Adaptations in biceps brachii motor unit activity after repeated bouts of eccentric exercise in elbow flexor muscles

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Submitted 7 October 2010; accepted in final form 19 January 2011

Dartnall TJ, Nordstrom MA, Semmler JG. Adaptations in biceps brachii motor unit activity after repeated bouts of eccentric exercise in elbow flexor muscles. J Neurophysiol 105: 1225–1235, 2011. First published January 19, 2011; doi:10.1152/jn.00854.2010.—The purpose of this study was to examine changes in motor unit activity in the biceps brachii muscle after an initial (Bout 1) and repeated (Bout 2) session of eccentric exercise separated by 1 wk. Eight subjects (aged 22 ± 2 yr) participated in experimental assessments of neuromuscular function obtained before, immediately after, 24 h after, and 7 days after each exercise bout. Each experimental session involved assessments of elbow-flexor force and biceps and triceps brachii electromyography during maximum voluntary isometric contractions (MVCs) and constant-force isometric contractions at five contraction intensities (5–50% MVC), along with indicators of muscle damage (muscle pain and passive tension). In addition, motor unit recordings were obtained before exercise, 7 days after Bout 1, and 24 h after Bout 2 to assess motor unit synchronization and recruitment thresholds. Following a single eccentric exercise session that elicited significant indicators of muscle damage, we found a 57% increase in motor unit synchronization 7 days later compared with before exercise, despite the recovery of maximal strength, soreness, and relaxed elbow-joint angle at this time. Furthermore, a second bout of the same eccentric exercise resulted in reduced indicators of muscle damage and a decline in the strength of motor unit synchronization (24 h after Bout 2) toward levels observed before both exercise sessions. In contrast, no changes in motor unit recruitment thresholds were observed 7 days after Bout 1 or 24 h after Bout 2 compared with before exercise. The increased motor unit synchronization 7 days after a single eccentric exercise session provides new evidence of changes in motor unit activity during the putative repair and regeneration phase following eccentric muscle damage.

muscle damage; single motor unit; synchronization

LENGTHENING ( ECCENTRIC) MUSCLE contractions are performed regularly in everyday lives, and are important considerations for training and rehabilitation because of their potential to produce large forces with a low metabolic cost (Abbott et al. 1952). However, one consequence of performing eccentric exercise is that it produces substantial muscle damage. This muscle damage manifests as a loss of muscle strength, a shift to a longer muscle length for peak force generation, and muscle soreness 1 or 2 days after the exercise (see Proske and Morgan 2001 for review). Furthermore, numerous changes are evident in the central nervous system (CNS) after eccentric muscle damage, such as a disturbance in proprioception (Brockett et al. 1997; Weerakkody et al. 2003), a reduction in maximal voluntary muscle activation (Prasartwuth et al. 2006), an increase in electromyography (EMG) at submaximal forces (Weerakkody et al. 2003; Semmler et al. 2007), and an increase in antagonist muscle coactivation (Leger and Milner 2001; Semmler et al. 2007). More recently, we have shown with intramuscular motor unit recordings that eccentric muscle damage alters the neural control of force for at least 24 h after the exercise (Dartnall et al. 2008; 2009). In particular, we found an increase in motor unit synchronization (Dartnall et al. 2008) and a reduction in motor unit recruitment threshold (Dartnall et al. 2009) in biceps brachii motor units when recorded immediately after and 24 h after unaccustomed eccentric exercise of the elbow flexor muscles. These studies suggest that the muscle damage from eccentric exercise produces short-term (≤24 h) changes in the CNS that act to increase motor unit activity during voluntary contractions.

When the muscle is damaged after unaccustomed eccentric exercise, there is a repair and regeneration process that occurs over days and weeks following the exercise, where histological appearance, total protein content, and muscle function typically return to normal (see Cutlip et al. 2009 for review). During this period, changes in the neuromuscular system are likely to contribute to an adaptation process, where a subsequent bout of eccentric exercise results in reduced indicators of muscle damage and soreness compared with the first bout, a process that has been termed the repeated-bout effect (Nosaka and Clarkson 1995). Although the consensus is that changes in the muscle are largely responsible for this adaptation process (see McHugh 2003), there is some limited evidence that the CNS may be involved. For example, there is a decrease in the EMG median frequency of the tibialis anterior muscle during a repeated bout of eccentric exercise (Warren et al. 2000), which may indicate a change in motor unit activity resulting from the muscle damage. Furthermore, several strength-training studies involving eccentric exercise have shown a larger increase in EMG throughout an eccentric, compared with a concentric, training protocol (Komi and Buskirk 1972; Hortobagyi et al. 1996a; 1996b). These surface EMG studies have led to the interpretation that changes in motor unit recruitment and synchronization may protect the muscle from subsequent damage by distributing the muscle torque over a greater number of motor units in the damaged muscle (Golden and Dudley 1992; Mair et al. 1995; Nosaka and Clarkson 1995; Warren et al. 2000). However, a number of different anatomical and physiological factors that are independent of motor unit activity influence the surface EMG (see Farina et al. 2004), which limits its usefulness as an index of motor unit activation. No studies to date have examined longer-term (>24 h) changes in

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motor unit activity after eccentric exercise when assessed with more direct intramuscular recording techniques.

The primary aim of the present study was to use intramuscular recordings to examine changes in motor unit activity after a single and repeated bout of eccentrics exercise. Because we have previously observed altered motor unit recruitment threshold and synchronization for at least 24 h after unaccustomed eccentric exercise (Dartnall et al. 2008; 2009), we expect longer-lasting changes in these features of motor unit activity that are evident before a subsequent bout of exercise is performed 1 wk later. A secondary aim was to examine whether there is a change in motor unit synchronization and recruitment thresholds 24 h after a second bout of eccentric exercise. Any changes in motor unit activity after eccentric exercise would provide new evidence of a neural adaptation that is evident 7 days after a single exercise session and would be a potential contributor to reduced muscle damage with subsequent sessions of exercise involving lengthening contractions (i.e., repeated-bout effect).

METHODS

Eight healthy young subjects [4 males, 4 females; age 22 ± 2 (SD) yr] volunteered for this study. No subject had participated in regular strength training for ≥6 mo before the study, and none had a history of musculoskeletal pain or injury in the left arm or shoulder. Written informed consent was obtained from all subjects before beginning the set of experiments. All experimental procedures conformed to the Declaration of Helsinki and were approved by the Human Research Ethics Committee at the University of Adelaide.

All subjects performed two types of contractions with the elbow flexor muscles, one involving isokinetic movements that produce muscle damage (eccentric exercise), and the other involving experimental tasks during isometric contractions. The arrangement used to measure isometric force and EMG during experimental tasks has been described previously (Dartnall et al. 2008; 2009). Subjects were seated in a chair with the left elbow joint flexed at 90 degrees and the forearm positioned vertically. The forearm was constrained in a seated in a chair with the left elbow joint flexed at 90 degrees and the forearm positioned vertically. The forearm was constrained in a seated in a chair with the left elbow joint flexed at 90 degrees and the forearm positioned vertically. The forearm was constrained in a seated in a chair with the left elbow joint flexed at 90 degrees and the forearm positioned vertically. The forearm was constrained in a seated in a chair with the left elbow joint flexed at 90 degrees and the forearm positioned vertically. The forearm was constrained in a seated in a chair with the left elbow joint flexed at 90 degrees and the forearm positioned vertically. The forearm was constrained in a seated in a chair with the left elbow joint flexed at 90 degrees and the forearm positioned vertically. The forearm was constrained in a seated in a chair with the left elbow joint flexed at 90 degrees and the forearm positioned vertically. The forearm was constrained in a seated in a chair with the left elbow joint flexed at 90 degrees and the forearm positioned vertically. The forearm was constrained in a seated in a chair with the left elbow joint flexed at 90 degrees and the forearm positioned vertically. The forearm was constrained in a seated in a chair with the left elbow joint flexed at 90 degrees and the forearm positioned vertically. The forearm was constrained in a seated in a chair with the left elbow joint flexed at 90 degrees and the forearm positioned vertically. The forearm was constrained in a seated in a chair with the left elbow joint flexed at 90 degrees and the forearm positioned vertically. The forearm was constrained in a seated in a chair with the left elbow joint flexed at 90 degrees and the forearm positioned vertically. The forearm was constrained in a seated in a chair with the left elbow joint flexed at 90 degrees and the forearm positioned vertically. The forearm was constrained in a seated in a chair with the left elbow joint flexed at 90 degrees and the forearm positioned vertically. The forearm was constrained in a seated in a chair with the left elbow joint flexed at 90 degrees and the forearm positioned vertically. The forearm was constrained in a seated in a chair with the left elbow joint flexed at 90 degrees and the forearm positioned vertically. The forearm was constrained in a seated in a chair with the left elbow joint flexed at 90 degrees and the forearm positioned vertically.

Biopolar electrodes (silver-silver chloride, 4-mm diameter) were used to record surface EMG signals from the biceps brachii and triceps brachii muscles. Electrodes were placed ~2 cm apart (center to center) midway between the biceps brachii muscle belly and tendon and over the long head of the triceps brachii muscle. A grounding strap was positioned around the wrist to serve as a common reference. The surface EMG signals were amplified (100–1,000×; V75-02, Coulbourn Instruments, Allentown, PA), band-pass filtered (13 Hz–1 kHz), and stored on computer for offline analysis. A force transducer (MLP-150, range 0–700 N; Transducer Techniques, Temecula, CA), located perpendicular to the forearm at the level of the wrist was used to detect isometric forces exerted during the maximum voluntary isometric contraction (MVC) and constant force tasks. The output from the force transducer was displayed on an oscilloscope and stored on computer for offline analysis.

To obtain bipolar recordings of the discharge of single motor units, three or four separate fine-wire needle electrodes were inserted 1–2 cm deep into the lateral head of the biceps brachii muscle for each motor unit recording session. Each intramuscular electrode was placed ~2–2 cm apart positioned vertically along the muscle. Each individual electrode consisted of three Formvar-insulated, stainless-steel wires (50 μm diameter; California Fine Wire, Grover Beach, CA) threaded through the lumen of a 27-gauge disposable hypodermic needle. Each electrode had a hook of ~2 mm at the recording end. Once the electrode was inserted into the biceps brachii, the needle was withdrawn, leaving the wires secured within the belly of the muscle. Two of the three wires were used to obtain bipolar recordings, with the third wire providing an alternative bipolar configuration to sample from other motor units within the same muscle as necessary. Single motor unit action potentials were detected from two separate needle electrodes at any given time, with the recordings optimized by fine manual adjustments of the wires. Motor units were detected online from one of the electrodes using an amplitude window discriminator (V21-10, Coulbourn Instruments) connected to a speaker to provide audio feedback of motor unit discharge during the low-force elbow-flexion contractions. Single motor unit recordings were amplified (1,000×; V75-02, Coulbourn Instruments), band-pass filtered (90 Hz–10 kHz; Coulbourn Instruments), displayed on an oscilloscope, and stored on computer for offline analysis.

Experimental Procedures

Relaxed elbow angle was measured as an indirect indicator of a change in passive muscle tension after eccentric exercise. The measurement was obtained from the midpoint of the acromion and coracoid processes to the lateral epicondyle then to the midpoint of the ulnar and radius styloid processes. A goniometer was used to measure the elbow angle while the subjects stood upright with their left arms relaxed by their sides and their palms facing medially. A decrease in elbow joint angle after eccentric exercise represented a more flexed elbow, indicative of an exercise-induced rise in passive tension of the elbow flexor muscles (Jones et al. 1987). As an indicator of delayed-onset muscle soreness (DOMS) induced by eccentric exercise, subjects rated the degree of soreness exhibited in the biceps during forced extension of the elbow joint. Soreness was rated using a visual analog scale where 0 represented no muscle soreness, and 10 corresponded to the most intense muscle soreness imaginable. Relaxed elbow angle and DOMS were measured before, immediately after, 24 h after, and 7 days after two bouts of eccentric exercise performed 1 wk apart.

For the experimental tasks, each subject performed four tasks requiring isometric contraction of the elbow flexor muscles: 1) MVC (N) at 90° elbow flexion, 2) a constant-force task, consisting of a steady submaximal isometric contraction at five different force levels at 90° elbow flexion, 3) a motor unit recruitment threshold task, to identify the lowest force at which a selected motor unit was recruited and derecruited during isometric contractions at 90° elbow flexion, and 4) a motor unit repetitive discharge task to assess motor unit synchronization, consisting of sustained tonic discharge of a pair of biceps brachii motor units at a constant, low-mean-discharge frequency during isometric contractions at 90° elbow flexion. Surface EMG was recorded during all four tasks, and single motor unit recordings were obtained in tasks 3 and 4. The motor unit tasks were performed on three separate occasions in the same subjects, which consisted of a baseline measure before the first session of eccentric exercise (referred to as Bout 1), a measure 7 days after Bout 1, and a final measure 24 h after the repeated bout of eccentric exercise (Bout 2). All other force tasks were performed before, immediately after, 24 h after, and 7 days after each bout of eccentric exercise.

MVC force. The MVC task involved a ramp increase in isometric elbow-flexion force from resting to maximum force over 3 s that was sustained for a further 3 s. Subjects were provided with verbal timing of the task by the experimenter and were able to monitor the force displayed on an oscilloscope placed in front of them at eye level. Verbal encouragement was provided by the experimenter to facilitate maximum force production during the sustained maximum contraction. The subject performed three isometric MVCs with at least 1 min rest between contractions, and the force was stored on a computer for offline analysis. This task was then repeated for elbow extension. The trial with the greatest force was considered the MVC.
force and then used as the reference for the constant-force task in each respective testing session.

**Constant-force task.** A horizontal line on the oscilloscope screen showed the required target for each submaximal force level. Isometric contractions were performed at target forces of 5, 10, 20, 35, and 50% MVC (% MVC refers to the MVC recorded at the beginning of the respective testing session). Subjects were instructed to exert a steady elbow-flexion force for 10 s at each target force. One trial was performed at each target force level, and the order of contractions was randomized. Each trial was visually scrutinized for erroneous force fluctuations attributable to concentration errors from the subject, and, if obvious deviations in force occurred, the trial was repeated at the appropriate force level.

**Motor unit recruitment task.** The discharges of single motor units were recorded from the biceps brachii while a steady elbow-flexion force, detected by a more sensitive force transducer (MLP-25, range 0–112 N, Transducer Techniques), was exerted by the subjects. Subjects were provided with a target line on the oscilloscope representing a ramp increase in force of 2% MVCs. With the aid of the target line, subjects slowly increased the elbow-flexion force until one motor unit began to discharge action potentials that were detected with an intramuscular electrode. The flexion force required to activate the motor unit was then held constant for 3 s and then lowered at the same rate it was increased. The subject was given feedback through audio speakers of the discharge events obtained from the single motor unit. This task was performed three times for each identified motor unit, with a period of at least 10-s rest between each trial. The mean force at which the motor unit first began to discharge action potentials over the three trials of increasing force was regarded as the motor unit recruitment threshold (Barry et al. 2007; Dartnall et al. 2009). Recruitment threshold was assessed in the same manner but was calculated as the mean force at which the motor unit ceased to discharge action potentials with decreasing force.

**Motor unit repetitive-discharge task.** The discharge of pairs of single motor units was recorded from the biceps brachii while subjects exerted a low-, or moderate-intensity elbow-flexion force. Elbow-flexion force was slowly increased by the subjects until at least one motor unit that discharged action potentials repetitively was detected with each electrode. Audio feedback of the discharge from one motor unit was provided to the subject, and a target force was made available on the oscilloscope screen, which represented the force required to sustain tonic discharge for 2–3 min. To enable at least one motor unit from each electrode to be accurately discriminated, the target force was occasionally adjusted during these trials. The mean force used throughout the trials to activate the motor units was noted, and the strength of the contraction relative to maximum force in that session (% MVC) was determined. Motor units were active at relatively low forces, and subjects rested for at least 2 min at the end of each trial, minimizing the effects of fatigue. After a period of rest, a different motor unit from one of the electrodes was selected for recording, either by moving the electrode at least 0.5 cm to detect a different motor unit waveform, or by recording from a new electrode. To reduce the likelihood of recording from the same motor unit on separate occasions, no more than three different motor units were recorded from a single electrode.

**Eccentric exercise.** Eccentric exercise with the elbow flexors of the left arm was used to induce an ~40% reduction of isometric MVC force in all subjects, as performed previously (Semmler et al. 2007; Dartnall et al. 2008; 2009). A constant reduction in strength was used to obtain a similar extent of muscle damage in all subjects, which reduces the variability in strength loss after eccentric exercise compared with protocols that use a set number of contractions, and likely reduces any potential sex differences in the extent of muscle damage. Subjects performed maximal voluntary eccentric contractions of the elbow flexors of the left arm on a Biodex isokinetic dynamometer (System 4; Biodex Medical Systems, New York, NY) at an angular velocity of 45°/s, with an angular displacement of the elbow joint from 90° to 180° (full extension). Each muscle contraction lasted 2 s followed by a 4-s rest period in which the subjects relaxed while the dynamometer arm returned automatically back to 90° of elbow flexion to begin the next repetition. Sets of 10 maximal isokinetic contractions with 30-s rest in between each set were performed. Subjects completed 3 sets of 10 contractions, at which time a brief isometric MVC at 90° was performed to monitor the reduction in elbow-flexor torque. The sets of eccentric contractions continued until there was a reduction in the isometric MVC torque of at least 40%. This required between 30–110 contractions in this group of subjects. A repeated bout of the same number of eccentric contractions for each subject was performed 7 days after the initial bout.

**Data Analysis**

All signals recorded were digitized (CED 1401, Cambridge, UK) onto a hard drive of a computer and sampled at 200 Hz (force), 2,000 Hz (torque and surface EMG), or 20 kHz (single motor unit recordings). Offline analysis was performed with Spike 2 data analysis software (Cambridge Electronic Design, Cambridge, UK) and custom-written scripts. For the MVC trial, the EMG was full-wave rectified and averaged over a 1-s epoch centered around the point at which the maximum force was achieved. For the constant-force task, a 10-s sample of force from the middle of each trial was used to calculate the mean and standard deviation of force. From these values the coefficient of variation of force (SD/mean force × 100) was obtained. The biceps and triceps surface EMG was rectified and averaged over a user-selectable window of 1 s corresponding to a stable portion of the EMG and force record. The EMG was then normalized to the maximum EMG recorded during the MVC performed on that day to facilitate comparisons made between subjects and across days. For the recruitment-threshold trials, the average rectified EMG from biceps and triceps brachii was quantified over 0.5 s before and after the time of the first action potential (recruitment threshold). For the motor unit synchronization trials, the average rectified biceps and triceps brachii EMG was quantified over the duration of each trial.

A computerized spike-sorting algorithm (Spike 2, version 6; Cambridge Electronic Design) was used to discriminate single motor unit discharges. Waveform shape was used to identify action potentials belonging to a particular motor unit of interest. For each trial of the synchronization task, the interspike intervals of identified motor units were examined to ensure discrimination accuracy. The mean, standard deviation (SD), and coefficient of variation (CV) of inter-spike intervals (ISIs) were obtained, and custom-designed software written in Matlab (Mathworks, Natick, MA) was used to determine the mean frequency of the discharge times (1,000/mean ISI). Cross-correlation analysis was used to assess the magnitude of motor unit synchronization in pairs of concurrently active motor units detected with separate electrodes. The cross-correlation histogram comprised 201 bins characterizing the discharge behavior of one motor unit for 100 ms before and after each discharge of the reference motor unit. The location and width of the central synchronous peak in the cross-correlogram was estimated using the cumulative sum (Ellaway 1978) technique. Methods described by Wiegner and Wierzbicka (1987) were used to determine statistical significance of the peak in the cross-correlation histogram. A standard peak width of 11 ms centered at 0 ms was used when no significant peak was observed to quantify the strength of synchronization in that motor unit pair. Cross-correlation histograms were not analyzed if the mean bin count was <4. Two commonly used indices were used to quantify the magnitude of the central synchronous peak in the cross-correlogram: J) the index common input strength (CIS), which represents the frequency of synchronous discharges in excess of those expected attributable to chance for a period in which both motor units are tonically active (expressed as pulses/s,pps) (Nordstrom et al. 1992), and 2) the index E (Datta et al. 1991; Datta and Stephens 1990), which corresponds to...
the total number of extra counts within the peak above those expected due to chance, relative to the total number of reference-unit discharges (expressed as pulses/trigger event, ppt).

Coherence analysis was performed in Matlab (Mathworks) on the coherence spectrum of the discharge times of the same pairs of motor units analyzed in the synchronization task (Farmer et al. 1993b; Rosenberg et al. 1989; Semmler et al. 2004). The discriminated motor unit data were divided into contiguous, nonoverlapping epochs of 1.28 s that comprised 256 bins. A value of 1 was assigned to each 5-ms bin when it contained a discriminated action potential, and, when no action potential was present, a value of 0 was given. These data were transformed into the frequency domain with a resolution of 0.8 Hz. The coherence data for each motor unit pair were obtained by averaging auto- and cross-spectra over the disjoint sections, resulting in a measure of linear association with values between 0 (completely independent) and 1 (completely dependent). Calculation of pooled coherence between the same pair of motor units was then performed (Amjad et al. 1997), allowing motor units to be combined under similar conditions. Significant values of coherence were those exceeding the 95% confidence level (Rosenberg et al. 1989) for the frequencies of interest (0–30 Hz). To allow for comparison of coherence between subjects and to facilitate statistical comparison for recordings on different days the estimates of coherence for each pair of motor units were normalized into z-scores (Rosenberg et al. 1989).

Statistical Analysis

Elbow-flexor force and biceps and triceps brachii surface EMG during maximum-flexion contractions were analyzed using a one-way repeated-measures ANOVA to assess the effect of each eccentric exercise bout (time effect: before, immediately after, 24 h after, and 7 days after). To analyze the CV for force and the average rectified biceps and triceps brachii surface EMG for the constant-force tasks, a two-way repeated-measures ANOVA for time and target force (5, 10, 20, 35, and 50% MVC) was used. A paired t-test with Bonferroni correction was used to examine differences for each dependent variable between each bout of eccentric exercise. For the motor unit tasks, the dependent variables were recruitment and derecruitment threshold, flexion force, CV of discharge rate, geometric mean of the discharge rate for motor unit pairs used for synchrony analysis, synchronization strength (CIS and E), synchronization peak width, and the strength of coherence. To allow for comparison of coherence on different days the estimates of coherence for each pair of motor units were normalized into z-scores (Rosenberg et al. 1989).

RESULTS

MVC Force, EMG, and Muscle Damage

An average of 56 ± 14 maximal isokinetic eccentric contractions were required to produce an ~40% decline in maximum strength and significant indicators of muscle damage after eccentric exercise in all subjects (Table 1). Each subject performed two eccentric exercise bouts 7 days apart that involved the same number of contractions on each occasion.

The amount of work performed by each subject in the first bout (72 ± 37 Nm.s) was similar to that in the second bout (68 ± 30 Nm.s, P = 0.16). For each exercise bout (Table 1), significant effects of time in the ANOVA were obtained for MVC force (Bouts 1 and 2, P < 0.001), relaxed elbow angle (Bout 1 only, P < 0.001), and DOMS (Bout 1 only, P < 0.001), whereas there was no change in biceps brachii MVC EMG with time after either bout (P = 0.80 and 0.73). After Bout 1, there was a significant decline in MVC force and a decrease in relaxed elbow-joint angle immediately after and 24 h after exercise, whereas significant muscle soreness was obtained 24 h after exercise (all P values <0.05). The only change in these indicators of muscle damage after Bout 2 was a significant decline in MVC force immediately after exercise (P < 0.0001) that recovered a day later (P = 0.36). Reduced muscle damage in Bout 2 compared with Bout 1 of eccentric exercise was shown by improved recovery of strength (P = 0.008) and reduced muscle soreness (P = 0.004) 24 h after exercise (Table 1). Despite significant differences in elbow-flexor strength between males and females (sex effect, P < 0.001), there was no difference between sexes in the response to eccentric exercise for any indicator of muscle damage (all sex × time interactions from Table 1 had P values >0.53). However, it is possible that potential sex differences in the response to muscle damage may be evident with improved statistical power, and future studies specifically designed to

| Table 1. MVC force, EMG, and indicators of muscle damage recorded before, immediately after, 24 h after, and 7 days after 2 bouts of eccentric exercise |
|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| Exercise        | Before          | After           | 24 h After       | 7 Days After     | % ΔA-B          | %ΔD1-B          | %ΔD7-B          |
| Elbow Flexor    | Bout 1          | Bout 2          | Bout 1           | Bout 2           | Bout 1          | Bout 2           | Bout 1          | 24 h After       | 7 Days After     | % ΔA-B          | %ΔD1-B          | %ΔD7-B          |
| Biceps MVC      | 201 ± 94        | 149 ± 73*       | 193 ± 101‡§      | 196 ± 85‡        | 0.60 ± 0.20     | 0.57 ± 0.17     | 0.56 ± 0.17     | 0.63 ± 0.15      | 6 ± 49           | 12 ± 71          | 30 ± 72         |
| EMG, mV         | 0.63 ± 0.15     | 0.66 ± 0.15     | 0.69 ± 0.15      | 0.67 ± 0.22      | Bout 1          | Bout 2          | Bout 1          | Bout 2           | Bout 1          | Bout 2          | Bout 1          |
| % ΔA-B          | 3 ± 7           | 4 ± 6           | 5 ± 6            | 3 ± 3            | -2 ± 4          | 3 ± 5           | 3 ± 3           | 1 ± 4            |
| Relaxed Elbow   | 151 ± 7         | 139 ± 6*        | 142 ± 10*        | 149 ± 11†‡       | 0.00 ± 0.00     | 0.4 ± 0.69      | 1.8 ± 1.00‡     | 0.00 ± 0.00†     | —               | —               |
| Angle, °        | 149 ± 11        | 146 ± 11        | 146 ± 8          | 150 ± 8          | Bout 2          | Bout 2          | Bout 2          | Bout 2           | 0.00 ± 0.00     | —               | —               |
| DOMS on a VAS, cm| Bouyt 1          | Bouyt 2          | Bouyt 1          | Bouyt 2          | Bouyt 1          | Bouyt 2          | Bouyt 1          | Bouyt 2           | 0.00 ± 0.00     | —               | —               |

Data are shown as means ± SD. %Δ values were calculated from individual subject data. MVC, maximum voluntary contractions; EMG, electromyography; DOMS, delayed-onset muscle soreness; VAS, visual analog scale; A–B, difference in before and after; D1-B, 24 h after bout; D7-B, 7 days after bout. *P < 0.05 compared with before eccentric exercise in the same bout. †P < 0.05 compared with immediately after eccentric exercise in the same bout. ‡P < 0.05 compared with 24 h after eccentric exercise in the same bout. §P < 0.05 compared with Bout 1 at the same time point.
examine sex differences after eccentric exercise will require increased subject numbers to address this issue.

Submaximal EMG and Force Fluctuations

Biceps and triceps brachii EMG and elbow-flexor force fluctuations during submaximal isometric contractions were quantified before and after the two bouts of eccentric exercise (Fig. 1). For each exercise bout, significant effects of time were obtained for CV of force (Bouts 1 and 2, \( P < 0.001 \)), biceps brachii EMG (Bouts 1 and 2, \( P < 0.001 \)), and triceps brachii EMG (Bout 1 only, \( P < 0.001 \)). Across all target forces after Bout 1, there was a significant increase in CV of force (Fig. 1A) and biceps brachii EMG (Fig. 1B) immediately after \( P < 0.0001 \) and 24 h after exercise (\( P = 0.005 \) and 0.016), whereas an increase in triceps brachii EMG (Fig. 1C) was only observed immediately after Bout 1 (\( P < 0.0001 \)). After Bout 2, there was a significant increase in CV of force (\( P < 0.0001 \)) and biceps brachii EMG (\( P < 0.0001 \)) immediately after exercise and an increase in CV of force 24 h later (\( P < 0.05 \)). Despite these increases, the second bout of eccentric exercise resulted in lower CV of force immediately after exercise compared with immediately after Bout 1 (\( P = 0.006 \)). Significant differences between target forces were obtained for CV of force (Bout 1 only, \( P < 0.0001 \)) and biceps brachii EMG (Bout 2 only, \( P = 0.0006 \)) but not triceps brachii EMG after either bout (\( P = 0.68 \) and 0.84).

Force fluctuations were still significantly greater 24 h after the first bout compared with before exercise but only at a target force of 5% MVC (\( P = 0.001 \)) and had recovered back to preexercise levels 7 days later. After Bout 2 there was no significant difference in CV of force for each target force during the constant-force task. When compared with before Bout 2, biceps brachii EMG was significantly greater at target forces of 35% MVC and 50% MVC immediately after the repeated bout (\( P = 0.0002 \) and <0.0001), and was significantly less 24 h (\( P = 0.0005 \) and <0.0001) and 7 days later (\( P = 0.0009 \) and 0.0003) compared with immediately after the exercise.

Motor unit Recruitment Task

For the motor unit recruitment task, a total of 206 motor units were obtained from 8 subjects in all recording sessions. A similar number of motor units were recorded from the biceps brachii in each subject for each of the three separate recording sessions (~8 in each session). For the biceps brachii motor units sampled in this population, no significant effect of time in the ANOVA was obtained for motor unit recruitment threshold (\( F_{2,220} = 0.03, P = 0.97 \)) or motor unit derecruitment threshold (\( F_{2,203} = 0.78, P = 0.46 \)) (Table 2). A significant effect of time was obtained for biceps brachii EMG at recruitment (\( F_{2,151} = 6.26, P = 0.002 \)) but not for triceps EMG at recruitment (\( F_{2,97} = 0.007, P = 0.99 \), Table 2). Biceps brachii EMG was not different 7 days after the first bout (\( P = 0.06 \)) but was significantly reduced 24 h after Bout 2 compared with before the first bout of eccentric exercise (\( P = 0.0005 \), Table 2).

Motor unit Synchronization Task

Figure 2 illustrates recordings obtained during the motor unit synchronization task before and after each bout of eccentric exercise in one subject. To activate the pair of motor units throughout the trial, the subject exerted a mean force of 37.8 N.
(9.8% MVC) before the first bout of exercise, 28.0 N (7.2% MVC) 7 days after the first bout, and 36.5 N (8.3% MVC) 24 h after the second bout of exercise. The geometric mean discharge rate for both motor units of the pair before exercise was 11.3 Hz, compared with 10.4 Hz 7 days after the initial bout, and 9.6 Hz 24 h after Bout 2. The geometric mean discharge rate variability was 13.6% before exercise, compared with 12.4% 7 days after the first bout, and 11.7% 24 h after Bout 2. The motor unit synchronization index CIS was 0.37 for the motor unit pair examined before the first bout of exercise, and 0.66 and 0.39 for the pairs studied 7 days after the first bout and 24 h after the second bout, respectively. The peak width of the central synchronous peak in the cross-correlogram of each pair studied was 13 ms before, 19 ms 7 days after the first bout, and 16 ms 24 h after Bout 2. For all motor unit pairs in this subject, the mean strength of motor unit synchronization was 0.42 before (n = 7), 0.60 7 days after the first bout (n = 7), and 0.43 24 h after the second bout (n = 8). The mean peak width for all cross-correlation histograms obtained from this subject was 14 ms before, 19 ms 7 days after Bout 1, and 17 ms 24 h after Bout 2.

For the motor unit synchronization task, a total of 133 motor unit pairs (206 individual motor units) were obtained from eight subjects in all recording sessions, with 46 motor unit pairs obtained before the first bout of exercise, 43 motor unit pairs 7 days after the first bout, and 45 motor unit pairs 24 h after the second bout.
recorded 24 h after the second bout of eccentric exercise. The number of motor unit pairs recorded in each subject in each of the three recording sessions was similar (~6).

For all motor unit pairs, the mean strength of motor unit synchronization was influenced by time for the synchronization index CIS (Fig. 3A; $F_{2,130} = 3.40, P = 0.036$) and the index E (Fig. 3B; $F_{2,130} = 3.55, P = 0.032$). Synchronization index CIS increased by 57% (0.16 pps, $P = 0.01$) 7 days after the first bout of eccentric exercise compared with before and was not significantly different 24 h after the second bout of exercise compared with before Bout 1 ($P = 0.39$) and 7 days after the first bout ($P = 0.09$). Similarly, synchronization index E increased by 48% (0.013 ppt, $P = 0.01$) 7 days after the first bout of eccentric exercise compared with before and was not significantly different 24 h after the second bout of exercise compared with before the first bout ($P = 0.27$) and 7 days after the first bout ($P = 0.14$). The duration of the synchronous peak was also influenced by time (Fig. 3C; $F_{2,130} = 5.99, P = 0.003$). The duration of the mean peak width significantly increased by 6 ms 7 days after the first bout compared with before the first bout ($P = 0.005$) and was 6 ms greater 7 days after Bout 1 compared with 24 h after Bout 2 ($P = 0.002$). Peak width was not different 24 h after Bout 2 compared with before Bout 1 ($P = 0.78$). A weak but significant positive linear association was found between synchrony index CIS and peak width before Bout 1 ($r^2 = 0.14, P = 0.01$), 7 days after Bout 1 ($r^2 = 0.16, P = 0.009$), but not 24 h after the repeated bout ($r^2 = 0.05, P = 0.15$). The mean elbow-flexion force exerted during the motor unit trials was not significantly different for the three testing sessions and was 5.9% MVC before, 7.3% MVC 7 days after Bout 1, and 6.4% MVC 24 h after Bout 2 (time effect: $F_{2,78} = 0.85, P = 0.43$; Fig. 3D).

The discharge characteristics were obtained from the same pairs of motor units examined in the synchronization task. The geometric mean discharge rate was influenced by time (Fig. 3E; $F_{2,131} = 11.08, P < 0.0001$); however, the geometric mean discharge rate variability was not (Fig. 3F; $F_{2,131} = 0.50, P = 0.61$). Post hoc analysis revealed that geometric mean discharge rate remained unchanged 7 days after the first bout compared with before ($P = 0.34$) but was significantly lower 24 h after the second bout of exercise compared with before Bout 1 (1.1 Hz, $P < 0.0001$) and 7 days after the first bout (0.9 Hz, $P = 0.0007$). Biceps brachii EMG recorded during the...
motor unit synchronization trials (data not shown) was influenced by time ($F_{2,75} = 3.79$, $P = 0.03$). Similar to biceps brachii motor unit discharge rate (Fig. 3E), EMG of the biceps brachii decreased 24 h after Bout 2 compared with the EMG recorded from the biceps brachii before the first bout of eccentric exercise ($P = 0.008$). No effect was found for time in the ANOVA for triceps brachii EMG recorded during the motor unit synchrony trials ($F_{2,48} = 0.02$, $P = 0.98$).

Coherence analysis was performed on the same 133 motor unit pairs that were used for the cross-correlation analysis measured before and after each exercise bout. Typically for biceps brachii motor units, a single large-amplitude peak occurred from 1–2 Hz in the coherence spectrum, with minimal coherence observed above 10 Hz (see Dartnall et al. 2008). Mean coherence $z$-scores were examined for the low- (0–5 Hz), medium- (5–10 Hz), and high-frequency (10–30 Hz) bands. No difference in mean coherence was observed at each of the time points (time effect; $F_{2,63} = 0.54$, $P = 0.59$), and this was similar for each frequency band (time $\times$ frequency band interaction; $F_{4,63} = 0.49$, $P = 0.74$). The mean coherence $z$-scores for the low-frequency band (0–5 Hz) were 4.1 before, 5.5 7 days after Bout 1, and 5.1 24 h after Bout 2. For the medium-frequency band (5–10 Hz), the mean coherence $z$-scores were 1.3 before, 1.3 7 days after the first bout, and 1.4 24 h after the repeated bout. The mean coherence $z$-scores for the high-frequency band were 1.4 before, 1.5 7 days after Bout 1, and 1.2 24 h after the second bout.

DISCUSSION

On the basis of our previous observations of a change in motor unit synchronization (Dartnall et al. 2008) and recruitment thresholds (Dartnall et al. 2009) that occurred for at least 24 h after unaccustomed eccentric exercise, we were interested in determining whether there were any longer-lasting (7 days) changes in motor unit activity and whether any of these effects were attenuated after a second bout of exercise. We found an increase in motor unit synchronization in biceps brachii motor units 7 days after the exercise despite the recovery of maximal strength, soreness, and relaxed elbow-joint angle at this time. Furthermore, a similar bout of eccentric exercise performed 1 wk after the first exercise session resulted in reduced symptoms of muscle damage, suggesting an adaptation in the neuromuscular system with repeated bouts of eccentric exercise. Following the second bout of exercise, there were divergent changes in motor unit synchronization and recruitment thresholds 24 h later, suggesting that different mechanisms are responsible for the change in these features of motor unit activity after repeated eccentric exercise. A summary of these changes in motor unit activity has been provided in Fig. 4, along with the motor unit synchronization (Dartnall et al. 2008) and recruitment threshold (Dartnall et al. 2009) data for up to 24 h after exercise from our previous studies.

Changes in Motor unit Activity after Eccentric Exercise

The time course of the effect of muscle damage can be described as an initial event that lasts 24–48 h and then a period of regeneration and repair that can last several days to weeks. The initial event results in several dramatic changes in the muscle, such as the disruption of sarcomeres, disruption of cytoskeletal elements involved in force transmission, damage to the muscle cell membrane, impaired excitation-contraction coupling, and loss of force production (see Proskove and Morgan 2001 for review). These effects of muscle damage were evident in the present study as a long-lasting decline in muscle strength, changes in resting elbow-joint angle reflecting a rise in passive muscle tension, increased muscle soreness several days after the exercise, and an increase in EMG and force fluctuations during low-force isometric contractions. Within this early period following unaccustomed eccentric exercise, we have recently shown alterations in motor unit activity that last for at least 24 h after the exercise bout (Fig. 4). For example, there is an increase in motor unit synchronization (Dartnall et al. 2008) and a reduction in motor unit recruitment threshold (Dartnall et al. 2009) in the biceps brachii muscle immediately after and 24 h after exercise, providing unequivocal evidence of nervous-system adjustments in the early stages of eccentric muscle damage.
In contrast to the initial muscle damage, there is a subsequent regeneration period that lasts from a few days to several weeks, in which changes in the neuromuscular system contribute to an adaptation process, presumably to repair the muscle and reduce the extent of damage during a subsequent bout of exercise. Several changes in the muscle are known to occur throughout this process, such as the removal of damaged “stress-susceptible” fibers (Armstrong et al. 1983), strengthening of the sarcolemma and remodeling of the intermediate filaments (Armstrong et al. 1991), and chronic inflammation of the muscle tissue, including an increased number of monocytes and macrophages (Jones et al. 1986; Round et al. 1987). In addition to these muscular changes, we provide evidence in the present study showing changes in the CNS during this putative regeneration and repair process. When we examined it 7 days after eccentric muscle damage, we observed an ~50% increase in motor unit synchronization in the biceps brachii muscle during low-force isometric contractions. This change in correlated motor unit activity was evident despite the recovery of all other assessments of neuromuscular function, such as maximal strength, submaximal EMG and force fluctuations, and motor unit recruitment and dererecruitment thresholds.

Motor unit synchronization, which represents the near-coincident discharges of action potentials in different motor units above a level that is expected by chance, is believed to occur because of common synaptic input that is delivered to the motoneurons at the level of the spinal cord (Sears and Stagg 1976; Kirkwood and Sears 1978). As such, variations in the amount of motor unit synchronization are interpreted as a change in the CNS strategy that is used to perform the task. For example, motor unit synchronization is increased during the performance of lengthening contractions (Semmler et al. 2002) and is greatest during attention-demanding contractions (Schmied et al. 2000), suggesting increased common neural input during these tasks. Several chronic adaptations in motor unit synchronization have also been observed, such as greater motor unit synchronization in strength-trained weightlifters and reduced synchronization in skill-trained musicians (Semmler and Nordstrom 1998; Fling et al. 2009). However, skill or strength training with isometric contractions do not seem to be important training strategies for modulating motor unit synchronization, as 4 wk of pinch-grip skill training (Griffin et al. 2006) did not alter motor unit synchronization when measured in a hand muscle. However, the increased motor unit synchronization observed after eccentric exercise in the present study suggests that lengthening contractions that induce muscle damage may be an important mediator of increased motor unit synchronization with exercise and training.

During voluntary contractions in fresh muscle, the common input responsible for motor unit synchronization is likely to occur through supraspinal sources, as motor unit synchronization is abolished after stroke (Datta et al. 1991; Farmer et al. 1993a) but is present in patients who have lost sensory feedback (Farmer et al. 1993b). However, when the muscle is damaged by lengthening contractions, there are likely to be mechanical and chemical changes in the muscle during the regeneration and recovery phase that influence both the spinal and cortical control of movement. Consequently, the increased motor unit synchronization could be due to physiological adjustments at the cortex or spinal cord that increase the number of common inputs that are shared by the motoneurons. Alternatively, the greater synchronization could be due to an increase in the amplitude of common excitatory or inhibitory postsynaptic potentials received by the motoneurons. It has previously been shown that common inhibitory inputs result in a broader central peak in the cross-correlation histogram (Turker and Powers 2001), which was observed in the present study 7 days after exercise (see Fig. 3), suggesting that this is a possible mechanism for the increased motor unit synchronization. Although it is not yet known how this might occur, it is conceivable that eccentric exercise may influence reciprocal or recurrent inhibition, given that there is an increase in antagonist muscle coactivation with eccentric muscle damage (Leger and Milner 2001; Semmler et al. 2007), and the pharmacological enhancement of recurrent inhibition results in increased motor unit synchronization (Mattei et al. 2003; Del Santo et al. 2006).

Motor unit Activity and the Repeated-Bout Effect

It is well known that a single bout of unaccustomed eccentric exercise provides protection against muscle damage from a repeated bout of the same exercise. For the elbow flexor muscles, this adaptation to a repeated bout of eccentric exercise has previously been shown to occur after as little as three days (Chen 2003; Chen and Nosaka 2006; Nosaka and Clarkson 1995) and can last for up to 4 wk or longer (Chen et al. 2009; Chen et al. 2007; Clarkson and Tremblay 1988; Ebbeling and Clarkson 1990; Newman et al. 1987). When we performed two sessions of the same eccentric exercise separated by 1 wk, the second session resulted in reduced signs of muscle damage, such as a faster recovery of muscle strength and reduced muscle soreness, despite the same amount of eccentric work performed in the two bouts. We also found reduced changes in neuromuscular function after the second exercise session, which included a smaller increase in force fluctuations during submaximal isometric contractions, and no change in antagonist muscle coactivation. Interestingly, we found a similar increase in biceps brachii EMG during the elbow-flexor contractions immediately after the two exercise bouts (Fig. 1), suggesting that an increased extensor torque through antagonist muscle coactivation is not responsible for this effect. Nonetheless, these data suggest that the eccentric exercise performed in the present study induced neuromuscular adaptations that resulted in reduced muscle damage with a subsequent session of the same exercise.

During the initial stages of muscle damage, we have previously shown that biceps brachii motor unit activity is increased 24 h after unaccustomed eccentric exercise, as demonstrated by increased motor unit synchronization (Dartnall et al. 2008) and a reduction in motor unit recruitment thresholds (Dartnall et al. 2009; see Fig. 4) that occurred 1 day after an initial eccentric exercise bout. If these changes in motor unit activity were associated with the magnitude of exercise-induced muscle damage, the expectation is that there would be smaller changes in motor unit activity after a second session of eccentric exercise. This hypothesis was supported by the recruitment threshold data. After the first exercise session, there was an ~40% reduction in mean motor unit recruitment threshold 24 h later (Dartnall et al. 2009; and see Fig. 4B), indicating that biceps brachii motor units were recruited earlier during the
isometric contraction after muscle damage. Despite a recovery 7 days after the first exercise session, motor unit recruitment thresholds did not change 24 h after the second exercise session, which is consistent with the finding of reduced muscle damage with repeated bouts of eccentric exercise. The only difference in muscle activation during the recruitment of motor units was a small reduction in biceps brachii EMG when assessed 24 h after Bout 2 compared with before Bout 1 (Table 2), which was also found after a single exercise session in our previous study (Dartnall et al. 2009). This effect could be due to increased inflammation and swelling 24 h after eccentric exercise (see Prosko and Allen 2005) producing a greater distance between the surface electrodes and the active motor units to reduce the action-potential amplitude. However, this possibility remains speculative as inflammation and swelling from muscle damage was not assessed in the present study.

In contrast to motor unit recruitment thresholds, motor unit synchronization was elevated immediately after the first bout of exercise (Dartnall et al. 2008) and remained elevated 7 days later (see Fig. 4A) despite the return to baseline levels of the indicators of muscle damage at that time (Table 1). This elevated synchrony 7 days after the first bout of eccentric exercise suggests a dissociation between damage and motor unit synchronization, as the muscle damage indicators had recovered at this time. When a second bout of eccentric exercise was performed, this resulted in reduced indicators of muscle damage and a decline in the strength of motor unit synchronization (24 h later) toward levels obtained before both exercise bouts. The different behavior of motor unit synchronization after Bout 2 of exercise compared with Bout 1 suggests that there is some feature of the damage or repair process that is different after each exercise bout. As the subjects performed the same exercise in each bout, the change in motor unit synchronization must not be some after-effect of the motor commands for the eccentric exercise task but presumably some consequence of the exercise (such as the extent of muscle damage) that differed between the two bouts. An alternative view is that the elevated motor unit synchronization 7 days after Bout 1 of exercise may have influenced the ability to detect an increase in motor unit synchronization after Bout 2. If, after the first bout of exercise, motor unit synchronization had recovered to levels observed before exercise, there would be an ~20% increase in synchrony 24 h after Bout 2, compared with an ~50% increase after Bout 1 (see Fig. 3). Under this scheme, the change in motor unit synchronization would be roughly proportional to the change in strength (Table 1) and force fluctuations (Fig. 1) observed after each bout. To more clearly address this issue, a longer delay between exercise bouts would be necessary to determine whether motor unit synchronization returns to levels observed before the first bout of exercise and to examine whether the change in motor unit synchronization is consistent with reduced muscle damage after repeated bouts of eccentric exercise.

On a theoretical level, the reduction in motor unit synchronization 24 h after the second exercise session (compared with preexercise levels) could occur through a decrease in the relative proportion of common inputs or an increase in the relative proportion of independent inputs to the motoneuron pool. Although the physiological mechanisms for this reduction in synchrony 24 h after Bout 2 are unknown, the narrower synchrony peak width 24 h after Bout 2 compared with 7 days after Bout 1 suggests the possibility of a decrease in common inhibitory inputs at this time (see Turker and Powers 2001). Alternatively, it has been shown that stretching of a muscle results in reduced spinal excitability and passive muscle tension (Guissard et al. 1988) that occurs through pre- and postsynaptic inhibitory mechanisms (Guissard et al. 2001). Assuming that the first bout of eccentric exercise results in changes in the mechanical properties of the muscle (see McHugh 2003), the lengthening contractions in the second bout may act as a stretching stimulus to reduce passive tension, resulting in a decrease in motoneuron excitability and a decrease in motor unit synchronization (via increased independent inhibitory inputs). Finally, with existing intramuscular recording techniques it is not presently possible to record from the same motor unit over repeated sessions. Therefore, the possibility remains that the reduction in motor unit synchronization observed 24 h after Bout 2 was influenced by a sampling bias by recording from a different population of motor units in each session. To account for this inherent limitation, we recorded from a large sample of motor units in each session that had a similar range of recruitment and derecruitment thresholds, suggesting that the population of motor units examined were equivalent in each session.

In conclusion, we performed intramuscular recordings of single motor units in the human biceps brachii muscle to examine the adjustments and adaptations in motor unit activity before and after repeated sessions of eccentric exercise. The main finding was an increase in motor unit synchronization 7 days after a single session of eccentric exercise. This represents the first evidence of changes in motor unit activation during the putative repair process following exercise-induced muscle damage. Furthermore, a second session of the same eccentric exercise resulted in reduced indicators of muscle damage and a decline in the strength of motor unit synchronization toward levels observed before exercise. The physiological mechanisms and functional significance of these findings remain to be determined.

ACKNOWLEDGMENTS
The authors thank John Cirillo and Nigel Rogasch for assistance with some of the experiments.

GRANTS
T. Dartnall was supported by a University of Adelaide Postgraduate Research Scholarship. This work was supported by the National Health and Medical Research Council of Australia, and equipment support was provided by the Faculty of Health Sciences at the University of Adelaide.

DISCLOSURES
No conflicts of interest, financial or otherwise, are declared by the authors.

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J Neurophysiol • VOL 105 • MARCH 2011 • www.jn.org


