Neurophysiological responses and adaptation following repeated bouts of maximal lengthening contractions in young and older adults

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ABSTRACT

A bout of maximal lengthening contractions is known to produce muscle damage, but confers protection against subsequent damaging bouts, both of which tend to be lower in older adults. Neural factors contribute to this adaptation, but the role of processes along the corticospinal pathway remains unclear. Twelve young (27±5 yrs) and eleven older adults (66±4 yrs) performed two bouts of 60 maximal lengthening dorsiflexions two weeks apart. Neuromuscular responses were measured pre, immediately-post and at 24 and 72 hours following both bouts. The initial exercise bout resulted in prolonged reductions in maximal voluntary torque (MVC; immediately post-exercise onwards, p<0.001) and increased creatine kinase (from 24 h-post onwards, p=0.001), with both responses being attenuated following the second bout (p<0.015), demonstrating a protective effect. The reduction in MVC following both bouts was smaller in older adults (p=0.005). Torque variability (p≤0.041) and H-reflex size (p=0.024) increased, whilst intracortical inhibition (SICI; p=0.019) and the silent period duration (SP) decreased (p=0.001) in both groups immediately post-exercise. The SP decrease immediately post-exercise was smaller following the second bout (p=0.021), and there was an association between the change in SICI and reduction in MVC 24 h post-exercise in young adults (R=−0.47, p=0.036). Changes in neurophysiological responses were limited to immediately post-exercise, suggesting a modest role in adaptation. Changes in neural inhibition in young are linked to the extent of MVC reduction, possibly mediated by the muscle damage-related afferent feedback. Importantly, older adults incurred less muscle damage, which has implications for exercise prescription in older age.

Key words: aging, corticospinal excitability, eccentric, H-reflex, repeated bout, TMS.
NEW & NOTEWORTHY

This is the first study to have collectively assessed the role of corticospinal, spinal and intracortical activity in muscle damage attenuation following repeated bouts of exercise in young and older adults. Lower levels of muscle damage in older adults are not related to their neurophysiological responses. Neural inhibition transiently changed, which might be related to the extent of muscle damage; however, the role of processes along the corticospinal pathway in the adaptive response is limited.
INTRODUCTION

A bout of unaccustomed maximal lengthening contractions produces structural damage to muscle fibres and the extracellular matrix, resulting in impaired neuromuscular function following exercise (56, 72). This is accompanied by an immediate reduction in voluntary and electrically-evoked force production, which persists for several days after exercise (83, 87). Despite the negative consequences that accompany a bout of unaccustomed lengthening contractions, this type of exercise provides a protective effect against subsequent bouts as evidenced by attenuation of torque loss, soreness and other muscle damage indices (36, 71). This phenomenon is commonly referred to as the repeated bout effect (RBE) and has been attributed to mechanical, cellular and neural factors (for review see Ref. 39).

Whilst studies have tended to focus on peripheral factors in response and adaptation to damaging exercise, the neural contribution remains far less explored. Following a bout of unaccustomed maximal lengthening contractions, a reduction in voluntary activation using motor nerve (30, 83) and transcranial magnetic stimulation (TMS; 30) has been observed, suggesting a neural contribution (36). Further evidence of neural adjustments in response to damaging exercise is exhibited by observed impairments in proprioception (6, 9), alterations in the force-electromyography (EMG) relationship (83, 89, 106), increased torque variability (89, 106), and modulation of motor unit behaviour (88), including increased motor unit synchronisation (16) and decreased recruitment threshold (16). However, if the damaging bout of exercise is repeated, the alterations in voluntary activation and motor unit behaviour are attenuated (15, 30, 36), suggesting modulation of spinal and supraspinal inputs to the motoneuron pool. However, the exact origin of the synaptic input causing adaptation at the level of the motoneuron pool is unclear (39). Animal data showed elevated brain cytokines following damaging exercise (8), and experiments in humans have suggested that modulation in cortical networks such as reduced intracortical inhibition (78) and increased somatosensory
cortical excitability (59) might occur. However, it remains unknown whether the abovementioned neurophysiological responses following damaging exercise would differ if the bout of exercise was repeated, when disruptions in peripheral biochemistry are expected to be smaller.

Another factor that is known to affect neuromuscular properties and could mediate the response to damaging exercise is age. Typically, healthy aging is accompanied by an age-related decrease in voluntary isometric strength (e.g. Ref. 77). However, there appears to be a preservation of this strength during lengthening contractions in older adults (82) and hence using lengthening contractions has been suggested to be a well-suited training stimulus for older adults (84). Despite this, older adults appear to exhibit a smaller manifestation of the RBE (31, 49), which could be related to the reduced capacity for neuromuscular adaptation. Indeed, healthy aging is characterised by many central nervous system (CNS) adjustments, including alterations in synaptic input during dynamic contractions originating from spinal and supraspinal centres (73, 93). These include increased inhibitory and decreased excitatory neurotransmitter activity in the motor cortex (62, 73), which have been linked to a reduction in the capacity for neural adaptation (11, 65). This capacity might be further impaired in older adults due to altered cortical sensorimotor integration of afferent input (95), which is attenuated following the release of inflammatory cytokines associated with muscle damage (8). Thus, discerning the aetiology of neuroplastic impairment in the elderly is important for informing neurorehabilitation (18), motor learning (86, 97) and adaptation to exercise (107).

The age-related alterations in CNS also result in impairments in motor function, including the ability to control force output (for review see Ref. 23), which has important implications in performance of functional daily tasks (24, 32, 58) and incidence of falls (43, 108). For example, older adults typically exhibit greater torque variability, particularly during dynamic contractions (45, 46, 93). Torque variability is increased following damaging exercise in
younger populations (89, 106). Whilst not investigated in older population, if similar or exaggerated increases in torque variability are shown, this could have implications for performance of daily tasks, such as greater risk of falls (10), following damaging exercise.

Accordingly, the present study aimed to: 1) assess neurophysiological (intracortical, corticospinal and spinal) responses following damaging exercise; 2) assess the neurophysiological contribution to the RBE; and 3) compare the extent of muscle damage and the associated modulation of neurophysiological responses and torque variability between young and older adults. We hypothesised that damaging exercise will modulate neurophysiological responses, but this modulation will be of smaller magnitude following the repeated bout. Given the alterations in sensorimotor integration with advanced age, we also hypothesised that older adults will exhibit a smaller RBE.
METHODS

Participants

Twelve young (27 ± 5, range 21-35 years; 180 ± 7 cm, 77.2 ± 9.6 kg; 2 females) and 11 older (66 ± 4, range 61-73 years; 177 ± 13 cm, 75.3 ± 12.1 kg; 3 females) adults took part in the study. Individuals were considered for the ‘older’ group if they were over 60 years of age (38). Participants were instructed to refrain from strenuous exercise for 48 hours before the first visit to the laboratory and throughout the duration of the study, and alcohol and caffeine for 24 and 12 hours before the visits to the laboratory, respectively. Prior to taking part in the study, participants responded to a questionnaire for contraindications to TMS (42) and a health screening questionnaire to ascertain contraindications to participation. The exclusion criteria included any neurological or neuromuscular disorders, musculoskeletal injury that may attenuate the ability to produce torque, taking any medication known to affect the nervous system and having any metal material in their body. To minimise the influence of sex hormones on TMS-induced responses (96), premenopausal females were only recruited if they were taking a monophasic contraceptive pill (2) and were only tested on the days of pill consumption. The study conformed to the standards of Declaration of Helsinki. Participants eligible for the study gave written informed consent prior to any element of the study proceeding. All procedures were approved by Northumbria University Ethics Committee.

Experimental protocol

Participants visited the laboratory seven times throughout the duration of the study, at the same time of day (±1 h) to limit diurnal variations (100). The purpose of the first session was familiarisation with the experimental procedures. Following familiarisation, participants returned to the laboratory to perform a bout of maximal lengthening contractions. Before,
immediately after, and 24 and 72 hours post the exercise bout, assessment of neuromuscular function was performed (Figure 1) including isometric maximal voluntary contraction (MVC), followed by assessment of torque variability at 5 and 20% isometric MVC, H-reflex, and responses to TMS (randomised order). Half of the sample in each group exercised with their dominant limb, whilst the other half of the sample exercised with their non-dominant limb (pseudorandomised) as per the lateral preference inventory (13) to discount the influence of limb dominance. Indeed, a separate analysis taking into account limb dominance showed no difference in measures of isometric MVC torque across all time points ($p = 0.297$). The whole protocol was repeated two weeks post the initial visit, including the exercise bout to assess the adaptive neural response.

**Procedures**

*The bout of maximal lengthening contractions*

The exercise bout consisting of maximal lengthening contractions was performed on an isokinetic dynamometer (Biodex System 4 Pro, New York, USA). Participants sat with hip and knee at 60 and 110° flexion, respectively. The foot was strapped securely to a metal plate attached to the lever arm of the motor with a velcro strap at the level of the talus and phalange bones, with particular attention made to minimise extraneous toe movement. The distal part of the thigh was strapped down with velcro to minimise abduction, adduction and flexion of the hip. Participants were instructed to focus solely on dorsiflexion and activation of tibialis anterior (TA). Visual feedback of the target torques was provided with a monitor placed approximately 1.5 m from the participant. Participants were required to perform 10 sets of 6 maximal lengthening contractions throughout 40° range of motion (from 10° dorsiflexion to 30° plantar flexion) by maximally resisting the motor of the device moving at 15°·s$^{-1}$ and
then relaxing throughout the passive dorsiflexion (shortening) phase. The exercise was performed through the identical participant-specific range-of-motion across both bouts to match the muscle length and muscle strain between the bouts.

Experimental setup

During the assessment of neuromuscular function, participants remained in the same dynamometer position as during exercise, but with the ankle fixed at anatomical zero (90°). Measures were performed on the limb that was exercised. This position was replicated during each visit to the laboratory to ensure consistent inferred muscle length to avoid influencing neural responses. Isometric MVC was performed twice separated by 30 seconds of rest. If the greatest instantaneous torque values differed for more than 5%, a third trial was performed. Verbal encouragement and real-time visual torque feedback was provided to ensure reliability of MVC recordings (28). All subsequent contractions with stimulations were performed at 10% isometric MVC (7).

Constant torque task

To investigate the effect of a bout of maximal lengthening contractions on torque variability, participants were asked to match the torque level to a horizontal line on the screen in front of them that was equal to 5 and 20% of isometric MVC and maintain torque production as close to the horizontal line as possible for 15 seconds. In the assessments post exercise, the constant torque task was performed with participants contracting at 5 and 20% isometric MVC. Additionally, participants performed a constant torque task at 20% isometric MVC relative to pre-exercise (absolute) isometric MVC. The absolute torque-matching at time points following exercise was only performed for 20% isometric MVC since pilot testing indicated
that the difference between relative and absolute values for lower contraction intensities was within the measurement error of isometric MVC. The location of the horizontal line on the screen remained constant across participants by alterations in the gain of the y-axis (−10 to 110% of participant’s MVC). The specific torque intensities were chosen as previous work indicated that following a bout of maximal lengthening contractions, torque variability changes are greater during low level contractions (89). Furthermore, any differences between young and older individuals are likely to be the greatest at, and possibly confined to, lower level contractions (e.g. Ref. 102).

Surface electromyography

Electromyographic (EMG) activity was recorded with a bipolar electrode arrangement (8 mm diameter, 20 mm inter-electrode distance; Kendall 1041PTS, Tyco Healthcare Group, USA) over the muscle belly of TA with the ground electrode placed over the lateral malleolus. The electrodes were placed according to SENIAM recommendations (34), at one-third of the length between the tip of the fibula and the tip of the medial malleolus. Prior to placement of electrodes, the recording site was shaved, abraded with preparation gel and wiped clean with an alcohol swab to ensure appropriate impedance (< 2 kΩ). The EMG signal was amplified (×1000), band pass filtered (20-2000 Hz; Neurolog System, Digitimer Ltd, UK), digitised (5 kHz; CED 1401, CED, UK), acquired and analysed offline (Spike2, v8, CED, UK).

Transcranial magnetic stimulation

Single and paired-pulse TMS were delivered using two magnetic stimulators (200\textsuperscript{2} and BiStim; Magstim Co., Ltd., Whitland, UK; maximal output of ~1.4 T) connected to a concave double-cone coil (110 mm diameter) positioned over the primary motor cortex with the
posterior-to-anterior oriented current. Initially, the coil was positioned over the reported optimal spot for stimulation of the TA muscle, roughly 0.5-1 cm lateral, and posterior, to the vertex (17). The coil was then moved in small steps around the initial position until the spot capable of evoking the biggest motor evoked potential (MEP) in TA was found (hotspot). Once identified, the location of the back of the coil was marked directly on the scalp with a permanent marker to ensure consistent placement across trials. The initial hotspot remained consistent throughout the assessments before and immediately, 24 h, and 72 h after the first bout of exercise, but was re-determined prior to the second bout of exercise. Following hotspot localisation, active motor threshold (AMT) was determined at 10% of isometric MVC with the ankle positioned at anatomical zero and defined as the intensity eliciting MEP amplitude $\geq 200 \mu V$ in 3 out of 5 trials (44). AMT was determined at the start of each individual assessment and did not differ between young and older individuals (38 ± 4 vs. 49 ± 4% of stimulator output, $p = 0.057$) or across visits ($p = 0.287$). Single pulse TMS was always delivered at an intensity of $1.2 \times$ AMT as it lies on the middle portion of the ascending part of the stimulus-response curve (33) and is thus sensitive to changes in corticospinal excitability. The conditioning stimuli of paired-pulse paradigms were performed at intensities of 0.7 and $0.6 \times$ AMT at inter-stimulus intervals of 2 and 10 ms for SICI and ICF, respectively (7). Ten single and ten paired stimuli were delivered in an alternating fashion during 10% isometric MVC, with the mean of 10 responses taken as a representative value.

**Percutaneous nerve stimulation**

Percutaneous electrical stimuli (1 ms pulse duration; Digitimer DS7AH, Hertfordshire, UK) over the fibular nerve (40 mm cathode/anode arrangement; Digitimer, Hertfordshire, UK) were performed to evoke H-reflex and M-wave in TA. Upon localization of the optimal site, it was marked with a permanent marker and the stimulating electrode was strapped to the
participant’s leg. The H-M recruitment curve was constructed during 10% isometric MVC by gradually increasing the intensity from H-reflex threshold by 0.5 mA increments to maximal H-reflex every 3 pulses. Once the H-reflex amplitude started to decrease after three consecutive increases in intensity, the amperage was increased in bigger steps (3 mA) until the EMG response plateaued. After that, the intensity was further increased by 30% to ensure supramaximal stimulation eliciting maximal compound action potential ($M_{\text{max}}$). The intensity required to elicit $M_{\text{max}}$ was lower for young, compared to older individuals (30 ± 4 vs. 50 ± 5 mA, $p = 0.003$), but did not differ across visits ($p = 0.614$).

*Creatine kinase*

Fingertip capillary blood samples (30 µl) were obtained at each time point and were immediately assayed for creatine kinase (CK) concentration based on reflectance in an automated system (range: 24.4 – 1500 IU.L$^{-1}$, coefficient of variation: 0.5% of reflectance; Reflotron, Roche Diagnostics, Germany). Due to technical issues, samples from 2 older individuals could not be analysed.

*Data analysis*

*MVC and work.* The greatest instantaneous torque value was taken as MVC. Peak torque (N.m) and total work (J) performed were recorded during each bout. Total work was also calculated for each set of maximal lengthening contractions. Additionally, total work during each bout was normalised to individual’s body mass. Because rotational forces change with body size due to cross-sectional area (body mass to the power of 2/3) and the change in lever arm (body mass of the power of 1/3) of the muscle, the allometric parameter of 1 was used to calculate total work done relative to body mass (40).
Evoked potentials. Peak-to-peak amplitudes of the evoked responses were calculated. MEPs and H-reflexes were expressed relative to M_max. SICI and ICF measures were quantified as a ratio between unconditioned and conditioned MEP amplitude. An increase in this ratio indicates a reduction in intracortical inhibition (SICI) and an increase in facilitation (ICF). The silent period duration following a MEP response was calculated from the stimulus artefact to the return of continuous EMG activity based on visual inspection (94). Voluntary EMG activity was quantified as root-mean-square EMG activity in the period 100 ms before stimulation was delivered and was normalised to M_max (RMS/M_max; 49).

Torque variability. Torque variability was quantified from the 10 seconds in the middle portion of the 15-second constant torque task (from 2.5 to 12.5 s) as the standard deviation (SD) and the coefficient of variation of torque (CV = [SD of torque / mean torque] * 100). EMG activity during torque-matching tasks was quantified as RMS in the same 10-second epoch where torque variability was assessed, and expressed relative to RMS during 500 ms around peak isometric MVC (RMS/RMS_max).

Statistical analyses. All analysis was performed using SPSS (v20, SPSS Inc., Chicago, IL, USA). Normality of data was assessed using Shapiro-Wilks test. If the data were not normally distributed, transformation using common logarithm was performed. Two significant outliers were identified via studentised residuals (> 3) in the young group for SICI and one for ICF, and were excluded from further analyses. Sphericity was assessed using Mauchly’s test of sphericity. In the case of violation, a Greenhouse-Geisser correction was employed. Differences in responses between groups to different bouts over time were assessed using 2 × 2 × 4 ANOVA (age × bout × time). A 2 × 2 ANOVA (age × bout) was used to assess the differences in peak lengthening MVC torque and total work performed between the two bouts of exercise. A 2 × 2 ANOVA (age × bout) was also used to assess differences between young and older adults at baseline. If significant interactions or main effects were found, the analysis
was continued using pairwise comparison with Bonferroni correction. To investigate the differences in adaptability (RBE) between young and older adults, the difference in isometric MVC from baseline to 24 and 72 hrs post-exercise for the second bout was divided by the difference in this measure following the first bout, and assessed using an independent samples T-test (51). To assess the neural contribution to exercise-induced disruption in neuromuscular function (isometric MVC) immediately and 24 h post-exercise, linear regression analyses were performed. Significance was set at alpha level of 0.05. Data are presented as mean ± SD, unless the data were transformed, in which case the geometric mean ± SD are presented.

Reliability. Neuromuscular responses are known to exhibit inherent variability. As such, interpreting the results within statistical measures of error has been recommended, allowing the contribution of real change and random variation to potential changes in neuromuscular function to be distinguished (27). For that reason, the baseline responses from each bout were used to determine test-retest reliability of electrophysiologic and mechanical variables. Typical error (TE) was calculated for the main variables of interest as the standard deviation of mean differences between the two pre-exercise values divided by the square root of 2, and was expressed as both absolute and relative (rTE) values (percentage of the mean). Bias between the two pre-exercise scores was assessed using paired samples T-test. Reliability indices for main variables of interest are displayed in Table 1.
RESULTS

Age differences at baseline

Younger individuals displayed greater isometric MVC compared to the older ($F_{1, 21} = 5.2$, $p = 0.032$, $\eta^2_p = 0.20$; Table 1), and lower torque variability during an isometric constant torque task at 5% ($F_{1, 21} = 11.7$, $p = 0.003$, $\eta^2_p = 0.36$) and 20% isometric MVC ($F_{1, 21} = 4.4$, $p = 0.048$, $\eta^2_p = 0.17$). There were no age-related differences in peak lengthening MVC ($p = 0.178$), lengthening to isometric MVC ratio ($p = 0.194$), RMS/$M_{\text{max}}$ ($p = 0.065$), $M_{\text{max}}$ ($p = 0.096$), $H_{\text{max}}$/max ($p = 0.787$), $\text{MEP}/M_{\text{max}}$ ($p = 0.080$), SICI ($p = 0.672$), ICF ($p = 0.233$) and SP ($p = 0.370$).

Exercise performance and markers of muscle damage

Following the initial bout of maximal lengthening contractions there was a significant reduction in isometric MVC torque ($F_{2.1, 43.8} = 75.8$, $p < 0.001$, $\eta^2_p = 0.78$), with the greatest reduction observed immediately post exercise after the initial bout regardless of age (young: ~28% reduction, Figure 2A; older: ~22% reduction, Figure 2B; $p < 0.001$ for both). The isometric MVC remained lower in young compared to older adults in the days following the exercise bout (age $\times$ time interaction: $F_{2.1, 43.8} = 6.0$, $p = 0.005$, $\eta^2_p = 0.22$). However, a smaller reduction in isometric MVC was demonstrated following the second bout of exercise in both groups (bout $\times$ time interaction: $F_{3, 63} = 4.1$, $p = 0.011$, $\eta^2_p = 0.16$). The RBE was similar between young and older adults as assessed by the relative difference in isometric MVC decline between bouts at 24 (90 vs. 91%; $p = 0.768$) and 72 hrs post-exercise (92 vs. 97%; $p = 0.234$).

The mean total work done across both damaging bouts in young adults was $5973 \pm 1028$ J, whereas older individuals did $4862 \pm 1320$ J. These values did not differ between the two bouts of exercise ($F_{1, 21} = 0.9$, $p = 0.360$, $\eta^2_p = 0.40$) or age groups ($F_{1, 21} = 3.5$, $p = 0.076$, $\eta^2_p$...
During both bouts, work decreased progressively across sets (main effect of time: $F_{3.2, 67.2} = 24.8$, $p < 0.001$, $\eta^2_p = 0.54$), but the decline did not differ between the groups (age $\times$ time interaction: $F_{3.8, 79.5} = 0.47$, $p = 0.750$, $\eta^2_p = 0.02$). On the other hand, when total work was normalised to body mass, younger adults ($75 \pm 10$ J.kg$^{-1}$) exhibited higher values compared to older ($64 \pm 12$ J.kg$^{-1}$; main effect of age: $F_{1, 21} = 5.6$, $p < 0.028$, $\eta^2_p = 0.21$).

An increase of CK concentration was observed following maximal lengthening contractions in both groups ($F_{1.5, 28.7} = 10.4$, $p = 0.001$, $\eta^2_p = 0.35$). The CK kinetics were different between the two exercise bouts (bout $\times$ time interaction: $F_{1.3, 25.0} = 2.3$, $p = 0.014$, $\eta^2_p = 0.25$), such that there was an increase in CK levels at 24 ($p = 0.030$) and 72 hours ($p = 0.012$) after the first bout, but only 24 hours ($p = 0.014$) and not 72 hours ($p = 0.097$) after the second bout (Table 2). The CK increase was also greater 24 hours following the first compared to the second bout ($p = 0.029$). There were no group differences.

**Corticospinal and spinal responses and adaptation**

There were no differences in prestimulus EMG activity across different time points or between the age groups ($p \geq 0.106$; Table 2). $H_{\text{max}}/M_{\text{max}}$ was modulated in response to the bouts of maximal lengthening contractions (main effect of time: $F_{3, 63} = 3.4$, $p = 0.024$, $\eta^2_p = 0.14$), such that it increased immediately post exercise relative to other time points ($p = 0.025 - 0.039$; Figure 3A and 3C). No differences were observed for $M_{\text{max}}$ across all time points ($p \geq 0.287$; Table 2).

$\text{MEP}/M_{\text{max}}$ was modulated differently between the age groups (age $\times$ time interaction: $F_{3, 63} = 6.5$, $p = 0.001$, $\eta^2_p = 0.24$) insofar as it increased at 24 ($p = 0.038$) and 72 hours ($p = 0.011$) post exercise bouts in young (Figure 3B), but remained unchanged in older adults ($p \geq 0.053$; Figure 3D). No differences in $\text{MEP}/M_{\text{max}}$ were observed between the bouts.
Changes in SICI were observed following the two bouts of exercise (main effect of time: $F_{3, 57} = 5.3, p = 0.003, \eta^2_p = 0.22$); an increase in the ratio of conditioned to unconditioned MEP was noted immediately post ($p = 0.019$; Figure 4A and 4C), suggesting reduced intracortical inhibition. For young individuals, a significant association was found between the post-exercise reduction in SICI and the extent of reduction in isometric MVC torque 24 hours post exercise ($R^2 = 0.22, R = -0.47, p = 0.036$; Figure 5). However, this association was not observed in older individuals ($R^2 = 0.01, R = -0.10, p = 0.645$). No other associations were shown between corticospinal and spinal responses and reductions in isometric MVC immediately post or in the days following exercise ($p \geq 0.246$). SP was modulated in response to exercise (main effect of time: $F_{3, 63} = 6.3, p = 0.001, \eta^2_p = 0.23$), such that it decreased immediately post exercise ($p = 0.044$), suggesting a reduction in inhibition (Figure 5B and 5D). This modulation of SP was greater during the first compared to the second exercise bout (main effect of bout: $F_{1, 21} = 6.3, p = 0.021, \eta^2_p = 0.23$). No differences in ICF were observed ($p \geq 0.245$; Table 2).

**Torque variability**

Torque variability was greater in older individuals compared to young at 5% ($F_{1, 21} = 13.8, p = 0.001, \eta^2_p = 0.40$), relative 20% ($F_{1, 21} = 12.3, p = 0.002, \eta^2_p = 0.37$) and absolute ($F_{1, 21} = 16.8, p = 0.001, \eta^2_p = 0.44$) 20% isometric MVC across all time points. At 5% isometric MVC, torque variability was modulated differently between the age groups across time points (age × time interaction: $F_{3, 63} = 3.3, p = 0.027, \eta^2_p = 0.13$). Post hoc testing showed there was an increase in torque variability immediately post both bouts of exercise for the older group ($p = 0.007$, Figure 6D), whereas it increased 24 hours following both bouts of exercise in the young ($p = 0.041$; Figure 6A). At both relative (main effect of time: $F_{3, 63} = 6.4, p = 0.001, \eta^2_p = 0.23$) and absolute (main effect of time: $F_{3, 63} = 3.4, p = 0.023, \eta^2_p = 0.14$) 20% isometric
MVC torque variability was modulated in response to exercise. At relative 20% isometric MVC, torque variability increased immediately post (p = 0.005) and 24 hours following both bouts of exercise (p = 0.015: Figure 6B and E), whereas it only increased immediately post exercise following both bouts of exercise at absolute 20% isometric MVC (p = 0.012; Figure 6C and F).

During a constant torque task, RMS/RMS_max was modulated in response to exercise at 5% (main effect of time: F 2.1, 43.1 = 18.0, p < 0.001, ηp^2 = 0.46), relative 20% (F 3, 63 = 114.7, p < 0.001, ηp^2 = 0.41) and absolute 20% (F 3, 63 = 30.5, p < 0.001, ηp^2 = 0.59) of isometric MVC insofar it increased immediately post both exercise bouts (p = 0.002, p < 0.001 and p = 0.001, respectively; Table 2). Across all time points RMS/RMS_max was greater in older compared to younger individuals at 5% (F 1, 21 = 6.8, p = 0.017, ηp^2 = 0.24), relative 20% (F 1, 21 = 14.5, p = 0.001, ηp^2 = 0.41) and absolute 20% (F 1, 21 = 12.0, p = 0.002, ηp^2 = 0.37) of isometric MVC (Table 2).

Similarly for RMS/MMax changes were noted at different time points at 5% (F 3, 63 = 5.4, p = 0.002, ηp^2 = 0.21) and absolute 20% (F 2.1, 44.9 = 5.3, p = 0.007, ηp^2 = 0.20) of isometric MVC during a constant torque task, increasing immediately post exercise in both bouts (p = 0.040 and p = 0.004, respectively), regardless of age. Across all time points RMS/MMax was greater in older compared to younger individuals at relative (F 1, 21 = 5.7, p = 0.026, ηp^2 = 0.22) and absolute 20% (F 1, 21 = 4.6, p = 0.044, ηp^2 = 0.18) of isometric MVC (Table 2).
DISCUSSION

The present study assessed corticospinal, spinal and intracortical activity in response to repeated bouts of maximal lengthening contractions in younger and older adults. A bout of maximal lengthening exercise caused a reduction in maximal torque, which was greater in young compared to older individuals. For both groups, this reduction was attenuated following a repeated bout. Corticospinal and spinal responses were modulated immediately following bouts of damaging exercise, suggesting that the observed changes were reactive to the muscle damage, rather than a protective mechanism for the repeated exercise. Older adults experienced less muscle damage, but this was not related to neurophysiological responses. These data extend our understanding about the role of the nervous system in muscle damage and repeated bout effect throughout the life span.

Exercise-induced muscle damage and the repeated bout effect

A bout of 60 maximal lengthening contractions caused a prolonged reduction in maximal torque producing capacity regardless of age. This reduction was comparable in magnitude to that observed previously in dorsiflexors with higher volumes of exercise (150 contractions; Refs. 67, 85, 86, 90). The lack of change in $M_{\text{max}}$ suggests that this prolonged depression of maximal torque is not due to changes in sarcolemma excitability, but rather due to disruption of excitation-contraction coupling processes (30). Twenty-four hours following exercise, isometric MVC was still reduced, suggesting that the bout of exercise was indeed damaging (105), as corroborated by the elevation in plasma CK. Furthermore, the reduction in isometric MVC was attenuated following the repeated exercise, confirming the occurrence of RBE (30, 36, 39).

The extent of muscle damage was greater in young compared to older individuals. This cannot be attributed to the total amount of work performed because this did not differ during the two
bouts of exercise between the age groups. However, older adults performed less total work per body mass, which could have contributed to less damage incurred in this group. The smaller degree of muscle damage in an aging population has been shown previously (31, 49–51) and attributed to preferential damage of type II fibres, with younger individuals often exhibiting a greater proportion of such fibres in comparison to older counterparts (67, 68). A preferential damage of type II fibres due to maximal lengthening contractions has been reported in animal (25, 54, 104) as well as human studies (26, 41). However, these inferences have been questioned due to reliance on animal studies and small effect sizes in humans (for review see Ref. 88). Nevertheless, the expression of RBE was similar between groups, suggesting that the adaptability of the aging neuromuscular system is preserved in response to damaging exercise of lower limbs.

Disruptions in motor performance and muscle activity

Muscle damage resulted in increased torque variability during submaximal isometric contractions immediately post exercise, as well as 24 hours after, corroborating previous work (14, 49, 53, 89). This behaviour was observed regardless of age and despite the greater torque variability of older adults at baseline, that has previously been related to the age-related increase in variability of the common synaptic input to motoneurons (12, 76). Thus, our results suggest that control of muscle force is equally perturbed in young and older adults following damaging exercise.

The increased variability following damaging exercise in the young and older groups could stem from increased discharge rate variability following exercise (16), or increased variability of common synaptic input to motoneurons (24) due to prolonged depression of low-frequency contractile properties (20). At the time of increased torque variability, greater amplitude of H-reflex was also observed. This behaviour could indicate an increase in gain around the short-
latency stretch reflex loop (21, 22). Such an increase in gain could also be linked to concurrent increases in EMG activity post-exercise during a constant torque task (21, 22). The increased torque variability post-exercise was not dependent on exercise bout or age, suggesting it is not a variable that is adaptive in this paradigm. Overall, decreased performance of the constant-torque task and the accompanied increased EMG activity, together with prolonged reduction in maximal torque producing capacity, suggest that the incurred muscle damage resulted in alterations in motoneuron pool activity.

The role of the central nervous system in the adaptive process to damaging exercise

It has been suggested that synaptic input from spinal and supraspinal centres might play a role in the adaptive process from damaging exercise (39). In the present study, neurophysiological responses were modulated in response to damaging exercise. Maximal H-reflex increased immediately following exercise, which agrees with studies performing isometric tasks to failure (55, 70, 98), but contrasts with studies showing H-reflex depression (103) or lack of change (74) following shortening and lengthening contractions. This discrepancy could be explained by methodological (109) or task differences (sustained vs. intermittent, contraction intensity) among studies. As H reflex only changed transiently post-exercise, and was not altered during the 72 hrs recovery period following either bout of maximal lengthening contractions, it is likely that the changes were not involved in the adaptive process(es) underpinning the RBE, but rather reflected the acute exercise stress. The transient change, however, could stem from reduced presynaptic inhibition of Ia afferent fibres (70), decreased recruitment threshold of motoneurons (15, 16), or a combination of these.

Changes in SICI and TMS-induced SP following damaging exercise are consistent with decreased CNS inhibition. No changes were observed in ICF, which concur with previous work following isometric contractions to task failure (60), but not others (3, 37, 101). In
young adults, the change in SICI following the first bout of damaging exercise was ~23%, similar to that observed in biceps brachii following damaging elbow flexion exercise when measured during a low intensity muscle contraction (78). However, there was only a ~5% change in SICI that was observed immediately after the second bout of exercise. This difference in the mean SICI change between the two bouts of exercise (~23 vs. ~5%) was also larger than the associated measurement error (17.8%; Table 1). The TMS-induced SP showed a greater decrease in duration following the first exercise bout relative to the second. This could be related to less damage in the second bout, causing less disruption in the characteristics of the TMS twitch (30), which has been suggested to potentially influence SP duration (94). Due to the timing of post-exercise assessment, i.e. within 20 minutes of exercise, fatigue could explain modifications in CNS inhibition (78). Reductions in intracortical inhibition have been observed after fatiguing exercise with the upper (4, 37, 60, 99), but not lower limbs (2, 29). Furthermore, SP duration has been shown to increase post fatiguing single-joint isometric exercise (2, 4, 29) rather than decrease, as seen in the present study. Thus, the attenuation of the change in SICI and SP following repeated exercise suggests that this behaviour could be related to the specific exercise task employed in the present experiment, and the degree of muscle damage induced. Indeed, damaging exercise has been demonstrated to cause a near-immediate release in biochemical substrates (e.g. prostaglandin, bradykinin) and inflammation-related factors (e.g. histamine, neuropeptides; 54, 75). Of particular interest is a large efflux of bradykinin that has been demonstrated immediately post damaging exercise, despite a delayed increase in CK (5), and has been shown to acutely increase the activity of group III and IV afferents (66). Thus, a change in SICI following damaging exercise might be a reflection of acute alterations in afferent feedback in response to damage (78). Since muscle damage was attenuated following the second bout of exercise, it is likely that disruption to biochemical homeostasis was smaller,
leading to smaller alterations in afferent feedback and thus, smaller modulation in CNS inhibitory measures. This notion is further supported by a significant association that was observed between the reduction in intracortical inhibition and the extent of muscle damage (i.e. reduction in isometric MVC at 24 hours post-exercise) in young adults. Interestingly, studies investigating the effect of fatigue-related group III and IV afferent feedback on neurophysiological responses showed an increase in long-interval intracortical inhibition following cycling exercise (90, 91), rather than a decrease that was observed in the present study and others (48, 78) following lengthening contractions. However, cycling is an activity that predominantly consists of shortening, rather than lengthening contractions. Thus, it is possible that modulation of inhibitory mechanisms is contraction-mode specific, as suggested by a recent study (48). Alternatively, afferent feedback might mediate specific inhibitory networks differently. Whilst long-interval intracortical inhibition represents the activity of gamma-aminobutyric acid (GABA) B-receptors (61), SICI is thought to reflect GABA-A receptors (52, 110). Thus, increased activity of group III and IV afferents could upregulate GABA-B receptor activity causing greater inhibition, whereas GABA-A receptors could be downregulated, resulting in less inhibition. The acute increase in biochemical substrates such as bradykinin and the associated increase in chemosensitive muscle afferents have also been shown to alter fusimotor reflexes, exciting the primary and secondary muscle spindle endings (19, 75). The increased firing of muscle spindle afferents could, via inhibitory pathways, suppress the corticospinal response immediately post-exercise (35, 92) and contribute to the delayed increase in corticospinal excitability following damaging exercise as observed in the present study. Older adults similarly exhibited a reduction in SP duration in response to repeated bouts of damaging exercise. However, the modulation in SICI was similar between the exercise bouts (~15 vs. ~13% following bout 1 and 2, respectively), these changes were within the
measurement error, and no association was noted with the extent of damage. This lack of modulation could be attributed to smaller levels of muscle damage incurred by older adults and thus, less disruption in biochemical homeostasis. Alternatively, the lack of change in SICI could be an age-specific response as older adults have been shown to exhibit attenuated afferent modulation of SICI, possibly due to altered cortical sensorimotor integration of afferent input (95).

Methodological considerations

Muscle damage and RBE are complex phenomena, and thought not only to be mediated by neural factors, but also mechanical and cellular (63). Whilst the latter are equally important in adjustments of the neuromuscular system following damaging exercise, the aim of the present study was to explore neural factors, specifically processes along the corticospinal pathway. As such, the present study cannot directly ascertain the interaction between neural, mechanical and cellular mediators of muscle damage and repeated bout effect.

The time of assessment immediately post-exercise was performed within 20 minutes following a bout of maximal lengthening contractions. This makes it difficult to deduce whether immediate post-exercise modulation in neuromuscular function and neurophysiological responses is due to damage, exercise-induced fatigue or both. Some previous investigations have performed assessments 2 hours post-exercise to try and differentiate between fatigue and damage effects on responses (20, 78). However, the available evidence also suggests that following maximal sustained isometric contractions, fatigue-related alterations in responses to TMS return to baseline values within a minute post exercise (1). Nevertheless, despite not delaying the post-exercise assessment, the differential changes in certain neurophysiological responses (e.g. CNS inhibition measures) following the
two exercise bouts suggest that those responses are specific to the exercise task performed in the presented experiment and are likely associated with muscle damage.

There was a significant bias in $M_{\text{max}}$ between the two baseline scores in young individuals. Whilst some adaptation from the first bout cannot be excluded as an explanation for the observed change, it is more likely related to non-physiological factors, such as changes at the skin-electrode interface (69) or subtle changes in electrode placement. This is further supported by a lack of bias in measures that were normalised to $M_{\text{max}}$, because $M_{\text{max}}$ represents the maximal excitation of the muscle that can be recorded, and a change in this measure will be accompanied by a corresponding change in raw amplitudes of other evoked responses (e.g. H-reflex and MEP).

The present study examined neurophysiological responses in TA following damaging dorsiflexor exercise. This specific musculature was studied due to its functional relevance to locomotion and activities of daily living, particularly in an older population (93). However, due to smaller muscle mass, the damaging exercise resulted in a relatively small systemic response (~2-3-fold CK increase 24 h post-exercise) compared to previous studies in biceps brachii (~10-fold; Ref. 30). Therefore, it is possible that the present findings pertaining to afferent feedback activity actually underestimate the effect on neural inhibitory measures.

**Conclusion**

A bout of damaging maximal lengthening contractions caused a prolonged reduction in voluntary torque-producing capacity, which was smaller and recovered faster after the second bout of exercise, confirming the RBE. Neurophysiological responses were modulated following damaging exercise. The reduction in CNS inhibition following damaging exercise might be associated with changes in afferent feedback as a result of muscle damage, but this was observed only in young individuals, possibly due to age-related changes in cortical
sensorimotor integration of afferent feedback. However, changes in neurophysiological responses were transient, not paralleling the prolonged reduction in voluntary torque producing capacity. Thus, the nervous system processes along the corticospinal pathway and within the intracortical circuitry play a limited role in the adaptive response to damaging exercise. The extent of muscle damage was smaller in older adults, but the expression of RBE was similar compared to young, and this was not related to neurophysiological responses of older individuals, contrary to our hypothesis. These data show that older adults incur less damage, but exhibit similar RBE, which has implications for exercise prescription and recovery in older age.
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**FIGURE CAPTIONS**

**Figure 1. The overview of the experimental protocol.** MVC = maximal voluntary isometric contraction torque; M1 = primary motor cortex; AMT = active motor threshold. Contractions at 5 and 10% isometric MVC were only performed with relative normalisation (see ‘Constant torque task’ for further details).

**Figure 2. Exercise performance and recovery.** Isometric maximal voluntary contraction (MVC) torque before, immediately post, and 24- and 72 hours following both bouts of exercise in young (A) and older adults (B); work performed during each set and the total work performed during each bout in young (C) and older individuals (D); and total work performed expressed relative to body mass in young and older adults (E). Dashed lines represent individual responses whereas the full line with circles denotes the mean. The grey shaded area represents the measurement error for isometric MVC based on the difference in variability between the pre-exercise values before each bout. Data for isometric MVC are presented as a relative change, but statistical analyses were performed on raw values. *p < 0.010 relative to pre-exercise of ‘BOUT 1’, *p ≤ 0.001 relative to pre-exercise of ‘BOUT 2’, #p < 0.05 relative to the other exercise bout (based on age × time and bout × time interactions); †p ≤ 0.021 relative to the first set (based on main effect of time), ‡p = 0.028 different between the age groups (based on main effect of age).

**Figure 3. H-reflex and corticospinal excitability.** The amplitude of maximal Hoffman reflex normalised to maximal compound action potential (Hmax/Mmax; A, C) and motor evoked potentials normalised to maximal compound action potential (MEP/Mmax; B, D) in young (A, B) and older (C and D) adults before, immediately post, and 24- and 72 hours following both bouts of exercise. Dashed lines represent individual responses whereas the full lines with circles denote the mean. The grey shaded area represents the measurement error based on the
difference in variability between the pre-exercise values befor e each bout. Differences in
MEP/M_{\text{max}} are presented based on age× time interaction; †p ≤ 0.039 relative to other time
points (based on main effect of time).

**Figure 4. Central nervous system inhibition.** Intracortical inhibition (SICI; A, C), and silent
period duration (B, D) before, immediately post, and 24- and 72 hours following both bouts of
exercise in young (A, B) and older adults (C, D). Dashed lines represent individual responses
whereas the full line with circles denotes the mean. The grey shaded area represents the
measurement error based on the difference in variability between the pre-exercise values
before each bout. †p ≤ 0.044 relative to pre-exercise (based on main effect of time); #p < 0.05
relative to the other exercise bout (based on main effect of bout).

**Figure 5. The relationship between intracortical inhibition and muscle damage.** The
change in short-interval intracortical inhibition immediately post exercise (A and D) was
associated with the extent of muscle damage as marked by a reduction in isometric maximal
voluntary (MVC) torque 24 hours post exercise (B and E), but this was only evident in young
(C), and not older (F) adults.

**Figure 6. Neuromuscular performance during a constant torque task.** Torque variability
(coefficient of variation, CV%) during a 5 (A, D), relative 20 (B, E) and absolute 20% (C, F)
of isometric maximal voluntary contraction before, immediately post, and 24- and 72 hours
following both bouts of exercise in young (A, B, C) and older (D, E, F) adults. Dashed lines
represent individual responses whereas the full line with circles denotes the mean. The grey
shaded area represents the measurement error based on the difference in variability between
the two pre-exercise values. Differences in at 5% isometric MVC are presented based on age ×
time interaction; †p ≤ 0.015 relative to pre-exercise (based on main effect of time).
Table 1. Baseline differences between the age groups (mean ± SD) and reliability indices for main variables of interest.

<table>
<thead>
<tr>
<th></th>
<th>Bout 1</th>
<th>Bout 2</th>
<th>P</th>
<th>Bias</th>
<th>TE</th>
<th>rTE (%)</th>
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<tbody>
<tr>
<td><strong>Electrophysiological variables</strong></td>
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<tr>
<td>M&lt;sub&gt;max&lt;/sub&gt; (mV)</td>
<td></td>
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</tr>
<tr>
<td>Young</td>
<td>6.5 ± 1.8</td>
<td>5.8 ± 1.5</td>
<td>0.049</td>
<td>0.7</td>
<td>0.7</td>
<td>11.9%</td>
</tr>
<tr>
<td>Older</td>
<td>4.9 ± 1.2</td>
<td>5.3 ± 1.8</td>
<td>0.174</td>
<td>-0.4</td>
<td>0.6</td>
<td>11.8%</td>
</tr>
<tr>
<td>H&lt;sub&gt;max&lt;/sub&gt;/M&lt;sub&gt;max&lt;/sub&gt;</td>
<td></td>
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<tr>
<td>Young</td>
<td>0.11 ± 0.06</td>
<td>0.12 ± 0.06</td>
<td>0.410</td>
<td>-0.01</td>
<td>0.03</td>
<td>27.3%</td>
</tr>
<tr>
<td>Older</td>
<td>0.11 ± 0.06</td>
<td>0.11 ± 0.08</td>
<td>0.474</td>
<td>0.00</td>
<td>0.02</td>
<td>18.2%</td>
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<tr>
<td>MEP/M&lt;sub&gt;max&lt;/sub&gt;</td>
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<tr>
<td>Young</td>
<td>0.20 ± 0.10</td>
<td>0.21 ± 0.10</td>
<td>0.805</td>
<td>-0.01</td>
<td>0.08</td>
<td>40.0%</td>
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<tr>
<td>Older</td>
<td>0.29 ± 0.11</td>
<td>0.26 ± 0.13</td>
<td>0.231</td>
<td>0.03</td>
<td>0.05</td>
<td>17.9%</td>
</tr>
<tr>
<td>SICI</td>
<td></td>
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<tr>
<td>Young</td>
<td>1.21 ± 0.22</td>
<td>1.09 ± 0.11</td>
<td>0.064</td>
<td>0.12</td>
<td>0.13</td>
<td>17.8%</td>
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<tr>
<td>Older</td>
<td>1.08 ± 0.18</td>
<td>1.05 ± 0.19</td>
<td>0.585</td>
<td>0.03</td>
<td>0.22</td>
<td>20.4%</td>
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<tr>
<td>ICF</td>
<td></td>
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<tr>
<td>Young</td>
<td>152 ± 28</td>
<td>159 ± 32</td>
<td>0.297</td>
<td>7</td>
<td>18</td>
<td>11.6%</td>
</tr>
<tr>
<td>Older</td>
<td>166 ± 39</td>
<td>171 ± 41</td>
<td>0.246</td>
<td>-5</td>
<td>9</td>
<td>5.4%</td>
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</table>

|                  |        |        |         |      |     |         |
| **Mechanical variables** |        |        |         |      |     |         |
| Isometric MVC (N·m) |        |        |         |      |     |         |
| Young‡           | 59.6 ± 10.4 | 58.7 ± 11.4 | 0.964  | 0.9  | 2.9 | 4.9%    |
| Older            | 48.6 ± 11.8 | 48.0 ± 12.4 | 0.567  | 0.6  | 2.4 | 4.9%    |
| Lengthening MVC (N·m) |        |        |         |      |     |         |
| Young            | 90.9 ± 22.3 | 90.1 ± 19.2 | 0.801  | 0.8  | 7.2 | 7.9%    |
| Older            | 78.6 ± 17.6 | 80.1 ± 18.4 | 0.356  | -1.5 | 3.6 | 4.5%    |
| Lengthening/isometric MVC |        |        |         |      |     |         |
| Young            | 1.5 ± 0.2 | 1.6 ± 0.3 | 0.607  | 0.1  | 0.1 | 9.3%    |
| Older            | 1.6 ± 0.3 | 1.7 ± 0.2 | 0.381  | 0.1  | 0.1 | 7.6%    |
| 5% MVC (CV%)     |        |        |         |      |     |         |
| Older            | 7.0 ± 2.9 | 9.4 ± 4.3 | 0.081  | -2.4 | 3.0 | 36.6%   |
| Young‡           | 2.2 ± 0.9 | 2.1 ± 0.7 | 0.685  | 0.1  | 0.6 | 27.3%   |
| **20% MVC (CV%)** |        |        |         |      |     |         |
| Older            | 2.6 ± 0.9 | 3.1 ± 1.1 | 0.113  | -0.5 | 0.6 | 20.7%   |

‡p < 0.05 compared to older adults (2 × 2 ANOVA); MVC = maximal voluntary isometric contraction; M<sub>max</sub> = maximal compound action potential, H<sub>max</sub>/M<sub>max</sub> = maximal H-reflex relative to maximal compound action potential; MEP/M<sub>max</sub> = motor evoked potential relative to maximal compound action potential; SICI = short-interval intracortical inhibition; ICF = intracortical facilitation; SP = silent period; TE = typical error; rTE = relative typical error. The P-value refers to the difference between bouts (paired samples T-test). N =12 for young and n = 11 for older, except for SICI (n = 10) and ICF (n = 11) in young (see ‘Statistical analyses’ section).
### Table 2. Maximal compound action potential, creatine kinase, intracortical facilitation and prestimulus electromyographic activity (mean ± SD).

<table>
<thead>
<tr>
<th></th>
<th>Pre</th>
<th>Post</th>
<th>24 h</th>
<th>72 h</th>
<th>Time relative to exercise</th>
<th>Pre</th>
<th>Post</th>
<th>24 h</th>
<th>72 h</th>
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<td></td>
<td></td>
<td></td>
<td>Bout 1</td>
<td>24 h</td>
<td>72 h</td>
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<td></td>
<td>Bout 2</td>
<td>24 h</td>
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<tr>
<td><strong>M&lt;sub&gt;max&lt;/sub&gt; (mV)</strong></td>
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<tr>
<td>Young</td>
<td>6.5 ± 1.8</td>
<td>6.0 ± 1.5</td>
<td>6.2 ± 1.8</td>
<td>5.9 ± 1.7</td>
<td>5.8 ± 1.5</td>
<td>5.4 ± 1.2</td>
<td>6.1 ± 2.1</td>
<td>5.6 ± 2.0</td>
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<tr>
<td>Older</td>
<td>4.9 ± 1.2</td>
<td>4.6 ± 1.4</td>
<td>5.4 ± 1.8</td>
<td>5.4 ± 1.9</td>
<td>5.3 ± 1.8</td>
<td>5.3 ± 1.6</td>
<td>6.0 ± 1.7</td>
<td>5.3 ± 1.7</td>
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<tr>
<td><strong>CK (IU.L&lt;sup&gt;-1&lt;/sup&gt;)</strong></td>
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<tr>
<td>Young</td>
<td>110 ± 99</td>
<td>130 ± 102</td>
<td>367 ± 371&lt;sup&gt;†&lt;/sup&gt;</td>
<td>298 ± 291&lt;sup&gt;†&lt;/sup&gt;</td>
<td>298 ± 291&lt;sup&gt;‡&lt;/sup&gt;</td>
<td>86 ± 38</td>
<td>112 ± 49</td>
<td>122 ± 71&lt;sup&gt;‡&lt;/sup&gt;</td>
<td>160 ± 148</td>
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<tr>
<td>Older</td>
<td>97 ± 59</td>
<td>119 ± 58</td>
<td>205 ± 114&lt;sup&gt;‡&lt;/sup&gt;</td>
<td>200 ± 89&lt;sup&gt;‡&lt;/sup&gt;</td>
<td>115 ± 37</td>
<td>126 ± 41</td>
<td>164 ± 51&lt;sup&gt;‡&lt;/sup&gt;</td>
<td>181 ± 74</td>
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<tr>
<td><strong>ICF (ε unconditioned motor evoked potential)</strong></td>
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<tr>
<td>Young</td>
<td>1.21 ± 0.22</td>
<td>1.08 ± 0.14</td>
<td>1.17 ± 0.20</td>
<td>1.05 ± 0.17</td>
<td>1.09 ± 0.13</td>
<td>1.08 ± 0.20</td>
<td>1.07 ± 0.11</td>
<td>1.09 ± 0.23</td>
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<td>Older</td>
<td>1.10 ± 0.18</td>
<td>1.10 ± 0.13</td>
<td>1.09 ± 0.13</td>
<td>1.04 ± 0.25</td>
<td>1.05 ± 0.19</td>
<td>1.10 ± 0.19</td>
<td>1.08 ± 0.19</td>
<td>1.07 ± 0.19</td>
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<td><strong>Prestimulus RMS (%M&lt;sub&gt;max&lt;/sub&gt;)</strong></td>
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<tr>
<td>Young</td>
<td>7.3 ± 2.2</td>
<td>8.2 ± 2.6</td>
<td>7.4 ± 2.4</td>
<td>7.7 ± 2.2</td>
<td>7.3 ± 2.9</td>
<td>9.0 ± 4.5</td>
<td>7.5 ± 2.3</td>
<td>8.5 ± 2.4</td>
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<tr>
<td>Older</td>
<td>10.7 ± 4.9</td>
<td>12.1 ± 6.1</td>
<td>11.1 ± 7.0</td>
<td>10.3 ± 6.6</td>
<td>9.8 ± 5.8</td>
<td>10.8 ± 6.1</td>
<td>8.5 ± 3.3</td>
<td>9.8 ± 4.8</td>
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<tr>
<td><strong>5% MVC RMS (% RMS&lt;sub&gt;max&lt;/sub&gt;)</strong></td>
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<tr>
<td>Young</td>
<td>8.4 ± 3.0</td>
<td>11.3 ± 2.4&lt;sup&gt;†&lt;/sup&gt;</td>
<td>7.4 ± 3.5</td>
<td>7.0 ± 2.9</td>
<td>7.9 ± 2.6</td>
<td>11.1 ± 3.7&lt;sup&gt;‡&lt;/sup&gt;</td>
<td>7.8 ± 2.7</td>
<td>8.0 ± 3.1</td>
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<tr>
<td>Older</td>
<td>10.9 ± 4.8</td>
<td>13.5 ± 5.4&lt;sup&gt;‡&lt;/sup&gt;</td>
<td>11.5 ± 4.2</td>
<td>11.3 ± 4.3</td>
<td>11.4 ± 3.6</td>
<td>14.7 ± 6.6&lt;sup&gt;‡&lt;/sup&gt;</td>
<td>10.6 ± 4.4</td>
<td>11.3 ± 4.5</td>
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<tr>
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<td>17.5 ± 6.1</td>
<td>19.5 ± 5.0&lt;sup&gt;†&lt;/sup&gt;</td>
<td>16.5 ± 4.2</td>
<td>15.6 ± 3.5</td>
<td>17.4 ± 4.4</td>
<td>22.6 ± 4.7&lt;sup&gt;‡&lt;/sup&gt;</td>
<td>17.3 ± 3.4</td>
<td>19.0 ± 5.7</td>
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<tr>
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<td>24.7 ± 6.7</td>
<td>33.5 ± 12.2&lt;sup&gt;‡&lt;/sup&gt;</td>
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<td>25.1 ± 7.9</td>
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<td>24.2 ± 6.7</td>
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<tr>
<td>Young</td>
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<td>22.7 ± 5.0&lt;sup&gt;†&lt;/sup&gt;</td>
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<td>16.3 ± 3.4</td>
<td>17.4 ± 4.4</td>
<td>25.6 ± 5.4&lt;sup&gt;‡&lt;/sup&gt;</td>
<td>19.2 ± 6.0</td>
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<td>36.6 ± 12.0&lt;sup&gt;‡&lt;/sup&gt;</td>
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<td>8.8 ± 2.3&lt;sup&gt;†&lt;/sup&gt;</td>
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<td>6.1 ± 2.7</td>
<td>8.3 ± 4.6&lt;sup&gt;‡&lt;/sup&gt;</td>
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<tr>
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<td>11.6 ± 6.8&lt;sup&gt;†&lt;/sup&gt;</td>
<td>11.2 ± 8.4</td>
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<td>12.5 ± 8.7&lt;sup&gt;‡&lt;/sup&gt;</td>
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<tr>
<td>Young</td>
<td>15.0 ± 5.1</td>
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<td>14.8 ± 4.2</td>
<td>15.4 ± 4.5</td>
<td>13.5 ± 4.8</td>
<td>17.0 ± 8.5</td>
<td>16.9 ± 4.4</td>
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<td>29.3 ± 18.1</td>
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<td>24.8 ± 16.3</td>
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<td>22.7 ± 11.6</td>
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<td>20.2 ± 8.1</td>
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<td><strong>A&lt;sub&gt;20% MVC RMS&lt;/sub&gt; (% RMS&lt;sub&gt;max&lt;/sub&gt;)</strong></td>
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<tr>
<td>Older</td>
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<td>31.9 ± 18.8&lt;sup&gt;‡&lt;/sup&gt;</td>
<td>25.7 ± 16.3</td>
<td>24.8 ± 16.3</td>
<td>22.2 ± 14.1</td>
<td>24.2 ± 12.3&lt;sup&gt;‡&lt;/sup&gt;</td>
<td>19.9 ± 6.5</td>
<td>20.2 ± 8.1</td>
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</table>

* p < 0.050 relative to ‘Pre’, †p = 0.029 relative to the other bout, ‡p < 0.050 compared to older (bout × time interaction); M<sub>max</sub> = maximal compound action potential; CK = creatine kinase; ICF = intracortical facilitation; Prestimulus RMS (% M<sub>max</sub>) = root mean square EMG activity in the 500 ms epoch prior to stimulation at 10% isometric MVC (expressed as a percentage of maximal compound action potential); RMS (% RMS<sub>max</sub>) = root mean square EMG activity in the 10-second epoch during a constant torque task (expressed as a percentage of root mean square EMG activity during 500 ms around peak isometric MVC); RMS (% M<sub>max</sub>) = root mean square EMG activity in the 10-second epoch during a constant torque task (expressed as a percentage of maximal compound action potential); R = relative, A = absolute. N =12 for young and n = 11 for older, except for and CK in older (n = 9) and ICF in young (n = 11) (see ‘Methods’ for further details).
**A** Pre vs. post

**B** Pre vs. post 24 h

**C** YOUNG

**D** BOUT 1

**E** Isometric MVC (N.m)

**F** OLDER

**G** BOUT 2

**H** Isometric MVC (N.m)

**I** OLDER

**J** Δ SICI at immediately post

**K** Δ MVC at post 24 h

**L** R² = 0.22

**M** R = 0.47

**N** p = 0.036

**O** R² = 0.01

**P** R = 0.10

**Q** p = 0.645