercise and at

later points in the recovery and might act to partly compensate for the loss in corticospinal excitability, however more research is required to confirm this assertion. Whilst neural drive recovered by 10 min, significant decreases in tetanic torque were observed at later points in the recovery (i.e. 10 and 20 min), which might explain the prolonged loss of torque. However, no specific process within the muscle was predominant in the force decline, thus no conclusions can be drawn as to the muscular mechanism/s responsible for the loss of torque following repeated, high-intensity contractions.

Caffeine improved muscle force production and neural drive (EMG/M) measured during MVC. However, its effects did not appear to affect corticospinal excitability or MN facilitation pathways, and it is likely that caffeine exerted its effects on neural mechanisms not tested within the present study, or that our tests were insufficient to detect small changes in corticospinal excitability and MN facilitation between the non-caffeine and caffeine conditions.

4.2 Future directions

The present experiments were conducted under controlled laboratory conditions involving single-joint isometric, muscle contractions, and it might be the case that different muscle groups respond differently to both the exercise and caffeine ingestion. For instance, compound multi-joint movements that require activation of numerous muscles in a skilled manner may require greater neural regulation and activation than isolated single-joint activities, and therefore might trigger greater changes in corticospinal and MN facilitatory processes. In addition, the ergogenic effects of caffeine might be exacerbated when greater neural drive is required, as is likely the case in compound multi-joint movements that involve activation of a larger total muscle mass. Finally, individual variations in responses to caffeine might also play a role, and thus should be considered when using caffeine as an ergogenic aid. Regarding peripheral changes, the prolonged loss of muscular force following repeated high-intensity

submaximal efforts appeared to be attributable to changes within the muscle itself. Thus, future work might investigate the precise mechanisms responsible for the long-lasting loss of force so that training interventions and both nutrition and supplement strategies can be developed to target those mechanisms and thus reduce recovery time following repetitive high-intensity efforts.

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Appendices

Appendix 1: Information Letter to Participants (Experiment 1)



Information Letter to Participants

The acute effects of a single strength training session on motor neurone excitability and facilitation systems

Thank you for expressing your interest in this research study. The purpose of this information letter is to explain the study that you may choose to participate in. Please take a few minutes to carefully read the information below, and do not hesitate to ask any questions regarding the study methods or equipment or safety concerns. If you agree to participate in the project, you will be asked to undertake a screening process to evaluate your suitability for the study.

Project background information

It is well known that the performance of maximal or near-maximal muscular contractions, such as those used during heavy strength training, causes fatigue. Whilst many of the physiological changes that underpin fatigue reside in the muscles themselves, at least part of the response resides in the central nervous system, which loses its ability to fully activate the muscles. Therefore, determining the mechanisms that underpin this loss of neural drive to the muscle is vital in order to develop strategies to reduce fatigue and thus improve exercise performance.

Previous research indicates that the transmission of information through the corticospinal (brain and spinal cord) tract declines profoundly during fatigue, particularly at the motor neurone, (MN; the nerve connecting the spinal cord directly to the muscle). However, the mechanisms responsible for the decline remain unclear. Two possible mechanisms have been suggested to influence corticospinal transmission efficiency during strength training and other intense exercise activities: 1) a decrease in persistent inward current formation; an amplifier for signals at the MN which improves the connectivity between the spinal cord and muscle and is needed for us to produce large forces but losses its ability to function when we fatigue, and 2) a functional change at the start of

the motor neurone (the axon hillock) because of a spillover of serotonin (5-HT), a neurotransmitter that often increases nerve function but may reduce it when spillover occurs.

Purpose

The overall purpose of this research is to: 1) investigate whether a loss in corticospinal transmission efficiency contributes to the fatigue that occurs during high-intensity, intermittent, voluntary muscular contractions, such as those performed during strength training or other forms of high-intensity exercise, and 2) to determine which mechanisms are responsible for the change in corticospinal transmission efficiency. This research will be the first of two studies designed to understand the effects of corticospinal fatigue in strength training.

Methods

All testing will be performed at the ECU Exercise Physiology laboratory (19.150) at Joondalup.

Participant Requirements

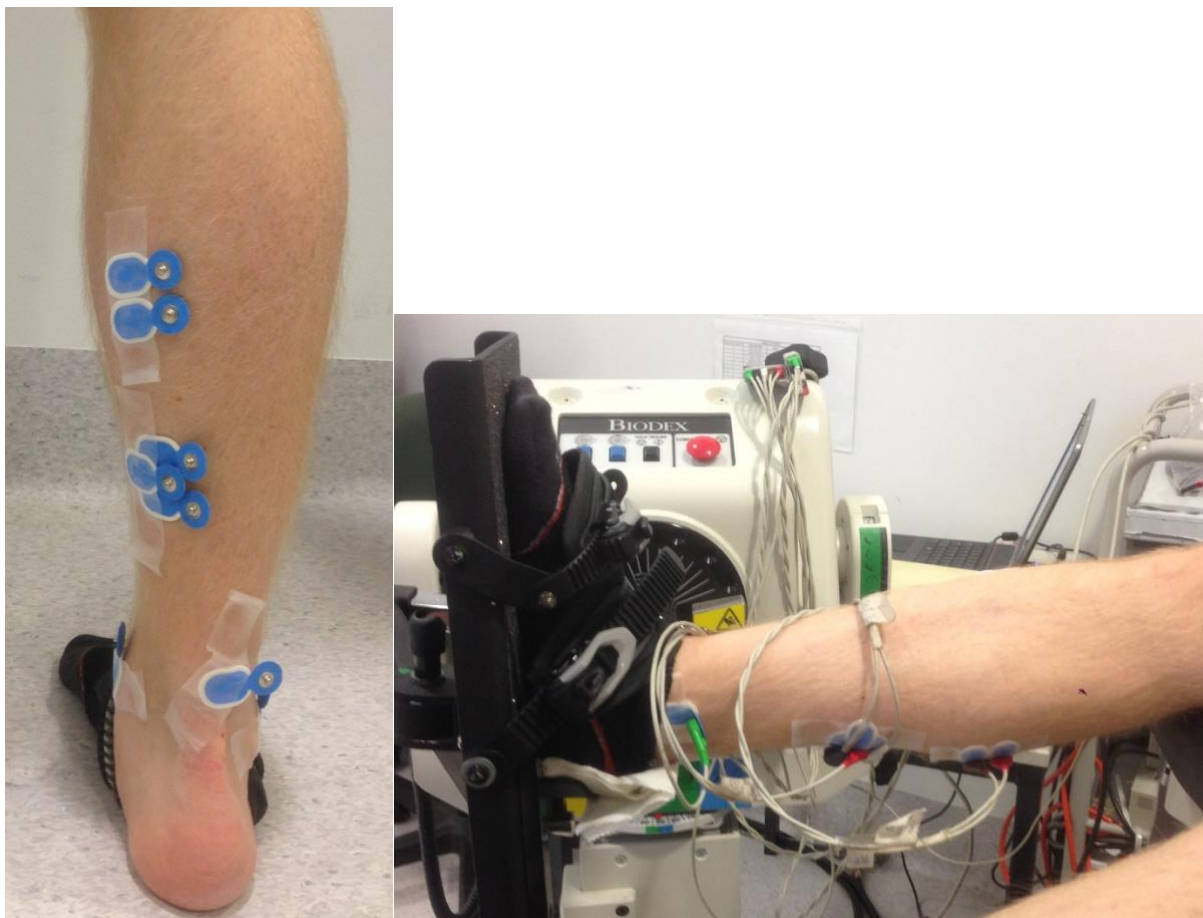
As a participant you must be between 18-45 years of age with a minimum of 1-year of experience in resistance training, no known neurological or cardiovascular diseases, not currently taking anti-depressant medication (i.e. serotonin and nor-adrenalin reuptake inhibitors) and consume a minimal (<200 mg/day) amount of caffeine (this will be determined by completing a caffeine consumption questionnaire). If you decide to participate in the study, you will be asked to report to the laboratory on five separate days at the same time of day with at least 72 hours between visits. You will be asked to abstain from consuming any foods, beverages or medications containing caffeine for at least 12 hours prior to testing sessions. You will also be asked not to consume any depressants (e.g. alcohol, medications etc.) for 24 hours prior to testing sessions. Additionally, you will be asked to refrain from performing intense exercise 48 hours prior to testing sessions.

What will I be required to do

In the first visit, you will be familiarised with all of the experimental equipment and procedures for the study including; (1) maximal voluntary isometric contractions of the calf muscles (MVC), (2) electromyography (EMG; passive recording of the electrical signals from your muscles), (3) transcranial magnetic (at the head) electrical stimulations, (4) electrical stimulation of the tibial nerve (located on the back of the knee joint), and (5) combined Achilles tendon vibration and tibial nerve electrical stimulation. In addition, you will be asked to perform a standardised warm-up consisting of 5-minutes of submaximal cycling on a stationary bike followed by five voluntary isometric muscle contractions of the calf muscles ranging from 20-100% of perceived maximal effort (*Familiarisation session*). The Familiarisation session will take approximately 60 minutes to

complete. The subsequent four visits will be to complete two separate protocols (Protocol A and Protocol B) with each protocol containing both a non-caffeine and caffeine ingestion condition to assess the potential changes in MN transmission efficiency resulting from fatiguing exercise.

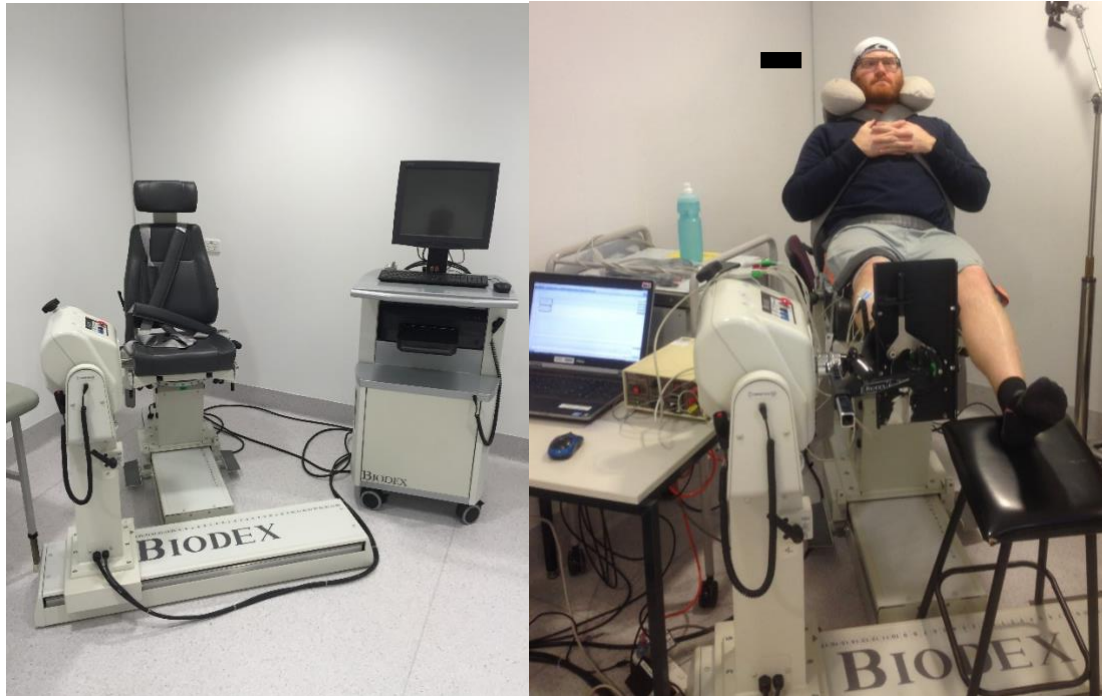
Upon arrival at the laboratory, you will be taken through a preparation period where we will position and apply skin-based self-adhesive EMG electrodes on the calf muscles of the right leg to record muscle activity. The area underneath the electrodes will be lightly abraded and cleaned with alcohol to minimise the risk of infection. After EMG preparation, the correct positioning for electrical stimulation of the tibial nerve will be established and the position will be marked with removable ink to ensure a consistent position throughout the experimental session. You will then complete the same standardised warm-up as in the Familiarisation session. This will take approximately 45-60 minutes.



Protocol A, Session 1: Transcranial (at the head) Magnetic Stimulation Without Caffeine:

Protocol A will be performed to determine whether corticospinal (brain and spinal cord) excitability/transmission is impaired during fatiguing exercise. During all testing you will be seated upright in a chair for testing of knee extension and other force measurements. Magnetic and electrical stimulation will occur at the head and at the nerve at the back of the knee respectively. These techniques will be performed at various intensities to find the point at which the calf muscle is best

recruited. Subsequent testing will then use these intensities to determine your ability to activate the muscle during fresh and fatigued states. Once the appropriate intensities are found, you will perform six maximal isometric voluntary contractions (MVCs) of the lower leg with overlaid transcranial stimuli, followed by three electrical nerve stimuli.



Exercise protocol

After a 2-min rest. You will perform 6 sets of 3-second contractions reaching a target level of 85% of your maximum force with a 90 s rest period between sets. Each set of contractions will be stopped when the required force level is not attained on 2 consecutive repetitions, i.e. each set will contain a different number of contractions. Transcranial (at the head) stimulation will be performed during the 1st, then every third and last contraction of each set. Upon completion of the 6 sets, you will perform six maximal voluntary contractions of the lower leg with overlaid transcranial stimuli, followed by three electrical nerve stimuli as done before the exercise protocol. You will then repeat the six MVCs and three nerve stimuli 10- and 20-minutes after the exercise protocol to determine the time it takes for the nervous system to recover.

Protocol A, Session 2: Transcranial Magnetic Stimulation With Caffeine:

Session 2 is designed to assess the effects of caffeine ingestion on fatigue-induced reductions in corticospinal excitability/transmission and force production. You will ingest 3 mg•kg⁻¹ of U.S.P grade caffeine supplied in tablet form then wait 1 h to allow the caffeine to be digested. You will then

perform the same procedure as done in Session 1 in order to determine whether caffeine reduces the neural fatigue that occurs with exercise.

Protocol B, Session 1: Vibration Plus Tibial Nerve Stimulation Without Caffeine:

Protocol B will be performed to determine whether fatigue in the corticospinal tract is caused by a decrease in spinal cord ‘amplification’, or a spillover of the neurotransmitter serotonin. You will perform the same standardised warm-up as in the familiarisation, followed by several low-level electrical stimuli delivered to the back of the knee at rest, three MVCs of the calf muscles with simultaneous electrical stimulation and one tendon vibration plus stimulation technique whereby electrical stimulation is applied to the back of the knee in one-second bursts, 5 times, with 1-s between each over a constant vibration of the tendon. These tests will be completed immediately before and immediately, 5-, 10- and 20-minutes after the fatiguing exercise which will be the same as Protocol A (see above).

Protocol B, Session 2: Vibration Plus Tibial Nerve Stimulation With Caffeine:

In Session 2, the effect of caffeine on fatigue-induced reductions in corticospinal excitability/transmission, spinal cord ‘amplification’ and force production will be examined. This session will be identical to Session 1 with the exception that you will ingest caffeine 1 h prior to testing i.e. you will perform the same warm-up, fatigue and test protocols as in Protocol B, Session 1. These sessions will last approximately 2.5 and 3 hours each for the non-caffeine and caffeine trials respectively.

Benefits of study participation

You will have the unique opportunity to learn about and observe advanced neurophysiological techniques that probe the activity of the intact human nervous system, particularly the central motor pathway controlling muscular force production and human movement. You will also have the opportunity to learn about the development of research strategies and research design as well as ask questions about research or any aspect of sports science, neuroscience and the role of neuroscience in sports science research. Additionally, you will get a free isometric ankle extension strength assessment.

Results from your participation will further our understanding of the neuromechanical control of muscle force. In addition, your results will aid in the development of interventions that minimise the loss of corticospinal excitability during fatiguing exercise and, thus, enhance physical performance of patients going through rehabilitation as well as athletes in competition.

Potential risks of study participation

There are some discomforts/risks associated with the procedures being employed in this study. Some of the procedures are considered uncomfortable, but they should not be painful. You will be asked to perform maximal muscular contractions of the calf muscles so there is a very small risk of fainting, exhaustion, cardiac events and musculoskeletal injury, as well as impairment to your ability to perform tasks such as driving after the cessation of exercise, however this risk will be minimised through the use of a standardised warm-up, test familiarisation and monitoring of physical symptoms throughout and the testing session.

Mechanical vibration of the Achilles tendon can cause discomfort, however, it should not be painful. During Achilles tendon vibration you may feel like your ankle joint is moving but this is only a sensation due to sensory feedback caused by the vibration itself. There is a chance for light bruising of the skin underneath the area in contact with the mechanical vibration device.

The use of transcranial magnetic stimulation (TMS) may cause small muscle twitches in the scalp, face or arms and also cause small movements of the limbs being targeted (i.e. calf muscles). Stimulation of these additional areas is sometimes unavoidable due to the overlapping of the motor areas in the brain. On rare occasions TMS may cause muscle tension headaches, which are usually mild and easily treated with aspirin. Accidental seizures with someone who has no known history of epilepsy and syncope are possible but have not been reported since safety standards were implemented in the mid-1990s, and only reported in high-frequency, high-intensity repetitive paired-pulse stimulation. In the present study we will be delivering single-pulse TMS at moderate intensities thus the likelihood of either occurring is low. No side effects have been reported with single-pulse TMS and the present study meets the current safety standards for this technique as discussed by Rossi et al. (2009) and Rossini et al. (2015).

Electrical stimulation of the peripheral tibial nerve can be uncomfortable, but should not be painful. Though highly unlikely, there is a very small risk that you may experience syncope (e.g. fainting) from the electrical stimulation. However, because we are using low electrical stimulation frequencies and moderate stimulation intensities the risk of syncope is very low. Additionally, the light skin abrasion performed prior to application of the skin-based EMG electrodes can increase the chance of skin infections. To minimise this risk, alcohol wipes will be applied to the skin after abrasion as well as after removal of electrodes.

Caffeine ingestion can cause restlessness, dizziness, anxiety, dehydration, higher body temperature, increased breathing and heart rate, and headaches. However, the doses we are using (6

mg/kg of bodyweight) are considered physiologically safe and water can be consumed ad libitum during testing sessions.

All experimental sessions will be completed under the supervision of a qualified researcher (Mr. Benjamin Kirk). We will remind you to tell us immediately if you experience any pain during the experimental procedures. If at any stage you are uncomfortable or want to stop the test, you will be free to withdraw from this study at any stage without reason or prejudice.

Confidentiality of information

Information collected will only be available to Mr. Benjamin Kirk and his team of researchers. Your personal data will be assigned an identification code, such that only those people directly involved in collecting information for the study will be able to recognise which person the information pertains to. So that personal information cannot be accessed by those external to the study, all personal data will be stored securely by filing data collection sheets (paper versions) on file in the School of Medical and Health Sciences and back-up copies on hard disk drive will be safeguarded by the research team on password-protected computers. All collected information will be retained for a period of 5 years after which the information will be destroyed.

Results of the research study

The results of this study will be used for the completion of a Masters by Research degree and may be presented at conferences/seminars and published in peer-reviewed academic journals, as magazine articles, as an online article or part of a book section or report. Additionally, the results of this study may be used in future projects. Published results will be summarised as average data for all participants and no individual data will be published or presented, meaning your personal information cannot be identified unless specific consent for this has been obtained. A copy of published results can be obtained from the investigator upon request.

Participant rights

Your participation in this study is voluntary. No monetary reward will be provided. You retain the right to withdraw from the study or refuse single measurements at any time, and no justification or explanation is needed if you choose not to participate. There will be no consequences for your withdrawal or refusal of single measurements. In addition, if you choose to withdraw from the study, you have the right to withdraw any data that has been collected during the research prior to publication. If you are a student, your grades/assessments will not be impacted should you decline to participate in this study, nor will it affect your relationship with Edith Cowan University. Reporting of the study findings will be done with complete confidentiality and your identity will not be disclosed to any party outside of the study at any time. You have the right to receive information regarding your

own data/results at any time during the study from a member of the research team. You also have the right to withdraw any personal information that has been collected during the research.

ECU Human Research Ethics Committee has approved this research project. Should you have any questions relating to any of the information provided above, please feel free to contact me or my research supervisor, Professor Anthony Blazeovich, for further explanation. If you have any concerns about this research, or would like to speak to an independent person, you may contact the ECU Human Research Ethics Committee. *All contact information is below.*

Sincerely,

Benjamin Kirk, B.Sc. (Hons)

Researchers and contact details

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

M.Sc. candidate and Lead Investigator: Mr. Benjamin Kirk

Supervisor: Professor Anthony Blazeovich

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

M.Sc. Candidate / Lead Researcher

Mr. Benjamin Kirk

Building 19 Room 126

School of Medical and Health Sciences

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270 Joondalup Drive

JOONDALUP, WESTERN AUSTRALIA 6027
6027

Supervisor

Professor Anthony Blazeovich

Building 19 Room 3101

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Phone: +61 422 413 142

Email: b.kirk@ecu.edu.au

Phone: (08) 6304 5472

Email: a.blazeovich@ecu.edu.au

Further details of supervisors and the School of Medical and Health Sciences are available at:

<http://www.ecu.edu.au/schools/exercise-and-health-sciences/overview>

Independent contact person – Edith Cowan University Research Ethics

Research Ethics Support Officer

Human Research Ethics Committee

Office of Research & Innovation

Edith Cowan University

270 Joondalup Drive

JOONDALUP, WESTERN AUSTRALIA 6027

Phone: (08) 6304 2170

Email: research.ethics@ecu.edu.au

The information above should be kept safe so you can refer to it if you need to.

The following page will be detached and kept by the research team.

The acute effects of a single strength training session on motor neurone excitability and facilitation systems

Informed consent form

Mr. Benjamin Kirk and the research team

Consent for study participation

I [PRINT NAME] _____ confirm that I have read the information provided, describing the purpose of the study, methodology (including possible risks) and participant rights. I confirm that any questions I have asked have been answered to my satisfaction by the investigator. I agree to voluntarily participate in this activity, realizing 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 the purpose of the study, what data is being collected and what will be done with the data upon completion of the research. I agree that research data obtained for the study may be published (e.g. peer-reviewed publications, conferences and text books) provided my name or other identifying information is not used. I understand that I can request a copy of this signed form if I wish to retain one for my records.

I request to have my data withdrawn prior to publication if I am excluded or withdraw from the project

☐

Participant signature

Date (DD/MM/YYYY)

Appendix 2: 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.

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/height²) greater Y N Unsure _____

than 30 kg/m²?

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. Do you have any known food, drink and/or medication allergies? | Y | N | _____ _____ |

PART C

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

Y N If so please list,

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 | _____ |
| Recurring neck pain that would make | Y | N | _____ |

exercise problematic, or where exercise may aggravate the pain?

PART C cont'd

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 _____

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

Y N _____

Do you have any medical implants (e.g. cardiac pacemaker, neurostimulators, etc.)?

Y N _____

Do you have any metallic implants (e.g. bone pins, neuros)?

Y N _____

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

Y N _____

Please provide information for an emergency contact below:

Emergency contact name: _____ *Relation:* _____
Home phone number: _____ *Mobile:* _____

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): _____

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 3: Transcranial Magnetic Stimulation Checklist



TMS Assessment Form and Checklist

Participant Name: _____

Date of Birth (DD/MM/YYYY): _____

Phone: _____

Please answer ALL questions to the best of your knowledge. **ALL INFORMATION FROM THIS QUESTIONNAIRE IS STRICTLY CONFIDENTIAL AND IS ONLY USED TO SCREEN FOR SAFE PARTICIPATION IN THIS STUDY.** Thank you for your cooperation.

Does the participant have one or more of the following conditions/implantable devices, which could be effected by TMS?

Positive answers to one or more of questions 1 through 13 may represent contraindications to TMS. A risk/benefit ratio will be considered by the Principal Investigator and the participant will be required to consult with their GP.

1) Do you have epilepsy or have you ever had a convulsion or a seizure? YES NO

2) Have you ever had a fainting spell or syncope? If yes, please describe. YES NO

| | | |
|--|-----|----|
| 3) Have you ever had severe (i.e., followed by loss of consciousness) | | |
| head trauma? | YES | NO |
| 4) Do you have any hearing problems or ringing in your ears? | YES | NO |
| 5) Are you pregnant or is there any chance that you might be? | YES | NO |
| 6) Do you have metal in the brain/skull (except titanium)? | | |
| (e.g. splinters, fragments, clips, etc.) | YES | NO |
| 7) Do you have cochlear implants? | YES | NO |
| 8) Do you have an implanted neurostimulator? | | |
| (e.g., DBS, epidural/subdural, VNS) | YES | NO |
| 9) Do you have a cardiac pacemaker or intracardiac lines or | | |
| metal in your body? | YES | NO |
| 10) Did you ever have a surgical procedure to your spinal cord? | YES | NO |
| 11) Do you have spinal ventricular derivations? | YES | NO |
| 12) Do you have a medication infusion device? | YES | NO |
| 13) Are you taking ANY medications? (Please list) | YES | NO |
| _____ | | |
| _____ | | |
| _____ | | |
| _____ | | |
| _____ | | |
| 14) Did you ever undergo TMS in the past? | YES | NO |
| 15) Did you ever undergo MRI in the past? | YES | NO |

Other: _____

The participant can proceed and undergo TMS: YES NO

Participant signature: _____

Date: _____

Adapted from:

Rossi et al. (2009). Safety, ethical considerations, and application guidelines for the use of transcranial magnetic stimulation in clinical practice and research. *Clinical Neurophysiology*, 10(12): 2008-2039.

Rossini et al. (2015). Non-invasive electrical and magnetic stimulation of the brain, spinal cord, roots and peripheral nerves: basic principles and procedures for routine clinical and research application. An updated report from an I.F.C.N. Committee. *Clinical Neurophysiology*. Advance online publication. doi: <http://dx.doi.org/10.1016/j.clinph.2015.02.001>