Neuromuscular changes and the rapid adaptation following a bout of damaging eccentric exercise.

Stuart Goodall[1], Kevin Thomas[1], Martin Barwood[2], Karen Keane[1], Javier T Gonzalez[3], Alan St Clair Gibson[4] & Glyn Howatson[1,5].

[1]Faculty of Health and Life Sciences, Department of Sport, Exercise & Rehabilitation, Northumbria University, Newcastle, UK.

[2]Department of Sport, Health and Nutrition, Leeds Trinity University, Leeds, UK.

[3]Department for Health, University of Bath, Bath, UK.

[4]Faculty of Health, Sport and Human Performances, University of Waikato, Hamilton, New Zealand.

[5]Water Research Group, School of Environmental Sciences and Development, Northwest University, Potchefstroom, South Africa.

Running Title: Neural function following eccentric exercise.

Keywords: fatigue, lengthening contractions, motor cortex, recovery, repeated bout, stimulation.

Words: 6,050.

Corresponding Author: Dr Stuart Goodall, Faculty of Health and Life Sciences, Northumbria University, Newcastle upon Tyne, UK, NE1 8ST; e-mail: stuart.goodall@northumbria.ac.uk ; fax: +44 (0)191 227 4713; telephone: +44 (0)191 227 4749.

Abstract

An initial bout of eccentric exercise is known to protect against muscle damage following a repeated bout of the same exercise, however, the neuromuscular adaptions owing to this phenomenon are unknown. **Aim:** To determine if neuromuscular disturbances are modulated following a repeated bout of eccentric exercise. **Methods:** Following eccentric exercise performed with the elbow-flexors, we measured maximal voluntary force, resting twitch force, muscle soreness, creatine kinase and voluntary activation using motor point and motor cortex stimulation at baseline, immediately post and at 1, 2, 3, 4 and 7 days post-exercise on two occasions, separated by 3 weeks. **Results:** Significant muscle damage and fatigue was evident following the first exercise bout; maximal voluntary contraction was reduced immediately by 32% and remained depressed at 7 days post-exercise. Soreness and creatine kinase release peaked at 3 and 4 days post-exercise, respectively. Resting twitch force remained significantly reduced at 7 days (−48%) whilst voluntary activation measured with motor point and motor cortex stimulation was reduced until 2 and 3 days, respectively. A repeated bout effect was observed with attenuated soreness and creatine kinase release and a quicker recovery of maximal voluntary contraction and resting twitch force. A similar decrement in voluntary activation was observed following both bouts; however, following the repeated bout there was a significantly smaller reduction in, and a faster recovery of voluntary activation measured using motor cortical stimulation. **Conclusion:** Our data suggest that the repeated bout effect may be explained, partly, by a modification in motor corticospinal drive.

Words: 247

Abbreviations

CK, creatine kinase; CNS, central nervous system; cSP, corticospinal silent period; DOMS, delayed onset muscle soreness; EMG, electromyography; ERT, estimated resting twitch; M_{max}, maximal M wave; MVC, maximum voluntary contraction; MEP, motor evoked potential; RBE, repeated bout effect; SIT, superimposed twitch; TMS, transcranial magnetic stimulation; VA, voluntary activation measured using motor point stimulation; VA_{TMS}, voluntary activation measured using motor cortex stimulation.

Introduction

Unaccustomed eccentric exercise that involves repetitive lengthening muscle actions has been shown to produce damage to ultrastructural and cytoskeletal components of skeletal muscle fibres (Lauritzen et al., 2009). In human models, such disruptions might contribute to an immediate decline in voluntary and evoked muscle force production, which persist for several days after exercise (Prasartwuth et al., 2005, Sayers et al., 2003). In addition to the long-lasting reduction in muscle strength, there is evidence of oedema, muscle stiffness (Lau et al., 2015), inflammation (Tidball, 2005) and soreness (Proske and Allen, 2005) all of which can be evident following damaging exercise. Although the contributions of these events to reduction in force production are yet to be fully elucidated, it is likely they are implicated in the disruption of the excitation contraction coupling process (Corona et al., 2010, Proske and Morgan, 2001). Much of the previous research has focussed on the peripheral response to muscle damage, specifically those associated with the effected skeletal muscle (Corona et al., 2010, Hyldahl et al., 2011, Lacourpaille et al., 2014, Lapier et al., 1995, Lauritzen et al., 2009). However, there are lines of research that have documented changes occurring within the central nervous system (CNS) following unaccustomed, eccentric exercise (Semmler, 2014). Specifically, following eccentric exercise, there is evidence showing reduced inhibition (Pitman and Semmler, 2012) and a reduced voluntary activation (VA; i.e., increased central fatigue) when measured at the motor nerve (Prasartwuth et al., 2005), which might be influenced by muscle length (Prasartwuth et al., 2006). Additionally, other work utilising motor cortical stimulation has provided evidence of acute (Loscher and Nordlund, 2002) and longer lasting (Endoh et al., 2005) central fatigue that was supraspinal in nature. Furthermore, there is some indication that elevations in post-exercise brain cytokines following strenuous eccentric exercise, might also modulate recovery of CNS impairment (Carmichael et al., 2006). In any case, the exact contributing mechanisms are not entirely clear, but the CNS is almost certainly involved with prolonged eccentrically induced strength loss.

Although there are negative consequences of repeated eccentric muscle actions, particularly when performed at a high intensity, a single bout of eccentric exercise can provide a profound protective effect against subsequent bouts. This has been consistently demonstrated by a large reduction in the magnitude of muscle damage and exercise-induced strength loss (Howatson et al., 2009, Howatson et al., 2007, Nosaka and Clarkson, 1995, Gonzalez et al., 2015). This phenomenon is commonly referred to as the repeated bout effect (RBE) and such a rapid, adaptive response, has

been attributed to a number of potential mechanisms involving mechanical, cellular and neural factors (McHugh, 2003). There is a growing body of evidence to suggest that cellular adaptations are contributing to this effect by altered skeletal muscle ultrastructure and remodelling from the initial bout (Hyldahl et al., 2015, Hyldahl et al., 2011, Yu et al., 2004). Additionally, due to the unique recruitment strategy of eccentric contractions (Duchateau & Enoka, 2016), evidence also suggests that neural adaptations are present which have been characterised by changes in muscle unit recruitment and/or synchronisation (Chen, 2003, Howatson et al., 2007, Warren et al., 2000) detected using surface electromyography (EMG). Although data from EMG studies are not without limitations (Farina et al., 2004), when coupled with the observations that corticospinal function is modulated in response to damaging eccentric contractions, it makes the expectation tenable that changes in neural, as well as muscular function, might contribute to the RBE. Such changes in neural function could be evidenced by comparing changes in VA after an initial and second bout of eccentric exercise.

Accordingly, the aims of this investigation were to determine if neuromuscular disturbances are modulated following a repeated bout of eccentric exercise. We hypothesised a significant disturbance in neuromuscular function following the initial bout of exercise would be attenuated in the repeated bout, thereby providing evidence that the CNS is implicated in the repeated bout phenomenon.

Methods

Participants

Eight males (mean ± SD age, 20 ± 2 years; body mass, 74.1 ± 9.9 kg; stature, 1.78 ± 0.05 m) volunteered to participate. It was anticipated that recruiting 8 participants would provide a statistical power of 80%; these numbers were calculated based on the expected attenuation in the primary index of the RBE, MVC, following a repeated bout of eccentric exercise (Howatson et al., 2007) and the typical error score for the measurement of elbow flexor MVC (Allen et al., 1995). All participants were healthy and absent of contraindications for motor cortical stimulation or neuromuscular impairments in the upper limb. The study, in accordance with the Declaration of Helsinki, was approved by the institutional ethics committee and prior to any testing participants provided written, informed consent. All participants were asked to refrain from performing

strenuous exercise for the week preceding the first trial and for the duration of the protocol thereafter.

Experimental Design

Participants visited the laboratory for up to 13 separate occasions at the same time of day (± 1 h), over a 4-week period. Following an initial familiarisation session, on day one of the first testing week baseline measures, including markers of muscle damage and neuromuscular function, were recorded prior to completion of a muscle damaging protocol. All variables were then re-assessed immediately-post, and at 1, 2, 3, 4 and 7 days following the damaging exercise. To allow for complete recovery, three weeks following the initial damaging bout all participants completed an identical damaging bout of eccentric exercise and all variables were re-assessed at the aforementioned time points to investigate the potential contributing mechanisms of the RBE. An overview of the experimental design can be seen in Figure 1.

Eccentric exercise

The protocol was designed to induce muscle damage in the elbow flexors of participant's left, non-dominant arm. An isokinetic dynamometer (System 4 Pro, Biodex Medical Systems Inc. NY, USA) was set up to exercise the elbow flexors as recommended by the manufacturer. Positions of the dynamometer's power head and seat were recorded for each participant for identical replication on subsequent visits. The dynamometer was set in the passive mode to move at $30°\cdot s^{-1}$, which started at full elbow flexion and finished at full extension. The damaging protocol consisted of 30 maximal eccentric contractions (5 sets of 6, separated by 90 s rest). With the hand supinated and the wrist in a neutral positon, participants were instructed to maximally resist the dynamometer arm through the entire anatomical range of motion and subsequently relax through the passive flexion phase (Howatson et al., 2005, Howatson et al., 2007). The damaging bouts of exercise were conducted with identical participant-specific range of motion to ensure the exercise regimen was at matched muscle lengths for both bouts. Peak torque (N·m) and total work (J) performed during each bout was recorded.

Markers of muscle damage

Perceived muscle soreness (DOMS)

A 200 mm visual analogue scale anchored with 'no pain' (0 mm) and 'extremely painful' (200 mm) was used to determine perceived muscle soreness. Participants were asked to rate passive soreness in the exercised arm with the shoulder flexed at 90° with the elbow extended, and active soreness when the elbow joint was actively moved through a full range of motion at their own pace.

Blood sampling and variables

A venous blood sample (~10 mL) was taken by a qualified phlebotomist from an antecubital vein in the non-exercising arm at each of the aforementioned time points. Whole blood was drawn into a EDTA vacutainer system (Becton, Dickinson and Company, Plymouth, New Zealand) and inverted to mix the anticoagulant then immediately centrifuged at 3000 × g for 10 min at 4°C (X-22R Beckman Coulter Allegra™, CA, USA). The supernatant was immediately aliquoted and stored at –80°C for subsequent analysis. Plasma concentrations of creatine kinase (CK) were quantified spectrophotometrically using an automated system (Roche Modular P, Roche Diagnostics, West Sussex, UK). Lower limit of activity was 7 $U \cdot L^{-1}$, and coefficient of variation for the system was 1.93%.

Force and EMG recordings

Isometric elbow flexion force of the exercised arm was measured using a calibrated load cell (NeuroLog NL62, Digitimer, Welwyn Garden City, UK). All force recordings were made while the participant sat at a purpose-made bench with the shoulder and elbow flexed at 90° with the forearm vertical and fully supinated, such that the palm was facing the participant. The load cell was adjusted to a height that was in the direct line of applied force and secured at the wrist via a non-compliant strap. After the skin was shaved, abraded and cleaned, EMG activity was recorded using surface electrodes (Kendall 1401PTS, Tyco Healthcare Group, MA, USA) placed over the tendon and middle of the muscle belly of the biceps brachii (long head), long head of the triceps brachii and brachioradialis muscles according to SENIAM guidelines. The positions of EMG electrodes were marked with indelible ink to ensure consistent placement on subsequent visits. Surface EMG signals were amplified (×100) and band-pass filtered (20-2000 Hz) using CED 1902 amplifiers (Cambridge Electronic Design, Cambridge, UK). Force and EMG signals were sampled at 250 and 4,000 Hz,

respectively, synchronised and stored on a computer using an analogue-to-digital converter (CED 1401, Cambridge Electronic Design) for later analysis (Spike2 v7.12, Cambridge Electronic Design).

Stimulation

Similar to the methods of Todd et al. (2004), three forms of stimulation were used; electrical stimulation of the brachial plexus, electrical stimulation of the biceps motor point and magnetic stimulation over the motor cortex. The evoked compound muscle action potentials in response to all forms of stimulation were recorded using surface EMG.

Stimulation of the brachial plexus. Single electrical stimuli of 100 μs duration were delivered through surface electrodes (32 mm diameter, CF3200, Nidd Valley Medical, Harrogate, UK) to the brachial plexus via a cathode in the supraclavicular fossa (Erb's point) and an anode over the acromion process, using a constant current stimulator (DS7AH Digitimer Ltd, Welwyn Garden City, Hertfordshire, UK). The initial stimulus (20 mA) was increased in steps of 20 mA until the amplitude of the biceps and triceps M-waves reached a maximum value (range, 120 - 280 mA). The final stimulation intensity was increased by a further 20% above the intensity required to produce the maximal compound muscle potential (M_{max}). The subsequent M_{max} amplitude was used to determine the intensity for motor cortical stimulation (see below).

Motor point stimulation. Single electrical stimuli 100 μs duration were delivered using the aforementioned electrical stimulator and surface electrodes over the biceps brachii and brachialis. With the elbow flexed at 90° the cathode was placed midway between the anterior edge of the deltoid and the elbow crease with the anode placed over the distal biceps tendon. The resting twitch of maximal amplitude was determined by step-wise increases in the stimulus intensity until elbow flexor twitch force failed to increase, despite an increase in stimulus intensity. The stimulation intensity (range, 120 - 310 mA) was set 20% above the level required to produce a maximal resting twitch.

Transcranial magnetic stimulation (TMS) of the motor cortex. TMS was delivered over the motor cortex using a Magstim 200^2 stimulator (The Magstim Company Ltd, Whitland, UK). Stimuli were delivered using a figure of eight coil (70 mm outer diameter) with the intersection of the coil placed tangentially to the scalp with the handle pointing backwards and laterally at a 45° angle away from

the midline. After finding the optimal stimulation site, MEPs were elicited in the left biceps with the direction of current flow preferentially activating the right motor cortex (postero-anterior intracranial current flow). Two, active motor thresholds were determined at the beginning of each day. Firstly, as a measure of motor pathway *responsiveness*, the stimulator output was set to evoke an MEP amplitude of >50% M_{max} in biceps and <20% M_{max} in triceps during brief contractions at 20% MVC (range, 50 - 80% stimulator output). Subsequently, for the measurement of *activation*, stimulator output was set to evoke the greatest superimposed twitch (SIT) amplitude during a 50% MVC, which corresponded to an MEP area ~80% M_{max} in biceps and <20% M_{max} in triceps (range, 55 - 80% stimulator output). The optimal coil position was marked on the scalp for consistent positioning throughout the experimental protocol.

Corticospinal responsiveness and intracortical inhibition. Corticospinal responsiveness (MEP/M_{max}) and intracortical inhibition (corticospinal silent period [cSP]) were measured whilst participants contracted at 20% MVC. Eight stimuli were presented 5 s apart and the mean response was used for analysis. At baseline these measures were recorded during a contraction held at 20% of the initial, non-fatigued MVC (absolute). At all post-exercise time points, measures were repeated during 20% of that days MVC force (relative) and at the pre-exercise (absolute) MVC force level.

Data Analysis

The characteristics of all force and EMG parameters were measured offline (Spike2, v7.12, Cambridge Electronic Design). For motor point stimulation, VA was quantified during a maximal contraction using the twitch interpolation method (Merton, 1954); whereby VA (%) = (1 – [SIT/resting twitch]) × 100. The same equation was used to quantify voluntary activation using TMS (VA_{TMS}) but the resting twitch was estimated rather than measured directly; this was necessary due to the differences that exist between cortical and motoneuronal excitability at rest compared to during contraction (Todd et al., 2004, Todd et al., 2003). A linear regression between the size of evoked twitches at 50, 75 and 100% MVC was determined and subsequently the amplitude of the estimated resting twitch (ERT), taken as the *y*-intercept of the regression, was calculated. VA_{TMS} (%) was subsequently quantified using the equation: (1 – [SIT/ERT]) × 100. Peak-to-peak amplitudes and areas of the evoked MEPs and M_{max} were measured offline. To account for any activity dependant changes (Cupido et al., 1996) and to ensure that the motor cortex stimulus during assessment of cortical voluntary activation was activating a high proportion of the biceps brachii motor units, the

area of biceps brachii MEP was normalised to that of the M_{max} elicited at the same contraction strength in each trial. The duration of the cSP evoked by TMS delivered during the 20% contraction was taken as the interval from stimulation artefact until the time at which post-stimulus EMG exceeded ± 2 SD of pre-stimulus EMG for at least 100 ms (Goodall et al., 2010).

Reliability Coefficients

The pre-exercise responses from each visit were used to determine test-retest reliability of the force and EMG responses. Typical error as a coefficient of variation (CV) was calculated for selected variables which demonstrate an acceptable level of reproducibility: MVC, CV = 13.4%; resting twitch force, CV = 12.7%; VA, CV = 1.7%, VA_{TMS}, CV = 2.1%; biceps M_{max}, CV = 21.8%; triceps M_{max}, CV = 24.9%; biceps MEP/M_{max}, 25.5%; biceps rmsEMG, CV = 14.4%; biceps cSP, CV = 14.7%.

Statistical Analysis

All data are reported as mean ± SD unless otherwise stated. The CK data were log transformed before analysis. Differences in the responses between bouts over time were analysed using a repeated measures ANOVA (bout, 2 × time, 7). Assumptions of sphericity were explored and controlled for all variables using the Greenhouse-Geisser adjustment, where appropriate. Where significant bout × time interactions were present, least significant difference *post-hoc* comparisons were used to identify differences between bouts in response to the damaging exercise (SPSS v21, IBM Co., NY, USA). The assumptions of these procedures, including data distribution, were verified as per the guidelines of Newell et al. (2010). The peak and total amount of work performed during each bout of eccentric exercise was assessed using paired samples t-tests. Statistical significance was established prior to all analyses and set at $P \leq 0.05$.

Results

Peak torque (64 ± 9 vs. 65 ± 14 N·m; P = 0.775) and the total amount of work performed (2058 ± 331 vs. 2092 ± 467 J; P = 0.780) during the muscle damaging protocol was similar in both bouts. The eccentric exercise reduced the force capability of the elbow flexors, induced muscle soreness and increased plasma CK, all of which followed a different time course in the days into recovery. Motor point and motor cortical stimulation was used to elicit muscle twitches and subsequently voluntary activation. EMG activity was also measured in response to motor nerve (M_{max}) and motor cortex

(MEP) stimulation. Compared to the initial bout, the repeated bout of eccentric exercise elicited changes in some of these variables. Representative examples are shown in Figure 2.

Markers of Muscle Damage. Perceived active muscle soreness was reduced following the second bout of exercise compared to the first ($F_{1,7}$ = 5.58, P = 0.050, partial η^2 = 0.44) and a significant interaction effect was present ($F_{6,42}$ = 4.23, P = 0.016). *Post hoc* comparisons revealed that soreness was greater in bout 1 at 1, 2, 3 and 4 days post-exercise compared to bout 2 (Figure 3A). Following the initial bout of eccentric exercise, MVC force was significantly reduced (F = 15.70, P < 0.001). Specifically, the largest reduction in MVC was observed immediately post-exercise (372 ± 46 vs. 242 ± 61 N; −35 ± 14%) and remained depressed until 7 days post-exercise. There was a greater loss in MVC following bout 1 compared to bout 2 ($F_{1,7}$ = 6.49, P = 0.038, partial η^2 = 0.48; Figure 3B). Similarly, there was a greater CK efflux in bout 1 compared to bout 2 (peak values 3,109 ± 5,263 vs. 347 ± 190 $IU \cdot L^{-1}$, mean difference = 2,762 $IU \cdot L^{-1}$; $F_{1,7}$ = 6.65, P = 0.037, partial η^2 = 0.49).

Neuromuscular Function. In line with the aforementioned reduction in MVC, the resting twitch force evoked from the biceps was reduced for the whole week following the first bout of eccentric exercise (F = 27.43, P < 0.001). Specifically, the greatest decrement in the potentiated twitch was observed immediately post-exercise (57 ± 13 vs. 15 ± 11 N; −72 ± 23%) and 7 days post-exercise this variable was still reduced by 42 ± 34% (30 ± 15 N). A similar reduction in the potentiated twitch force was observed immediately-post both bouts of eccentric exercise ($F_{1,7}$ = 1.2, P = 0.309, partial η^2 = 0.17), however, in bout 2 the recovery of peripheral fatigue in the days post-exercise was accelerated compared to bout 1 ($F_{6,42}$ = 4.55, P = 0.039). *Post hoc* comparisons revealed that the potentiated twitch was higher in bout 2 compared to bout 1 at 7 days post-exercise (30 ± 15 vs. 48 ± 8 N; Figure 4A). The ERT was reduced following exercise in bout 1 (F = 12.10, P < 0.001) with the greatest decrement immediately post-exercise (49 ± 18 vs. 22 ± 9 N; −55 ± 13%), which remained depressed 7 days post-exercise (39 ± 19 N; −8 ± 33%). There was no bout ($F_{1,7}$ = 0.133, P = 0.728, partial η^2 = 0.22) or interaction effect ($F_{6,42}$ = 0.43, P = 0.674) (Figure 4B).

During maximal voluntary contractions following eccentric exercise the force evoked by motor point and motor cortex stimulation increased and VA was reduced (F > 10.69, P < 0.001). VA (measured using motor point stimulation) was reduced immediately following the exercise in bout 1 (−28 ± 25%) and had recovered 2 days post-exercise (Figure 5A). A similar decrement in VA measured using

motor point stimulation, was observed in bout 2 compared to bout 1 ($F_{1,7}$ = 1.78, P = 0.223, partial η^2 = 0.20). VA_{TMS} was reduced immediately post-exercise (−19 ± 13%); however, this was not recovered until 3 days post-exercise (Figure 5B). There was a smaller reduction in VA_{TMS} following the exercise in bout 2 compared to bout 1 ($F_{1,7}$ = 18.78, P = 0.003, partial η^2 = 0.73) and an accelerated recovery was observed ($F_{6,42}$ = 3.23, P = 0.011). Specifically, VA_{TMS} was recovered 1 day post-exercise in bout 2 and these data were different from bout 1 immediately post-, 1 and 2 days post-exercise. Following the exercise in bout 2 the amount of force evoked by motor cortical stimulation during maximal contractions was less than in bout 1 ($F_{1,7}$ = 6.42, P = 0.039, partial η^2 = 0.48; Figure 6B).

Corticospinal Responsiveness. The resting M_{max} evoked in the biceps ($F_{1,7}$ = 4.24, P = 0.079, partial η^2 = 0.38) and triceps ($F_{1,7}$ = 0.03, P = 0.860, partial η^2 = 0.05) did not differ between bouts. Similarly, the biceps MEP/M_{max} ratios for amplitude ($F_{1,7}$ < 0.86, P > 0.387) and area ($F_{1,7}$ < 0.11, P > 0.710) determined during contractions at 100, 75 and 50% MVC, did not differ between bouts (Table 1). The MEP/M_{max} ratio (Table 2) and cSP measured in the biceps during a contraction at 20% MVC did not differ between bouts at the absolute ($F_{1,7}$ < 1.68, P > 0.236) or relative contraction intensities ($F_{1,7}$ < 0.91, P > 0.373).

Discussion

This is the first study to investigate the modulation of the neuromuscular and corticospinal responses to a repeated bout of damaging eccentric exercise. A significant disruption in neuromuscular function was observed following the initial bout of damaging exercise. We confirmed a repeated bout effect by the faster recovery in muscle force and reduced soreness; this rapid adaptation was associated with an attenuation of supraspinal fatigue, and a faster recovery of both supraspinal fatigue and peripheral function. These data extend our understanding regarding the neural contributions to the repeated bout effect, suggesting that the repeated bout effect is partly mediated by changes within the central nervous system.

Acute impairments in neuromuscular function following eccentric exercise

It has previously been suggested that a loss in maximal voluntary force is one of the most valid and reliable markers of muscle damage in humans (Warren et al., 1999). In the present study, elbow flexor MVC was reduced by 38% immediately following eccentric exercise and was not recovered until 7 days post-exercise. Presumably this result is a consequence of disruption to the processes

involved in excitation-contraction coupling and it is a finding commonly observed (Clarkson and Hubal, 2002, Endoh et al., 2005, Prasartwuth et al., 2005). Perceived soreness increased in the days following the initial bout and peaked 2 days post-exercise. Immediately following the initial bout, a profound change in peripheral function was evident where resting twitch force evoked by biceps motor point stimulation was reduced by 76%. The reduction persisted in the days following the exercise and was still depressed by 50%, 7 days post-exercise. Similar observations of prolonged reductions in maximum voluntary force and peripheral function have been previously reported after varying muscle damage protocols (Prasartwuth et al., 2005, Sayers et al., 2003). The resting twitch estimated using cortical stimulation was also reduced following the exercise and subsequent days, but to a lesser extent than that found with direct stimulation of the biceps motor point. Similar to Prasartwuth et al. (2005), the change in the ERT in this investigation was more closely aligned with the change in MVC (R^2 = 0.92), rather than the peripheral twitch evoked by electrical stimulation (R^2 = 0.78). The reductions in peripheral function are unlikely attributable to changes in sarcolemma excitability, as the maximal M-wave in response to muscle damaging exercise remained unchanged (Prasartwuth et al., 2005, Sayers et al., 2003). More likely, the reduction in the peripheral twitch is due to the presence of disrupted sarcomeres within myofibrils (Lauritzen et al., 2009, Proske and Morgan, 2001) and damage to components of the excitation-contraction coupling process (Corona et al., 2010, Warren et al., 2001). These lines of investigation collectively suggest that such disruptions in contractile apparatus can explain the long-lasting decrements in peripheral function following damaging eccentric exercise.

Although there seems to be good evidence that there is post-synaptic disruption in the ability to generate force following eccentric exercise (Corona et al., 2010, Proske and Allen, 2005, Proske and Morgan, 2001, Warren et al., 2001), the extent to which the CNS might contribute to the delayed recovery and ability to generate force has received less attention. Using the twitch interpolation technique, we found that VA was reduced immediately following the muscle damaging protocol and did not recover until 48 h post-exercise. These data are in line with Prasartwuth et al. (2005), who reported reductions in VA immediately post-exercise, which recovered by 24 h post. Thus, the loss in voluntary force following eccentric exercise is partly due to mechanisms relating to central fatigue. We also observed a significant reduction in VA_{TMS} which persisted until 72 h post-exercise indicating a persistent suboptimal output from the motor cortex. Immediately post-exercise in the initial bout, the relative size of superimposed twitches elicited by TMS were increased by ~65%

demonstrating the cortical stimulus was able to evoke additional output from the M1 despite maximal voluntary effort. Two days following eccentric exercise the relative size of the superimposed twitches elicited during maximal contractions was still increased from baseline by one quarter. Prasartwuth et al. (2005) did not show this response; following eccentric exercise a reduction in VA_{TMS} was not found and a failure in corticospinal drive relating to factors 'upstream' of the motor cortex were discounted. A critical point that should be highlighted is that post-exercise reductions in force were similar, but the exercise stimulus was vastly different. Prasartwuth et al. (2005) used submaximal contractions (30% of predicted eccentric MVC) until isometric MVC was reduced by 40%, which was attained by a huge range of contractions (40-350) within the cohort. We observed the same reductions in MVC, but prescribed a standardised exercise bout of 30 maximal eccentric contractions for all participants. Conceptually, the prolonged reduction in VA_{TMS} in the current investigation could be attributable to the *maximal* intensity of eccentric exercise, which might pose a greater challenge to supraspinal drive when compared to submaximal efforts (Prasartwuth et al., 2005). Although both studies achieved similar reductions in maximum force, the mechanisms responsible for the exercise-induced force loss appear to be different and should be considered in future investigations aiming to elucidate exercise-induced force loss following resistance exercise.

Based on their data, Prasartwuth et al. (2005) proposed that the disparate results between motor point and motor cortical stimulation were attributable to inhibition in the motor cortex and/or motorneurones that limits voluntary drive to the muscle following eccentric exercise. To test this postulate we investigated if the cSP, a surrogate of corticospinal inhibition, was affected in response to exercise induced muscle damage. The cSP was not affected at any time point following the eccentric exercise. In contrast, Pitman and Semmler (2012) reported a reduction in short interval intracortical inhibition immediately following maximal eccentric exercise performed with the elbow flexors and attributed this finding to a short term change in afferent feedback within the muscle resulting from the damage. Thus, it is possible the eccentric muscle damage elicits changes in inhibition, but these were not identified by a change in the cSP in the present study. Inhibition at a cortical and spinal level cannot be excluded as potential mechanisms that might explain our observations. Moreover, spinal excitability and inhibition should not solely be determined with measurement of the H-reflex as this measure is not purely monosynaptic and can be modulated by several factors that might be post- and/or presynaptic. Specifically, presynaptic inhibition can lead

to changes in H-reflex amplitude without any change in motoneuron excitability (Knikou, 2008, McNeil et al., 2013). To understand the role of spinal inhibition and excitability following eccentric exercise, future investigations need to apply H-reflex conditioning and/or to measure responses following cervicomedullary stimulation.

Although somewhat speculative, an alternative mechanism that might explain the observations associated with supraspinal fatigue is related to the post-exercise inflammatory response. An inflammatory response following eccentric exercise has previously been shown (Paulsen et al., 2012, Tidball, 2005). Specifically, the elevations of inflammatory cytokines are potent effectors of CNS function (Carmichael et al., 2006, de Rivero Vaccari et al., 2015). It has been reported that increased brain concentrations of the cytokine interleukin 1 beta (IL-1β), whether injected directly into cerebral tissue or elevated in response to inflammation, can induce negative behavioural responses (Dantzer, 2004) and fatigue (Sheng et al., 2001, Swain et al., 1998). Moreover, increased brain IL-1β within the cerebellum and cortex has been shown following downhill running, in conjunction with the delayed recovery of running performance for up to 48 h post-exercise (Carmichael et al., 2005) which is comparable with the length of time that supraspinal fatigue was evident in the present study. Although not observed in hominids, it is plausible that such a cytokine mediated inflammatory response could have contributed to supraspinal fatigue following eccentric exercise in the present study and this postulate warrants further investigation.

Taken together, our data show that a prolonged reduction in neuromuscular function following an acute bout of eccentric exercise is predominately due to peripheral fatigue, likely stemming from disruptions in contractile apparatus. Importantly, mechanisms of central fatigue are also involved during this prolonged recovery. The motor cortex does not optimally drive the target muscle for up to 48 h post-exercise, which might be linked to the inflammatory response elicited by muscle damaging exercise.

The repeated bout effect and changes in neuromuscular function

The total work performed during the muscle damaging protocol was similar in each bout, demonstrating the exercise stimulus was not different and produced no discernible training effect with regards to the volume of work that could be achieved. In line with the classic repeated bout response, we observed an attenuated response in the primary markers of muscle damage following

the second bout of eccentric exercise (Howatson et al., 2007, Lau et al., 2015, Nosaka et al., 2001). Specifically, the plasma concentration of CK and ratings of muscle soreness were lower, whilst the recovery of maximal force generating capacity was accelerated. Furthermore, the faster recovery of *involuntary* force production (potentiated twitch amplitude) following bout 2 lends support to the notion that the repeated bout is mediated by a mechanical adaptation (McHugh, 2003). The increased appearance of desmin (Yu and Thornell, 2002), the principal component in the Z-band structure, seems to contribute to the improved integrity of passive structures which is evidenced by a thickening of the Z-band following the initial bout, and probably plays an important contributing role to the RBE (Yu et al., 2004). Furthermore, increased dynamic stiffness such as a reduced myotendinous junction displacement during the second bout (Lau et al., 2015), along with extracellular matrix remodelling (Hyldahl et al., 2015, Janecki et al., 2011, Pousson et al., 1990) have been demonstrated during and following repeated bouts of eccentric exercise. Therefore, a stiffer muscle-tendon unit from the aforementioned remodelling, could increase the integrity of connective tissue (Lapier et al., 1995), and maintain active and passive structures within the sarcomeres. When translated, forces during the eccentric stretch of sarcomeres, can be better tolerated because of greater passive tension (generated by the non-contractile elements of muscle, particularly at longer muscle lengths), acting to provide greater protection against damage from a subsequent bout of eccentric activity (Lacourpaille et al., 2014, Lau et al., 2015).

No changes were evident in corticospinal excitability when tested at the absolute or relative force levels (Table 2). It is possible that the MEP data presented in the absolute condition are biased due to the increased background EMG following the protocol, as MEPs increase with contraction strength with no change in the M_{max} (Sidhu et al., 2009). Despite this, our relative data confirm there was no change in corticospinal excitability with the RBE and future investigations should be aware of measuring responses at absolute and relative force levels. Rather, our data suggest that a modification in neural drive to the exercising muscle might contribute to the repeated bout effect. Specifically, supraspinal fatigue was evident following the initial bout of eccentric exercise, however, following the repeated bout, the motor cortex was better able to drive the muscle and an attenuated level of supraspinal fatigue was evident immediately post-exercise and during the days into recovery. This adaptive response, shown by stimulation of M1, is the first direct evidence showing that neural drive is altered following the repeated bout and provides support for aforementioned studies using EMG in isolation (Chen, 2003, Howatson et al., 2007, Warren et al.,

2000), which is not without limitations (Farina et al., 2004). Despite some evidence to suggest there are no changes in firing frequency during low level eccentric contractions (Petersen et al., 2007), eccentric muscle contractions have been shown to have a unique recruitment strategy (Duchateau and Enoka, 2016, Nardone et al., 1989, Howell et al., 1995). It is for this reason why neural adaptations have been one of the proposed mechanisms that mediate the repeated bout effect (Howatson et al., 2007, McHugh, 2003). Indeed, neural adaptations accompany all types of strength training (Carroll et al., 2011), however, there is some evidence to show a decrease in frequency content of the EMG signal following a repeated bout of eccentric exercise (Chen, 2003, Howatson et al., 2007). This alteration has been suggested to be a de-recruitment of faster motor units or the preferential recruitment of additional slower motor units and/or increased motor unit synchronisation during the repeated bout (Hortobagyi et al., 1996), thereby serving to better distribute the workload across the muscle fibres (Nosaka and Clarkson, 1995). EMG activity was not measured *during* the muscle damaging protocols in the present study, however, previous studies (Chen, 2003, Howatson et al., 2007) did show a reduction in EMG frequency content, and as such, should not (despite the limitations) be ruled out as a potential contributor to the RBE in the current study. Furthermore, the primary argument against a neural mechanism is the presence of a repeated bout following exercise evoked using electrical stimulation, whereby conscious neural control is absent (McBride, 2003, Sacco and Jones, 1992). However, the critical element to consider is that although the brain-to-muscle motor path is by-passed, afferent feedback is not, and subsequent modulation of motor engrams and recruitment strategies cannot be excluded (Bard et al., 1995). Thus, the attenuated level of supraspinal fatigue following the second bout of eccentric exercise might be a consequence of the altered recruitment strategy during the repeated activity that reduced the magnitude of damage.

Interestingly, when measuring voluntary activation, the results obtained with motor point stimulation do not follow those obtained using motor cortex stimulation. The technical aspects owing to the measurement of voluntary activation have been discussed previously (Taylor, 2009). However, despite these technical limitations, it is critical to provide a plausible explanation for the apparent change in failure to drive the muscles from the motor cortex, without a change in drive from the entire neuraxis. Our data suggest that a sub-optimal output from the cortex was manipulated somewhere along the pathway to the motoneurons, possibly modulated at a spinal level. The unique recruitment strategy that accompanies the performance of an eccentric

contraction, as discussed above, indicates involvement of more functional regions within the brain (Duclay et al., 2008, Fang et al., 2004). Moreover, the response of neural excitability during eccentric contractions is somewhat unclear with investigations reporting reductions (Gruber et al., 2009), no change (Hahn et al., 2012) and increases (Abbruzzese et al., 1994). A period of training with eccentric contractions has been shown to increase neural drive from supraspinal centers (Duclay et al., 2008), with concomitant increases in antagonist EMG activity (Linnamo et al., 2002) and reduced spinal inhibition (Aagaard et al., 2000). Thus, repeated eccentric contractions seem to manipulate neural excitability. The present study demonstrated an increased corticospinal drive, however, this was not following a period of training, but after a repeated bout of maximal eccentric contractions. Thus, the RBE phenomenon involves neurally induced adaptations, which are likely to influence voluntary activation data when using motor cortical and motor point stimulation. Future investigations should aim to understand the role of spinal and/or cortical adaptations following repeated bouts of eccentric exercise.

Conclusion

In summary, an initial bout of eccentric exercise produces a marked and long-lasting reduction in neuromuscular function. The large reductions in peripheral twitch characteristics rather than maximal M-waves, suggests a significant disturbance in excitation-contraction coupling. Furthermore, mechanisms associated with central fatigue are evident and motor corticospinal output remains suboptimal for 48 h post-exercise. We confirmed a repeated bout effect via the faster recovery in muscle force and reduced soreness and this was associated with an attenuated level of supraspinal fatigue. This work provides new information regarding neural contributions to the repeated bout effect, specifically showing a faster recovery of centrally mediated mechanisms of fatigue after a second bout of eccentric exercise. Importantly, our data suggest that changes eliciting the repeated bout phenomenon may be attributed, at least in part, to a modification in motor corticospinal drive.

References

Aagaard, P., Simonsen, E. B., Andersen, J. L., Magnusson, S. P., Halkjaer-Kristensen, J. & Dyhre-Poulsen, P. 2000. Neural inhibition during maximal eccentric and concentric quadriceps contraction: effects of resistance training. *J Appl Physiol,* **89,** 2249-57.

Abbruzzese, G., Morena, M., Spadavecchia, L. & Schieppati, M. 1994. Response of arm flexor muscles to magnetic and electrical brain stimulation during shortening and lengthening tasks in man. *J Physiol,* **481,** 499-507.

Allen, G. M., Gandevia, S. C. & McKenzie, D. K. 1995. Reliability of measurements of muscle strength and voluntary activation using twitch interpolation. *Muscle Nerve,* **18,** 593-600.

Bard, C., Fleury, M., Teasdale, N., Paillard, J. & Nougier, V. 1995. Contribution of proprioception for calibrating and updating the motor space. *Can J Physiol Pharmacol,* **73,** 246-54.

Carmichael, M. D., Davis, J. M., Murphy, E. A., Brown, A. S., Carson, J. A., Mayer, E. & Ghaffar, A. 2005. Recovery of running performance following muscle-damaging exercise: relationship to brain IL-1beta. *Brain Behav Immun,* **19,** 445-52.

Carmichael, M. D., Davis, J. M., Murphy, E. A., Brown, A. S., Carson, J. A., Mayer, E. P. & Ghaffar, A. 2006. Role of brain IL-1beta on fatigue after exercise-induced muscle damage. *Am J Physiol Regul Integr Comp Physiol,* **291,** R1344-8.

Carroll, T. J., Selvanayagam, V. S., Riek, S. & Semmler, J. G. 2011. Neural adaptations to strength training: moving beyond transcranial magnetic stimulation and reflex studies. *Acta Physiol,* **202,** 119-40.

Chen, T. C. 2003. Effects of a second bout of maximal eccentric exercise on muscle damage and electromyographic activity. *Eur J Appl Physiol,* **89,** 115-21.

Clarkson, P. M. & Hubal, M. J. 2002. Exercise-induced muscle damage in humans. *Am J Phys Med Rehabil,* **81,** S52-69.

Corona, B. T., Balog, E. M., Doyle, J. A., Rupp, J. C., Luke, R. C. & Ingalls, C. P. 2010. Junctophilin damage contributes to early strength deficits and EC coupling failure after eccentric contractions. *Am J Physiol Cell Physiol,* **298,** C365-76.

Cupido, C. M., Galea, V. & McComas, A. J. 1996. Potentiation and depression of the M wave in human biceps brachii. *J Physiol,* **491,** 541-50.

Dantzer, R. 2004. Cytokine-induced sickness behaviour: a neuroimmune response to activation of innate immunity. *Eur J Pharmacol,* **500,** 399-411.

de Rivero Vaccari, J. P., Dietrich, W. D. & Keane, R. W. 2015. Therapeutics targeting the inflammasome after central nervous system injury. *Transl Res,* **167,** 35-45.

Duchateau, J. & Enoka, R. M. 2016. Neural control of lengthening contractions. *J Exp Biol,* **219,** 197-204.

Duclay, J., Martin, A., Robbe, A. & Pousson, M. 2008. Spinal reflex plasticity during maximal dynamic contractions after eccentric training. *Med Sci Sports Exerc,* **40,** 722-34.

Endoh, T., Nakajima, T., Sakamoto, M. & Komiyama, T. 2005. Effects of muscle damage induced by eccentric exercise on muscle fatigue. *Med Sci Sports Exerc,* **37,** 1151-1156.

Fang, Y., Siemionow, V., Sahgal, V., Xiong, F. & Yue, G. H. 2004. Distinct brain activation patterns for human maximal voluntary eccentric and concentric muscle actions. *Brain Res,* **1023,** 200-12.

Farina, D., Merletti, R. & Enoka, R. M. 2004. The extraction of neural strategies from the surface EMG. *J Appl Physiol,* **96,** 1486-95.

Gonzalez, J. T., Barwood, M. J., Goodall, S., Thomas, K. & Howatson, G. 2015. Alterations in Whole-Body Insulin Sensitivity Resulting From Repeated Eccentric Exercise of a Single Muscle Group: A Pilot Investigation. *Int J Sport Nutr Exerc Metab,* **25,** 405-10.

Goodall, S., Ross, E. Z. & Romer, L. M. 2010. Effect of graded hypoxia on supraspinal contributions to fatigue with unilateral knee-extensor contractions. *J Appl Physiol,* **109,** 1842-51.

Gruber, M., Linnamo, V., Strojnik, V., Rantalainen, T. & Avela, J. 2009. Excitability at the motoneuron pool and motor cortex is specifically modulated in lengthening compared to isometric contractions. *J Neurophysiol,* **101,** 2030-40.

Hahn, D., Hoffman, B. W., Carroll, T. J. & Cresswell, A. G. 2012. Cortical and spinal excitability during and after lengthening contractions of the human plantar flexor muscles performed with maximal voluntary effort. *PLoS One,* **7,** e49907.

Hortobagyi, T., Hill, J. P., Houmard, J. A., Fraser, D. D., Lambert, N. J. & Israel, R. G. 1996. Adaptive responses to muscle lengthening and shortening in humans. *J Appl Physiol,* **80,** 765-72.

Howatson, G., Gaze, D. & van Someren, K. A. 2005. The efficacy of ice massage in the treatment of exercise-induced muscle damage. *Scand J Med Sci Sports,* **15,** 416-22.

Howatson, G., Goodall, S. & van Someren, K. A. 2009. The influence of cold water immersions on adaptation following a single bout of damaging exercise. *Eur J Appl Physiol,* **105,** 615-21.

Howatson, G., Van Someren, K. & Hortobagyi, T. 2007. Repeated bout effect after maximal eccentric exercise. *Int J Sports Med,* **28,** 557-63.

Howell, J. N., Fuglevand, A. J., Walsh, M. L. & Bigland-Ritchie, B. 1995. Motor unit activity during isometric and concentric-eccentric contractions of the human first dorsal interosseus muscle. *J Neurophysiol,* **74,** 901-4.

Hyldahl, R. D., Nelson, B., Xin, L., Welling, T., Groscost, L., Hubal, M. J., Chipkin, S., Clarkson, P. M. & Parcell, A. C. 2015. Extracellular matrix remodeling and its contribution to protective adaptation following lengthening contractions in human muscle. *FASEB J,* **29,** 2894-904.

Hyldahl, R. D., Xin, L., Hubal, M. J., Moeckel-Cole, S., Chipkin, S. & Clarkson, P. M. 2011. Activation of nuclear factor-kappaB following muscle eccentric contractions in humans is localized primarily to skeletal muscle-residing pericytes. *FASEB J,* **25,** 2956-66.

Janecki, D., Jarocka, E., Jaskolska, A., Marusiak, J. & Jaskolski, A. 2011. Muscle passive stiffness increases less after the second bout of eccentric exercise compared to the first bout. *J Sci Med Sport,* **14,** 338-43.

Knikou, M. 2008. The H-reflex as a probe: pathways and pitfalls. *J Neurosci Methods,* **171,** 1-12.

Lacourpaille, L., Nordez, A., Hug, F., Couturier, A., Dibie, C. & Guilhem, G. 2014. Time-course effect of exercise-induced muscle damage on localized muscle mechanical properties assessed using elastography. *Acta Physiol,* **211,** 135-46.

Lapier, T. K., Burton, H. W., Almon, R. & Cerny, F. 1995. Alterations in intramuscular connective tissue after limb casting affect contraction-induced muscle injury. *J Appl Physiol,* **78,** 1065-9.

Lau, W. Y., Blazevich, A. J., Newton, M. J., Wu, S. S. & Nosaka, K. 2015. Reduced muscle lengthening during eccentric contractions as a mechanism underpinning the repeated-bout effect. *Am J Physiol Regul Integr Comp Physiol,* **308,** R879-86.

Lauritzen, F., Paulsen, G., Raastad, T., Bergersen, L. H. & Owe, S. G. 2009. Gross ultrastructural changes and necrotic fiber segments in elbow flexor muscles after maximal voluntary eccentric action in humans. *J Appl Physiol,* **107,** 1923-34.

Linnamo, V., Strojnik, V. & Komi, P. V. 2002. EMG power spectrum and features of the superimposed M-wave during voluntary eccentric and concentric actions at different activation levels. *Eur J Appl Physiol,* **86,** 534-40.

Loscher, W. N. & Nordlund, M. M. 2002. Central fatigue and motor cortical excitability during repeated shortening and lengthening actions. *Muscle Nerve,* **25,** 864-72.

McBride, T. A. 2003. Stretch-activated ion channels and c-fos expression remain active after repeated eccentric bouts. *J Appl Physiol,* **94,** 2296-302.

McHugh, M. P. 2003. Recent advances in the understanding of the repeated bout effect: the protective effect against muscle damage from a single bout of eccentric exercise. *Scand J Med Sci Sports,* **13,** 88-97.

McNeil, C. J., Butler, J. E., Taylor, J. L. & Gandevia, S. C. 2013. Testing the excitability of human motoneurons. *Front Hum Neurosci,* **7,** 152.

Merton, P. A. 1954. Voluntary strength and fatigue. *J Physiol,* **123,** 553-64.

Nardone, A., Romano, C. & Schieppati, M. 1989. Selective recruitment of high-threshold human motor units during voluntary isotonic lengthening of active muscles. *J Physiol,* **409,** 451-71.

Newell, J., Aitchison, T. & Grant, S. 2010. *Statistics for Sports and Exercise Science: A practical approach.,* Harlow, Pearson Education.

Nosaka, K. & Clarkson, P. M. 1995. Muscle damage following repeated bouts of high force eccentric exercise. *Med Sci Sports Exerc,* **27,** 1263-9.

Nosaka, K., Sakamoto, K., Newton, M. & Sacco, P. 2001. The repeated bout effect of reduced-load eccentric exercise on elbow flexor muscle damage. *Eur J Appl Physiol,* **85,** 34-40.

Paulsen, G., Mikkelsen, U. R., Raastad, T. & Peake, J. M. 2012. Leucocytes, cytokines and satellite cells: what role do they play in muscle damage and regeneration following eccentric exercise? *Exerc Immunol Rev,* **18,** 42-97.

Petersen, N. T., Butler, J. E., Carpenter, M. G. & Cresswell, A. G. 2007. Ia-afferent input to motoneurons during shortening and lengthening muscle contractions in humans. *J Appl Physiol,* **102,** 144-8.

Pitman, B. M. & Semmler, J. G. 2012. Reduced short-interval intracortical inhibition after eccentric muscle damage in human elbow flexor muscles. *J Appl Physiol,* **113,** 929-36.

Pousson, M., Van Hoecke, J. & Goubel, F. 1990. Changes in elastic characteristics of human muscle induced by eccentric exercise. *J Biomech,* **23,** 343-8.

Prasartwuth, O., Allen, T. J., Butler, J. E., Gandevia, S. C. & Taylor, J. L. 2006. Length-dependent changes in voluntary activation, maximum voluntary torque and twitch responses after eccentric damage in humans. *J Physiol,* **571,** 243-52.

Prasartwuth, O., Taylor, J. L. & Gandevia, S. C. 2005. Maximal force, voluntary activation and muscle soreness after eccentric damage to human elbow flexor muscles. *J Physiol,* **567,** 337-48.

Proske, U. & Allen, T. J. 2005. Damage to skeletal muscle from eccentric exercise. *Exerc Sport Sci Rev,* **33,** 98-104.

Proske, U. & Morgan, D. L. 2001. Muscle damage from eccentric exercise: mechanism, mechanical signs, adaptation and clinical applications. *J Physiol,* **537,** 333-45.

Sacco, P. & Jones, D. A. 1992. The protective effect of damaging eccentric exercise against repeated bouts of exercise in the mouse tibialis anterior muscle. *Exp Physiol,* **77,** 757-60.

Sayers, S. P., Peters, B. T., Knight, C. A., Urso, M. L., Parkington, J. & Clarkson, P. M. 2003. Short-term immobilization after eccentric exercise. Part I: contractile properties. *Med Sci Sports Exerc,* **35,** 753-61.

Semmler, J. G. 2014. Motor unit activity after eccentric exercise and muscle damage in humans. *Acta Physiol,* **210,** 754-67.

Sheng, W. S., Hu, S., Ding, J. M., Chao, C. C. & Peterson, P. K. 2001. Cytokine expression in the mouse brain in response to immune activation by Corynebacterium parvum. *Clin Diagn Lab Immunol,* **8,** 446-8.

Sidhu, S. K., Bentley, D. J. & Carroll, T. J. 2009. Cortical voluntary activation of the human knee extensors can be reliably estimated using transcranial magnetic stimulation. *Muscle Nerve,* **39,** 186-96.

Swain, M. G., Beck, P., Rioux, K. & Le, T. 1998. Augmented interleukin-1beta-induced depression of locomotor activity in cholestatic rats. *Hepatology,* **28,** 1561-5.

Taylor, J. L. 2009. Point: the interpolated twitch does/does not provide a valid measure of the voluntary activation of muscle. *J Appl Physiol,* **107,** 354-5.

Tidball, J. G. 2005. Inflammatory processes in muscle injury and repair. *Am J Physiol Regul Integr Comp Physiol,* **288,** R345-53.

Todd, G., Taylor, J. L. & Gandevia, S. C. 2003. Measurement of voluntary activation of fresh and fatigued human muscles using transcranial magnetic stimulation. *J Physiol,* **551,** 661-71.

Todd, G., Taylor, J. L. & Gandevia, S. C. 2004. Reproducible measurement of voluntary activation of human elbow flexors with motor cortical stimulation. *J Appl Physiol,* **97,** 236-42.

Warren, G. L., Hermann, K. M., Ingalls, C. P., Masselli, M. R. & Armstrong, R. B. 2000. Decreased EMG median frequency during a second bout of eccentric contractions. *Med Sci Sports Exerc,* **32,** 820-9.

Warren, G. L., Ingalls, C. P., Lowe, D. A. & Armstrong, R. B. 2001. Excitation-contraction uncoupling: major role in contraction-induced muscle injury. *Exerc Sport Sci Rev,* **29,** 82-7.

Warren, G. L., Lowe, D. A. & Armstrong, R. B. 1999. Measurement tools used in the study of eccentric contraction-induced injury. *Sports Med,* **27,** 43-59.

Yu, J. G., Carlsson, L. & Thornell, L. E. 2004. Evidence for myofibril remodeling as opposed to myofibril damage in human muscles with DOMS: an ultrastructural and immunoelectron microscopic study. *Histochem Cell Biol,* **121,** 219-27.

Yu, J. G. & Thornell, L. E. 2002. Desmin and actin alterations in human muscles affected by delayed onset muscle soreness: a high resolution immunocytochemical study. *Histochem Cell Biol,* **118,** 171-9.

Author Contributions

All experiments were performed in the Neurophysiology Laboratory at Northumbria University, Newcastle upon Tyne, UK; SG, KT and GH contributed to the conception/deign of the work, acquired the data, whilst contributing to analysis and interpretation of the work. MB, KK, JG, ASG acquired the data, whilst contributing to analysis and interpretation of the work. All authors and have drafted/revised the intellectual content and approved the final version. All listed authors qualify for authorship.

Funding

No funding was received for this study.

Acknowledgements

This work was conducted and supported by Northumbria University in its entirety. The authors wish to acknowledge the help from Mr Dean Crangle for assistance with data analysis and Dr Janet Taylor for feedback on an early draft of the results.

Table & Figure Legends

Table 1. Maximal responses to motor nerve stimulation and contraction specific MEP/M_{max} values pre and post the muscle damaging protocol in bout 1.

Table 2. Corticospinal responsiveness measured before and after two bouts of eccentric exercise at absolute and relative intensities.

Figure 1. Assessment of neuromuscular function and corticospinal responsiveness. Participants visited the laboratory 12 times, at the same time of day (± 1 h) over a 4-week period. On the Monday of week 1, baseline measures were recorded prior to the completion of a muscle damaging protocol. All variables were then re-assessed immediately post (1 h) and then for 7 days post-exercise. First, peak MVC of the non-dominant elbow flexors, from 3 attempts, was measured. Once established, a 20% target line was set and whilst participants held a contraction at this intensity TMS was delivered to evoke motor evoked potentials in the biceps. A block of 8 stimuli, separated by 6 s, were delivered to determine corticospinal responsiveness and the corticospinal silent period. Following this block, voluntary activation was assessed by motor point and motor cortex stimulation. Supramaximal brachial plexus stimulation was also delivered during a similar sequence of three contractions and at rest following the MVC; at each time point this procedure was repeated 3 times with 90 s left between the MVCs, stimulus timing is shown by the downward arrows. Following these contraction sets, participants were set up on the isokinetic dynamometer to perform the muscle damaging protocol. Immediately following the exercise participants were removed from the dynamometer and moved to the isometric testing rig for all post-assessments.

Figure 2. Twitch and EMG responses from a representative participant. Panel A shows typical traces of the resting twitch and the superimposed twitch force elicited during maximal contractions with motor point and motor cortical stimulation. Pre-exercise data is shown on the left and then responses immediately post the first and second bouts of eccentric exercise are to the right, respectively. A reduction in resting twitch force was evident immediately post bout 1; the twitches elicited with motor point and motor cortex stimulation increased, thereby reducing voluntary activation. However, following bout 2, the superimposed twitch force elicited with motor cortex stimulation was attenuated and there was a preservation of voluntary activation. Panel B shows typical traces of the maximal M-mave (M_{max} upper traces) evoked with brachial plexus stimulation and motor evoked potentials (MEP, lower traces) evoked with motor cortex stimulation during a 50% MVC. There were no changes in EMG traces at any point. The timing of all stimuli is indicated by the dashed line on each trace.

Figure 3. Delayed onset muscle soreness (VAS; A) and maximal voluntary contraction (MVC, B), measured from the elbow flexors at pre-exercise baseline and following both bouts of eccentric exercise. Data are means $\pm$ S.E.M. for 8 participants. ** $P < 0.05$ bout 1 vs. bout 2; * $P < 0.05$ bout 1 vs. bout 2 at the respective time point.

Figure 4. Potentiated twitch force measured using motor point stimulation (A) and the estimated resting twitch force derived using motor cortex stimulation (B), measured from the elbow flexors at pre-exercise baseline and following both bouts of eccentric exercise. Data are means $\pm$ S.E.M. for 8 participants. *** $P < 0.05$ interaction bout 1 vs. bout 2; * $P < 0.05$ bout 1 vs. bout 2 at the respective time point.

Figure 5. Voluntary activation measured using motor point stimulation (A) and transcranial magnetic stimulation (B), measured from the elbow flexors at pre-exercise baseline and following

both bouts of eccentric exercise. Data are means ± S.E.M. for 8 participants. *** $P < 0.05$ interaction bout 1 vs. bout 2; * $P < 0.05$ bout 1 vs. bout 2 at the respective time point.

Figure 6. Superimposed twitch (SIT) responses to motor point (A) and motor cortex (B) stimulation during MVCs performed with the elbow flexors at pre-exercise baseline and following both bouts of eccentric exercise. Data are means ± S.E.M. for 8 participants. ** $P < 0.05$ bout 1 vs. bout 2.