Eccentric Muscle Damage Has Variable Effects on Motor Unit Recruitment Thresholds and Discharge Patterns in Elbow Flexor Muscles

Tamara J. Dartnall, Nigel C. Rogasch, Michael A. Nordstrom, and John G. Semmler

Discipline of Physiology and Research Centre for Human Movement Control, School of Molecular and Biomedical Science, The University of Adelaide, Adelaide, Australia

Submitted 2 December 2008; accepted in final form 4 May 2009

Eccentric muscle damage has variable effects on motor unit recruitment thresholds and discharge patterns in elbow flexor muscles. J Neurophysiol 102: 413–423, 2009. First published May 6, 2009; doi:10.1152/jn.91285.2008. The purpose of this study was to determine the effect of eccentric muscle damage on recruitment threshold force and repetitive discharge properties of low-threshold motor units. Ten subjects performed four tasks involving isometric contraction of elbow flexors while electromyographic (EMG) data were recorded from human biceps brachii and brachialis muscles. Tasks were 1) maximum voluntary contraction (MVC); 2) constant-force contraction at various submaximal targets; 3) motor unit recruitment threshold task; and 4) minimum motor unit discharge rate task. These tasks were performed on three separate days before, immediately after, and 24 h after eccentric exercise of elbow flexor muscles. MVC force declined (42%) immediately after exercise and remained depressed (29%) 24 h later, indicative of muscle damage. Mean motor unit recruitment threshold for biceps brachii was 8.4 ± 4.2% MVC, (n = 34) before eccentric exercise, and was reduced by 41% (5.0 ± 3.0% MVC, n = 34) immediately after and by 39% (5.2 ± 2.5% MVC, n = 34) 24 h after exercise. No significant changes in motor unit recruitment threshold were observed in the brachialis muscle. However, for the minimum tonic discharge rate task, motor units in both muscles discharged 11% faster (10.8 ± 2.0 vs. 9.7 ± 1.7 Hz) immediately after (n = 29) exercise compared with that before (n = 32). The minimum discharge rate variability was greater in brachialis muscle immediately after exercise (13.8 ± 5.1%) compared with that before (11.9 ± 3.1%) and 24 h after exercise (11.7 ± 2.4%). No significant changes in minimum discharge rate variability were observed in the biceps brachii motor units after exercise. These results indicate that muscle damage from eccentric exercise alters motor unit recruitment thresholds for ≥24 h, but the effect is not the same in the different elbow flexor muscles.

INTRODUCTION

The regulation of the mechanical output of a muscle is dependent on motor unit recruitment and rate coding. The contribution of these two mechanisms to force output varies depending not only on the function and the size of the muscle, but also on its degree of use or muscle fiber composition (Kukulka and Clamann 1981). The recruitment of motor units is based on the size principle (Henneman 1957), in which small motor neurons are recruited before larger motor neurons in a relatively fixed order. Although the size-based order of recruitment is quite robust, the threshold force of motor unit recruitment in humans can vary depending on the task performed. For example, motor unit recruitment can be altered when contractions are performed at different muscle lengths (Pasquet et al. 2005), during shortening and lengthening contractions (Pasquet et al. 2006; Tax et al. 1989), and at different contraction velocities (Desmedt and Godaux 1977). Along with the task-related adjustments, interventions such as fatigue (Carpentier et al. 2001) and immobilization (Duchateau and Hainaut 1990) act to compress the range of motor unit recruitment during isometric contractions. Furthermore, the rate at which motor units begin to discharge action potentials repetitively is an important determinant of contractile force, as any change in the minimum motor unit discharge rate is likely to influence the extent of summation of the involved motor units, which will have important consequences for steady force production. This is particularly important at low forces, because each newly recruited motor unit contributes relatively more to the net force compared with contractions at high forces (Fuglevand et al. 1993).

One form of exercise that occurs during normal everyday activities is eccentric exercise, which involves the active lengthening of muscle during tasks such as walking down stairs, running, and lowering of an object held in the hand. Unlike concentric or isometric exercise, eccentric exercise is known to produce significant muscle damage and delayed onset muscle soreness (DOMS) (for review see Proske and Morgan 2001). The effects of muscle damage result in a reduction in maximal force production, a rise in whole-muscle passive tension, and a shift in the length–tension relation for peak force generation to longer optimal muscle lengths (for review see Proske and Allen 2005). Eccentric exercise is also known to influence the performance of submaximal tasks, where there is a more than proportional increase in electromyographic (EMG) amplitude at low forces (Dundon et al. 2008; Semmler et al. 2007; Weerakkody et al. 2003). Several lines of evidence suggest that an increase in motor unit activity is responsible for the altered EMG–force relation after eccentric exercise. For example, we have recently shown that mean motor unit discharge rate and synchronization in the biceps brachii muscle are increased for ≥24 h after eccentric exercise (Dartnall et al. 2008). Furthermore, eccentric exercise is characterized by pronounced low-frequency fatigue (Jones et al. 1989; Newham et al. 1983), which is associated with increased EMG at low forces (Dundon et al. 2008) and accompanied by increased mean motor unit discharge rates (de Ruiter et al. 2005). These findings suggest that the physiological and mechanical events associated with muscle damage are likely to alter the activity of low-threshold motor units.

Address for reprint requests and other correspondence: J. G. Semmler, School of Molecular and Biomedical Science, The University of Adelaide, Adelaide, South Australia 5005, Australia (E-mail: john.semmler@adelaide.edu.au).
The purpose of this study was to compare changes in motor unit recruitment threshold and the minimum tonic discharge rate of motor units before, immediately after, and 24 h after eccentric exercise. Because single motor units are the smallest functional elements of muscle, the strategy used to activate motor units is critical for the accurate performance of fine motor tasks, particularly at low forces. Based on our previous observations on the EMG–force relation (Semmler et al. 2007), motor unit synchronization (Dartnall et al. 2008), and low-frequency fatigue (Dundon et al. 2008) after eccentric exercise, we expect to see reduced motor unit recruitment thresholds and increased minimum discharge rates during low-force contractions, which persist for ≥24 h after eccentric muscle damage. We have examined these features of motor unit activity in the biceps brachii and brachialis muscles, to determine whether the pattern of change in motor unit activation is similar for these two elbow flexor muscles that have different mechanical actions at the elbow joint. Long-lasting changes in motor unit activity, such as an increase in motor unit recruitment, may help to explain the adaptation that results in increased resistance to muscle damage following repeated bouts of eccentric exercise (for review see McHugh 2003).

**Methods**

Ten healthy subjects (six males, four females; age 23.7 ± 5 yr, mean ± SD) with no history of musculoskeletal pain or injury in the left arm or shoulder were recruited for this study. They had not participated in regular strength training for ≥6 mo. Prior to the beginning of the experiment written informed consent was obtained from all subjects. All experimental procedures conformed to the Declaration of Helsinki and were approved by the Human Research Ethics Committee at the University of Adelaide.

Subjects were seated in an experimental chair with the elbow joint at 90° flexion and the left forearm positioned vertically. The forearm was constrained in this position in a device designed to measure isometric elbow flexion force in the sagittal plane. Throughout the experiment two wide nylon straps were used to secure the forearm in a supinated position.

Surface EMG signals were recorded with bipolar electrodes (silver/silver chloride, 4-mm diameter) placed about 2 cm apart (center to center) midway between the biceps brachii muscle belly and tendon and over the medial head of the triceps brachii muscle. A grounding strap was positioned around the wrist to act as a reference. The surface EMG signals were amplified (×100–1,000; V75-04, Coulbourn Instruments, Whitehall, PA), band-pass filtered (13 Hz to 1 kHz), and recorded on digital tape.

To obtain bipolar recordings of the discharge of single motor units, two to three fine-wire intramuscular electrodes were inserted percutaneously 1–2 cm deep into the lateral head of the biceps brachii and brachialis muscle. The intramuscular fine-wire electrode was inserted into the brachialis muscle from the lateral aspect of the arm about 3 cm above its point of insertion with care taken to avoid the biceps brachii muscle. Each electrode consisted of three Formvar-insulated, stainless steel wires (50-μm diameter; California Fine Wire, Grover Beach, CA). The three wires were threaded through the lumen of a disposable 27-gauge hypodermic needle with a hook of about 2 mm at the recording end of the electrode. The electrode was inserted into the biceps brachii or brachialis muscle and the needle withdrawn, leaving the hooked wires within the belly of the muscle. Bipolar recordings were obtained from two of the wires, with the third wire providing an alternative bipolar configuration to sample from other motor units within the same muscle as necessary. Fine manual adjustment of the wires was made to optimize the detection of action potentials from a single motor unit. An amplitude window discriminator (V21-10, Coulbourn Instruments) was used to detect motor units on-line from one of the electrodes with the output pulse connected to a speaker to provide audio feedback of motor unit discharge during the contractions. Single motor unit recordings were amplified (×1,000; V75-02, Coulbourn Instruments), band-pass filtered (90 Hz to 10 kHz; V75-48, Coulbourn Instruments), displayed on an oscilloscope, and recorded on digital tape.

**Experimental procedures**

Each subject performed four tasks requiring isometric contraction of the elbow flexor muscles: 1) maximum voluntary contraction (MVC) to assess muscle strength; 2) a constant-force task to quantify EMG and force fluctuations at five submaximal force levels; 3) a motor unit recruitment threshold task to identify the lowest force at which a selected motor unit was recruited and derecruited; and 4) a discrete isometric force task to identify the minimum tonic discharge rate and variability of the active motor unit. Surface EMG from biceps and triceps brachii was recorded during all four tasks and single motor unit recordings were obtained in the motor unit tasks from biceps brachii and brachialis muscles. These tasks were performed on three separate days in the same subjects, which consisted of a baseline measure before exercise, a second session immediately after eccentric exercise (this took place ~1 wk after the first session), and a third session 24 h later. Each experimental session lasted 2–3 h. The effects of fatigue and muscle damage on motor unit activity were expected to be seen immediately after eccentric exercise. Several studies have shown that a 30% decline in maximum isometric strength following fatiguing concentric exercise recoveries within 2 h (Dundon et al. 2008; Walsh et al. 2004). Therefore the data obtained 24 h after exercise are likely to examine the effects of exercise-induced muscle damage without fatigue.

**MVC Force.** The MVC task consisted of a ramp increase in elbow flexion force from zero to maximum force over a 3-s period and then sustained for a further 3 s. Verbal timing of the task was provided by the experimenter and the subject was able to monitor the force displayed on an oscilloscope placed in front of them at eye level. The experimenters provided verbal encouragement during the sustained maximum contraction to facilitate maximum force production. The subject performed three MVCs with ≥1-min rest between contractions and the force was recorded on tape. This task was then repeated in the extension direction. The trial with the greatest force was considered the MVC force and used as the reference for the elbow flexor constant force task in each respective testing session.

**Constant-Force Task.** A force transducer (MLP-150, range 0–700 N; Transducer Techniques, Temecula, CA), located perpendicularly to the forearm at the level of the wrist, was used to detect forces exerted during the MVC and constant-force tasks. The output from the force transducer was displayed on an oscilloscope, where a horizontal line on the oscilloscope screen showed the required target for each contraction intensity. Isometric contractions were performed at target forces of 5, 10, 20, 35, and 50% MVC (%MVC refers to MVC recorded at the beginning of the respective testing session). A larger number of contractions biased toward low force levels were selected because it was expected that they would encompass the forces used during the motor unit task. For each target force subjects were instructed to exert a steady elbow flexion force for 10 s. The order of contractions was randomized and one trial was performed at each target force level. Erroneous force fluctuations due to concentration errors from the subject were scrutinized and if obvious errors occurred the trial was repeated at the appropriate force level. A total of four trials before, four trials immediately after, and three trials 24 h after eccentric exercise were repeated due to concentration errors.

**Recruitment Threshold Task.** The discharges of single motor units were recorded from the biceps brachii and brachialis muscles.
while subjects exerted a steady elbow flexion force detected by the more sensitive force transducer (MLP-25, range 0–112 N; Transducer Techniques). The subject was given audio feedback through the computer speakers of the discharge from the motor unit. A target line was provided on the oscilloscope screen, which represented the slope of the force increment (2% MVCs) that subjects were instructed to produce to recruit and sustain a motor unit discharge for 3 s. With the aid of the target line, subjects slowly increased the elbow flexion force until a motor unit began to discharge action potentials that were detected with the intramuscular electrode. When the motor unit was activated the force was then held constant for 3 s and then the elbow flexor force was lowered at the same rate as it was increased (Fig. 1). This was repeated three times for each identified motor unit with a period of 10-s rest between each trial. Recruitment threshold was determined from the mean force at which the motor unit first began to discharge action potentials over the three trials of increasing force (Barry et al. 2007). Derecruitment threshold was determined in the same manner, but was calculated as the force when the motor unit ceased to discharge action potentials with decreasing force.

**MINIMUM TONIC DISCHARGE RATE TASK.** Subjects slowly increased the elbow flexion force at the same rate as in the recruitment threshold task until the same motor unit was recruited. The force just above recruitment threshold was then held constant for about 10 s and then lowered 1% MVC and held constant for another 10 s (see Fig. 4). This was repeated until the motor unit ceased to discharge action potentials repetitively, defined as no action potentials discharging for ≥1 s. The minimum tonic discharge rate and variability were obtained from the lowest force trial in which the motor unit maintained tonic discharge for 10 s. The force used to activate the motor units at their lowest tonic discharge rate was noted and the strength of the contraction relative to maximum force in that session (%MVC) was determined. Subjects rested for several minutes at the end of each trial. After the rest period, a different motor unit was selected, either by recording from a new electrode or by moving the electrode ≥0.5 cm to obtain a completely different motor unit waveform.

**ECCENTRIC EXERCISE.** In the second of the three experimental sessions conducted with each subject, controlled eccentric exercise with the elbow flexor muscles of the left arm was used to induce a roughly 40% reduction of isometric MVC force immediately prior to the four isometric force tasks. A prolonged reduction in muscle strength is regarded as one of the most valid and reliable indicators of the extent of muscle damage in humans (Warren et al. 1999). We used a protocol that resulted in similar declines in muscle strength between subjects, as opposed to alternative protocols that use a similar number of contractions that can produce large differences in muscle strength deficits after eccentric exercise (Hubal et al. 2007). In addition, this eccentric exercise protocol has been shown to produce a shift in optimal muscle length, a reduction in relaxed elbow joint angle (increased passive tension), and DOMS for several days following the exercise (Prasartwuth et al. 2005, 2006), which is indicative of muscle damage.

The subject was seated at a bench that consisted of an adjustable-height seat and a padded support for the left (nondominant) upper arm that was positioned 45° from the torso. The forearm was held vertically as the subject rested her/his upper arm on the support. The experimenter placed a load in the subject’s hand and the subject lowered this weight from about 45° flexion to full extension (range of 135°) by eccentric contraction of the elbow flexor muscles. The load was set to about 40% of the subject’s isometric MVC at 90° flexion. Each eccentric contraction lasted 2 s and was followed by a 4-s rest period. During the rest period the experimenter removed the load and the subject returned her/his arm to 45° flexion. Eccentric contractions were performed in sets of ten in time with a metronome followed by a 20-s rest period. This procedure continued until there were visible signs of tremor during the eccentric contractions and verbal communication from subjects that they were having difficulty controlling the load. At this point, the subjects moved to the testing apparatus and brief MVCs were performed to monitor the reduction in elbow flexor force. The eccentric contractions continued until there was a reduction in isometric MVC force exceeding 40%. This required between 30 and 110 eccentric contractions in this group of subjects.

**Data analysis**

All signals recorded on tape were digitized (CED 1401, Cambridge Electronic Design [CED], Cambridge, UK) onto a hard drive of a computer. Signals were sampled at 200 Hz (force), 2,000 Hz (surface EMG), or 20 kHz (single motor unit recordings). Spike2 data analysis software (CED) and custom-written scripts were used to perform off-line analysis. For the MVC trial, the EMG was full-wave rectified and averaged for a 1-s epoch centered around the point at which the maximum force was achieved. A 10-s sample of force from the middle of each trial for the constant-force task was used to determine the mean and SD of force, from which the coefficient of variation (CV) of force was calculated ([SD/mean force] × 100). The EMG from the biceps and triceps brachii was rectified and averaged over a user-selectable window of 1 s corresponding to a stable portion of the EMG and force record. To facilitate comparisons between subjects and across days the EMG was normalized to the maximum EMG obtained during the MVC performed on that day. Triceps brachii EMG data were excluded from all recording sessions in two subjects due to poor electrode contact following the eccentric exercise protocol.

To discriminate single motor unit discharges a computerized spike-sorting algorithm (Spike2, version 5) was used. Waveform shape was used to identify action potentials belonging to a particular motor unit. For the recruitment threshold trials, the discharge rate at recruitment was calculated for the first ten action potentials, provided that the interspike interval (ISI) between the first and second discharges was <0.5 s. 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 minimum discharge rate trials, the ISIs of the identified motor unit over the full 10-s duration were examined to ensure discrimination accuracy. The motor unit waveforms were sorted using Spike2, with operator intervention on a spike-by-spike basis in some trials to ensure high discrimination accuracy. Custom-designed software written in Matlab (The MathWorks, Natick, MA) was used to determine the mean, SD, and CV of ISIs and the mean frequency of the discharge times (1,000/mean ISI). The average rectified EMG from biceps and triceps brachii was also quantified over the full 10-s duration of the minimum discharge rate task.

**Statistical analysis**

Elbow flexor force and biceps brachii EMG during maximum flexion contractions were analyzed with a one-way repeated-measures ANOVA to assess the effect of eccentric exercise, based on the different Exercise States (before, immediately after, and 24 h after eccentric exercise). To analyze the CV for force as well as the average rectified biceps and triceps brachii EMG for the constant-force tasks, a two-way repeated-measures ANOVA for Exercise State and Target Force (5, 10, 20, 35, 50% MVC) was used. For the recruitment threshold task, the dependent variable was motor unit Threshold (recruitment and derecruitment), motor unit discharge rate, and biceps and triceps brachii EMG at recruitment. For the minimum discharge rate task the dependent variables were motor unit discharge rate (calculated from ISIs), motor unit discharge rate variability (calculated as CV of ISI), force at minimum discharge rate, and biceps and triceps brachii EMG. For these dependent variables in each motor unit task, significant differences were assessed using a two-way ANOVA for Exercise State (before, immediately after, and 24 h after) and Muscle (biceps brachii and brachialis). Degrees of freedom for the main independent variable and residual are reported, along with the
corresponding F value. Significant effects in the ANOVA were analyzed further with Fisher’s LSD (Protected Least Significant Difference) post hoc test, which performed all possible comparisons based on the type of factor examined. Statistical significance was set at $P < 0.05$ for all comparisons and all values for the text and figures are reported as means ± SD.

**RESULTS**

**Maximal voluntary contraction**

Elbow flexor MVC force was significantly reduced immediately after the repeated eccentric contractions in all subjects and had only partially recovered 24 h later (Table 1). This required an average of 78 ± 23 eccentric contractions with a mean exercise load of 10.5 ± 3.8 kg. No significant change was observed in biceps brachii EMG during the elbow flexor MVCs when measured before, immediately after, and 24 h after the exercise (Table 1; $P = 0.14$).

**Submaximal EMG activity after eccentric exercise**

To confirm the findings of previous studies, we measured biceps and triceps brachii EMG at a range of submaximal target forces before, immediately after, and 24 h after eccentric exercise. Across all force targets, biceps brachii EMG increased immediately after eccentric exercise and remained significantly elevated 24 h after, compared with that before eccentric exercise [Exercise State effect: $F_{(2,90)} = 71.2$, $P < 0.001$; Table 1]. The increase in biceps brachii EMG after exercise was greatest at low force levels compared with that before exercise [Exercise State × Target Force interaction: $F_{(8,90)} = 5.7$, $P < 0.001$]. The increase in biceps brachii EMG measured immediately after eccentric exercise was threefold greater at 10% target force, 2.4-fold greater at 20% target force, 2.6-fold greater at 35% target force, and 1.7-fold greater at the 50% target force compared with the EMG at the same target force before eccentric exercise (all $P$ values <0.01). Biceps brachii EMG was also significantly larger immediately after eccentric exercise compared with that at 24 h after eccentric exercise at the 35% ($P < 0.01$) and 50% ($P < 0.001$) target forces and significantly larger 24 h after eccentric exercise compared with that before eccentric exercise at the 35% ($P < 0.001$) and 50% ($P < 0.01$) target forces. For triceps brachii EMG during submaximal elbow flexor contractions, a significant effect between recording sessions [Exercise State effect: $F_{(2,70)} = 13.5$, $P < 0.001$] and subsequent post hoc analysis showed that triceps brachii EMG was about twofold larger immediately after ($P = 0.001$) compared with that before exercise. The triceps brachii EMG 24 h after exercise was not significantly different from that before exercise ($P = 0.05$).

**Submaximal force fluctuations after eccentric exercise**

Force fluctuations were quantified as the CV of force at each of the submaximal target forces. For all target forces combined, post hoc analysis indicated that the CV of force was significantly greater immediately after eccentric exercise compared with that before ($P < 0.001$) and 24 h after ($P = 0.001$) eccentric exercise [Exercise State effect: $F_{(2,90)} = 32.0$, $P < 0.001$; Table 1]. The CV of force was also significantly greater 24 h after compared with that before the exercise ($P = 0.004$). There was no significant difference in CV of force for each target force during the constant force contraction [Exercise State × Target Force interaction: $F_{(8,90)} = 1.1$, $P = 0.37$].

**Recruitment threshold task**

Figure 1 shows motor unit recordings obtained from the biceps brachii muscle during the recruitment threshold task before and immediately after eccentric exercise in one subject. In this subject, 40 eccentric contractions with a 6.7-kg load induced a 40% reduction in MVC force that recovered to a 35% reduction in MVC 24 h after eccentric exercise. For the two biceps brachii motor units in this figure, recruitment threshold for motor unit 1 was 7.7% MVC and its derecruitment threshold was 5.3% MVC before eccentric exercise. For motor unit 2 studied immediately after eccentric exercise, recruitment threshold was 2.6% MVC and derecruitment threshold was 1.1% MVC. For all biceps brachii motor units obtained from this subject, recruitment threshold decreased from an average of 8.0 ± 4.9% MVC ($n = 8$ motor units) before eccentric exercise to 4.4 ± 2.1% MVC ($n = 7$ motor units) immediately after. Mean motor unit derecruitment threshold decreased from 5.3 ± 4.2% MVC ($n = 8$ motor units) before the exercise to 3.1 ± 1.9% MVC ($n = 7$ motor units) immediately after.

For the motor unit recruitment task, a total of 197 motor units were obtained from ten subjects in all recording sessions, which consisted of 102 motor units from the biceps brachii and 95 from brachialis. A similar number of motor units were recorded from both muscles in each subject in each of the three recording sessions (~7 in each session).

For the population of motor units sampled, the motor unit recruitment threshold for the biceps brachii muscle ranged from 1 to 17% MVC before, 0–13% MVC immediately after, and 2–10% MVC 24 h after eccentric exercise. The range of motor unit recruitment thresholds for the brachialis muscle was

<table>
<thead>
<tr>
<th>Table 1. MVC force and EMG obtained before, immediately after, and 24 h after eccentric exercise.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before</td>
</tr>
<tr>
<td>Elbow flexor MVC force, N</td>
</tr>
<tr>
<td>Biceps MVC EMG, mV</td>
</tr>
<tr>
<td>Submax biceps EMG, % max</td>
</tr>
<tr>
<td>Submax triceps EMG, % max</td>
</tr>
<tr>
<td>Force fluctuations, % CV</td>
</tr>
</tbody>
</table>

Values are means ± SD. MVC, maximum voluntary contraction; EMG, electromyography; CV, coefficient of variation. *$P < 0.05$ compared with before eccentric exercise; #$P < 0.05$ compared with immediately after eccentric exercise. Table includes only main effects of exercise condition (pooled across all force levels) for submax biceps and triceps EMG and force fluctuations.
0–20% MVC before eccentric exercise, 0–16% MVC immediately after eccentric exercise, and 0–9% MVC 24 h after eccentric exercise. The mean and distribution of these motor unit populations in the biceps brachii and brachialis muscles are shown in Fig. 2. For all time points, the relative force at which motor units were recruited was different between muscles \(F_{(1,191)} = 18.7, P < 0.001\); Fig. 2], with the mean recruitment threshold significantly higher for the biceps brachii (6.2 ± 3.6% MVC) compared with that for the brachialis muscle (3.9 ± 4.1% MVC). For biceps brachii, post hoc analysis indicated that the mean motor unit recruitment threshold (normalized to MVC at each time point) was 40% lower immediately after eccentric exercise (5.0 ± 3.0% MVC, 7.1 ± 4.7 N) compared with that before eccentric exercise (8.4 ± 4.2% MVC, 20.9 ± 12.3 N) \(F_{(2,99)} = 11.5, P < 0.001\); Fig. 2A]. The mean motor unit recruitment threshold for biceps brachii was still significantly reduced by 39% 24 h after eccentric exercise (5.2 ± 2.5% MVC, 9.4 ± 5.4 N) compared with that before eccentric exercise \(F_{(2,92)} = 1.2, P = 0.29\); Fig. 2B and D]. The variability (SD) of motor unit recruitment threshold in the three trials was similar in each recording session \(F_{(2,191)} = 0.4, P = 0.65\). However, the SD of motor unit recruitment thresholds was lower in biceps brachii compared with that in biceps brachii \(F_{(1,191)} = 35.1, P < 0.001\]. For all recording sessions, the SDs of motor unit recruitment thresholds were 1.5% MVC in biceps brachii and 0.3% MVC in brachialis.

For the first ten action potentials at recruitment, we found no difference in mean discharge rate \(F_{(1,191)} = 0.5, P = 0.5\) and variability \(F_{(1,191)} = 2.1, P = 0.15\) between the two muscles. However, motor units in both muscles displayed increased mean discharge rates at recruitment after eccentric exercise (12.8 ± 3.5 Hz) compared with that before (10.9 ± 2.3 Hz, \(P < 0.001\]) and 24 h after (11.1 ± 2.5 Hz, \(P = 0.002\]) eccentric exercise \(F_{(2,191)} = 7.8, P < 0.001\]. Mean motor unit discharge rate variability at recruitment was not different after exercise in the two muscles \(F_{(2,191)} = 1.0, P = 0.4\).

The biceps brachii EMG at recruitment was increased after exercise \(F_{(2,116)} = 19.4, P < 0.001\], but this was not influenced by the muscle from which the motor unit was being recorded \(F_{(1,116)} = 0.05, P = 0.9\]. When recording motor units in both muscles, there was a significant increase in biceps brachii EMG immediately after exercise (11.2 ± 8.0% max) compared with that before (6.0 ± 4.7% max, \(P < 0.001\]) and 24 h after (3.6 ± 2.0% max, \(P < 0.001\)). Similarly, triceps brachii EMG at recruitment was increased after exercise \(F_{(2,116)} = 20.0, P < 0.001\], but this effect was not the same when recording motor units from the different muscles \(F_{(2,116)} = 3.6, P = 0.03\]. When recording motor units from biceps brachii (Fig. 2E), triceps brachii EMG at recruitment was significantly greater immediately after (\(P < 0.001\]) and 24 h after (\(P = 0.04\]) compared with that before exercise. For brachialis motor units (Fig. 2F), triceps brachii EMG was significantly greater immediately after exercise compared with that before \(P < 0.001\] and 24 h after exercise \(P < 0.001\]. Furthermore, the magnitude of triceps brachii EMG was twofold greater immediately after exercise when recording brachialis motor units compared with biceps brachii motor units \(P < 0.001\).

On average, the derecruitment thresholds were about 2% MVC lower than recruitment thresholds and the pattern of change in derecruitment thresholds with exercise were similar to those for the recruitment thresholds. There was no significant difference in motor unit derecruitment threshold between muscles \(F_{(1,191)} = 0.009, P = 0.93\]; Fig. 2B]. However, derecruitment threshold was influenced by exercise for all motor units combined \(F_{(2,191)} = 6.4, P < 0.01\]. Post hoc analysis indicated that the mean motor unit derecruitment threshold for biceps brachii and brachialis muscles was 38% lower (2.9 ± 2.7% MVC) immediately after eccentric exercise compared with that before (4.7 ± 4.8% MVC, \(P = 0.004\]) eccentric exercise. The mean motor unit derecruitment threshold for all motor units was still signifi-
Significantly reduced by 42% (2.7 ± 2.3% MVC) 24 h after eccentric exercise compared with that before exercise (P = 0.002).

Minimum discharge rate task

Figure 3 shows recordings obtained before and immediately after eccentric exercise from the brachialis muscle during the motor unit minimum discharge rate task (same subject as in Fig. 1, but different motor units). The last three phases of the task are shown where the target force was reduced by 1% MVC on each occasion. The three tasks shown correspond to 1) 1% MVC above minimum discharge rate (1% MVC above MDR); 2) minimum discharge rate (MDR); and 3) 1% MVC below minimum discharge rate (1% MVC below MDR). For the motor unit studied before eccentric exercise, the MDR was 9.7 Hz with a CV of 9.0% recorded at an elbow flexor force of 7.5% MVC (Fig. 3A). For the motor unit studied after eccentric exercise, the MDR was 10.2 Hz with a CV of 9.4% (Fig. 3B) at an elbow flexor force of 3.4% MVC.

For the minimum discharge rate task, a total of 184 motor units were obtained from ten subjects across all recording sessions, with 63 motor units obtained before, 61 motor units immediately after, and 60 motor units obtained 24 h after eccentric exercise. A similar number of motor units (~6) were recorded in each subject in each of the three recording sessions. For all motor units, minimum discharge rate [Exercise State effect: F(2,178) = 6.2, P = 0.003] and minimum discharge rate variability [Exercise State effect: F(2,178) = 7.1, P = 0.001] were both influenced by exercise (Fig. 4). For both muscles combined, post hoc analysis indicated that the mean minimum discharge rate and variability were 11–12% higher immediately after (10.8 ± 2.0 Hz, 12.9 ± 3.2%) compared with those before eccentric exercise (9.7 ± 1.7 Hz, 11.5 ± 2.7%; both P < 0.01). Both the minimum discharge rate (9.8 ± 2.0 Hz, P = 0.9) and variability (11.1 ± 2.1%, P = 0.4) returned to baseline levels 24 h later. However, minimum discharge rate variability was not the same for the two muscles.
50% lower immediately after eccentric exercise. Records are from different motor units recorded from brachialis muscle before (A) and immediately after eccentric exercise (B) while discharging at MDR (middle) and 1% MVC above (left) and 1% MVC below MDR (right). Records include instantaneous discharge rate for the motor unit (top), original recording of the same motor unit for that time point with superimposed motor unit action potential waveforms (middle), and elbow flexor muscle force (bottom). Dashed horizontal line represents zero force. MDR was slightly higher for the motor unit recorded after eccentric exercise, and the elbow flexor force was substantially lower.

DISCUSSION

Single motor unit recordings were obtained in the present study to examine the behavior of human motor neurons after eccentric exercise. Records are from different motor units recorded from brachialis muscle before (A) and immediately after eccentric exercise (B) while discharging at MDR (middle) and 1% MVC above (left) and 1% MVC below MDR (right). Records include instantaneous discharge rate for the motor unit (top), original recording of the same motor unit for that time point with superimposed motor unit action potential waveforms (middle), and elbow flexor muscle force (bottom). Dashed horizontal line represents zero force. MDR was slightly higher for the motor unit recorded after eccentric exercise, and the elbow flexor force was substantially lower.

Throughout the minimum discharge rate task we quantified the magnitude of biceps and triceps brachii EMG during the biceps and brachialis motor units trials (Fig. 4). When recording motor units in both muscles, there was a significant increase in biceps brachii EMG immediately after exercise (11.1 ± 8.7% max) compared with that before (5.7 ± 5.0% max, P < 0.001) and 24 h after (3.6 ± 2.5% max, P < 0.001) exercise [Exercise State effect: F(2,118) = 17.3, P < 0.001]. There was no difference in biceps brachii EMG during the minimum discharge rate trials when recording biceps or brachialis motor units [Muscle effect: F(1,118) = 1.2, P = 0.2]. Similarly, triceps brachii EMG was greater immediately after exercise (5.6 ± 3.8% max) compared with that before (3.6 ± 2.0% max, P < 0.001) and 24 h after (4.0 ± 2.1% max, P = 0.008) exercise [Exercise State effect: F(2,118) = 8.7, P < 0.001]. However, triceps brachii EMG varied depending on the muscle from which the motor unit was being recorded [Muscle effect: F(5,118) = 8.1, P = 0.005]. When recording from brachialis motor units (Fig. 4F), triceps brachii EMG was about twofold larger immediately after eccentric exercise compared with that before (P < 0.001) and 24 h after (P < 0.001) exercise. No difference in triceps brachii EMG was observed between the three recording sessions when minimum discharge properties were recorded from the biceps brachii muscle [Exercise State effect: F(2,66) = 0.9, P = 0.4; Fig. 4E].
muscles, and an increase in discharge rate variability in the brachialis muscle immediately after eccentric exercise. Furthermore, motor unit recruitment threshold remained depressed 24 h after exercise, suggesting that long-lasting features of muscle damage must be responsible for this effect. We therefore provide the first evidence of altered motor unit recruitment thresholds for 24 h after eccentric exercise.

Muscle damage was induced in the present study by repetitive lengthening contractions of the elbow flexor muscles to achieve a similar (~40% MVC) reduction in strength in all individuals. In agreement with previous studies (Semmler et al. 2007; Weerakkody et al. 2003), this procedure resulted in increased biceps brachii EMG during submaximal isometric contractions (Table 1). Previous studies have shown that the increased EMG is largest 2 h after eccentric exercise, but is not evident after a similar period of concentric exercise (Dundon et al. 2008; Semmler et al. 2007), suggesting that metabolic fatigue alone does not contribute to this effect. At least three factors are thought to contribute to an increase in EMG after eccentric exercise. First, we have recently shown that motor unit synchronization is increased after eccentric muscle damage (Dartnall et al. 2008), which can produce an increase in EMG through summation of the correlated action potentials at the surface of the muscle (Yao et al. 2000; Zhou and Rymer 2004). This summation effect could be exacerbated by the slowing of muscle fiber conduction velocity that occurs with muscle fatigue (Bigland-Ritchie et al. 1981). Second, eccentric exercise is accompanied by low-frequency fatigue, which is a disproportionate loss of force at low compared with high frequencies of activation (Edwards et al. 1977). The increased low-frequency fatigue after eccentric exercise has been associated with increased biceps brachii EMG at low forces (Dundon et al. 2008), suggesting that increased motor unit activity is necessary to compensate for the force loss. Third, an increase in antagonist muscle coactivation would result in additional elbow flexor EMG to maintain the required elbow flexor force. Several previous studies have shown increased antagonist muscle coactivation after eccentric exercise (Dundon et al. 2008;
Leger and Milner 2001; Turner et al. 2008), although the magnitude of the absolute change in triceps EMG (~5% of max EMG) does not fully account for the greater (10–20% max EMG) increase in elbow flexor EMG after muscle damage (Semmler et al. 2007).

Along with the biceps brachii, the increased EMG after eccentric exercise is also observed in other major elbow flexor muscles. Under conditions similar to those of the current study, we have recently shown increased EMG during constant-force isometric contractions in biceps brachii, brachialis, and brachioradialis muscles (Semmler et al. 2007), which all contribute to flexion torque at the elbow joint. This finding suggests that the increased biceps brachii EMG observed here is unlikely to be due to a reduction in activity of other elbow flexor muscles after muscle damage. For the contraction levels during the motor unit recordings in the present study (0–20% MVC), the greatest change in EMG after eccentric exercise is observed in the biceps brachii muscle (three- to fourfold greater), whereas the change in EMG at higher forces (35–50% MVC) is greatest in the brachialis muscle (twofold greater; Semmler et al. 2007). Furthermore, based on measures of physiological cross-sectional area and moment arms, the biceps brachii and brachialis muscles make the largest contribution to flexion torque at the elbow joint (An et al. 1981) and are thus likely to experience the most damage following eccentric exercise. For these reasons, we have obtained a large sample of motor units from these two major elbow flexor muscles in each subject to examine any change in motor unit activity before and after eccentric muscle damage.

**Reduced motor unit recruitment threshold**

Motor unit recruitment threshold, which represents the force at which the motor unit discharges its first action potential, can vary depending on the speed and type of muscle contraction. For example, motor unit recruitment threshold is progressively decreased with an increase in the rate of force development (Desmedt and Godaux 1977) and is lower during dynamic contractions compared with that during isometric contractions (Pasquet et al. 2006; Tax et al. 1989). To account for these differences, we have quantified motor unit recruitment threshold in the biceps brachii and brachialis muscles during isometric contractions using a controlled ramp increase in force at equivalent speeds in each recording session. We have also normalized the recruitment threshold force to the maximum force obtained in each session, so that any change in recruitment threshold cannot be attributed to the change in strength of the muscle. We found that there was a roughly 40% reduction in the relative recruitment threshold in the biceps brachii muscle after eccentric exercise that remained depressed 24 h later. The decrease in relative recruitment threshold of motor units in biceps brachii after eccentric exercise suggests that there is likely to be increased recruitment of low-threshold motor units when the muscle is damaged, contributing to the observed increase in biceps brachii EMG for low force contractions.

The factors that determine the recruitment threshold of a motor unit include the size of the motor neuron and the distribution of synaptic inputs to the motor neuron pool. From a large sample of motor units in the present study, the motor unit recruitment thresholds in the biceps brachii and brachialis muscles were all <20% MVC, suggesting that a similar population of motor units (and therefore motor neurons) were examined in the three recording sessions. Assuming no change in recruitment order after eccentric muscle damage, the reduction in relative motor unit recruitment threshold observed here suggests that low-threshold motor units in biceps brachii are contributing relatively less to the total force for ≥24 h after eccentric muscle damage. Although it is known that muscle fatigue could contribute to this reduction in motor unit recruitment threshold (Carpentier et al. 2001; Enoka et al. 1989), the rapid recovery of strength within 2 h (Dundon et al. 2008; Walsh et al. 2004) suggests that metabolic fatigue cannot be responsible for this effect, particularly 24 h after the exercise. Furthermore, experimentally induced muscle pain does not alter recruitment of low-threshold motor units (Sohn et al. 2000), so delayed onset muscle soreness is unlikely to contribute to the reduction in recruitment thresholds at any time point after exercise.

There remain at least two plausible explanations for the reduction in relative recruitment threshold of biceps brachii motor units after eccentric muscle damage. First, the reduction in recruitment threshold observed in the present study could be due to increased antagonist muscle activity, which would reduce the net flexion torque at the elbow joint given an equivalent level of elbow flexor muscle activity before and after exercise. In support of this, we found a twofold increase in triceps brachii EMG during the recruitment threshold task for biceps brachii motor units after exercise. Furthermore, the antagonist EMG remained elevated 24 h later, which could partially explain the lack of recovery in motor unit recruitment threshold at this time point. Second, there is substantial (≥50%) and long-lasting (hours to days) loss of force due to low-frequency fatigue after eccentric exercise (Dundon et al. 2008; Jones et al. 1989; Newham et al. 1983). We have recently shown that low-frequency fatigue after eccentric exercise is associated with an increase in EMG at low but not at high forces (Dundon et al. 2008), suggesting that this is a likely mechanism responsible for the increased motor unit recruitment in eccentrically damaged muscles.

In contrast to the biceps brachii muscle, a reduction in motor unit recruitment threshold after eccentric exercise was not observed in the brachialis muscle. Despite a small (25%) reduction in brachialis motor unit recruitment threshold after eccentric exercise, this decline did not reach statistical significance. The lack of change in motor unit recruitment threshold for brachialis motor units cannot be attributed to changes in coactivation because a greater triceps brachii EMG was observed during the recruitment threshold task for brachialis (compared with biceps brachii) motor units after exercise, which should reduce elbow flexor torque and facilitate the reduction in recruitment threshold for brachialis motor units after exercise. We can identify several reasons why recruitment threshold may be altered in biceps brachii but not brachialis motor units after eccentric exercise. First, we know from previous work that there is a greater change in biceps brachii EMG compared with that in brachialis EMG at low forces after eccentric exercise (Semmler et al. 2007), which may suggest that this intervention results in greater damage to low-threshold motor units in the biceps brachii muscle. Second, eccentric muscle damage could produce a shift in activity among elbow flexor muscles during the brachialis recruitment threshold task,
with an increased reliance on biceps brachii and brachioradialis muscles to the elbow flexor force. In the present study, there was no difference in biceps brachii EMG during the recruitment threshold task for biceps brachii and brachialis motor units, suggesting that task-related changes in biceps brachii EMG do not contribute to this effect. Although we did not measure EMG activity from the brachioradialis muscle, our previous study showed that brachioradialis EMG displays the smallest facilitation of all elbow flexor muscles during constant-force isometric contractions after eccentric exercise. However, it is not clear whether this also holds true for the ramp isometric contractions during the recruitment threshold task of the present study.

Altered motor unit discharge rate and variability

The force produced by a muscle is influenced by motor unit mean discharge rates and variability. These features of motor unit activity are particularly important at low forces because each motor unit contributes relatively more to the net force and this effect declines with increasing contraction intensity (Fuglevand et al. 1993). The minimum rate at which most motor neurons discharge action potentials repetitively during voluntary contractions is 6–10 Hz (Barry et al. 2007; Sogaard et al. 1996; Van Cutsem et al. 1997), with the highest values for more proximal muscles such as biceps brachii (Denier van der Gon et al. 1985). However, minimum discharge rates seem to be relatively fixed within a single muscle because there is no change with advancing age (Barry et al. 2007) or after a period of immobilization (Duchateau and Hainaut 1990), even though there may be alterations in the contractile properties of the involved motor units. Using a carefully controlled assessment, we provide evidence of a change in minimum motor unit discharge rates after eccentric muscle damage. For motor units in the biceps brachii and brachialis muscles, we found a 1-Hz increase in minimum discharge rate immediately after eccentric exercise that returned to baseline levels 24 h later. In support of these findings, we also found an increase in initial motor unit discharge rate during the recruitment threshold task, suggesting that the neural drive necessary to maintain tonic motor unit discharge in biceps brachii and brachialis muscles has increased after eccentric muscle damage.

The coefficient of variation (CV) of discharge rate is a measure of the relative motor unit discharge rate variability and has a significant effect on the force fluctuations during steady contractions (Enoka et al. 2003). The CV for discharge rate is typically reported at a value of about 10–20% (Nordstrom et al. 1992; Semmler and Nordstrom 1998) and declines with an increase in mean discharge rate (Person and Kudina 1972). Strength training and practice can reduce discharge rate variability, which is accompanied by improvements in steady motor performance (Kornatz et al. 2005). At the minimum discharge rate, we found that motor units in the biceps brachii and brachialis muscles discharged action potentials regularly before exercise (CV = 11–12%), but this behavior was altered after eccentric muscle damage, particularly in the brachialis muscle. We found that motor units in the brachialis muscle discharged less regularly immediately after muscle damage and this was accompanied by increased elbow flexor force fluctuations during low force isometric contractions after eccentric exercise.

The variability in motor unit discharge rate has been attributed to synaptic noise and its interaction with the trajectory of the motor neuron afterhyperpolarization (Calvin and Stevens 1968; Matthews 1996). Similarly, the minimum discharge rate is also likely to be influenced by random voltage fluctuations in the membrane potential resulting from synaptic noise (MacDonell et al. 2008), in that greater membrane potential variability would require increased descending drive to maintain repetitive motor neuron discharge. We found a similar pattern of change in motor unit discharge rate and variability immediately after exercise, suggesting that changes in synaptic noise to elbow flexor motor neurons may contribute to both of these changes in motor unit activity. Although several mechanisms are possible, we expect that the increase in synaptic noise is a consequence of greater excitatory and inhibitory inputs to the motor neuron pool after exercise. For example, it is known that fatigue is accompanied by increased afferent feedback from group III–IV afferents that are sensitive to changes in the mechanical state and the metabolic environment of the muscle (for review see Gandevia 2001). Furthermore, the mechanical disruption of the muscle fibers following eccentric exercise results in the increased release of inflammatory mediators that are detected by group IV afferents (Marqueste et al. 2004). Both of these factors could contribute to increased motor unit discharge rate variability immediately after exercise. Furthermore, we found that the change in minimum discharge rate and variability after exercise was more pronounced in the brachialis muscle during the minimum discharge rate task. Despite similar levels of biceps brachii EMG, there was a substantial increase in antagonist EMG during the brachialis muscle motor unit trials, suggesting that an increase in antagonist coactivation could have contributed to the increased discharge rate and variability in the brachialis muscle.

In summary, we have recorded single motor unit activity from biceps brachii and brachialis muscles to examine changes in low-threshold motor unit activity after eccentric muscle damage. We found a decrease in motor unit recruitment threshold in the biceps brachii muscle, increased minimum discharge rates in both muscles, and an increase in minimum discharge rate variability in the brachialis muscle immediately after eccentric exercise. The decline in motor unit recruitment threshold for the biceps brachii muscle persisted for ≥24 h after exercise. Because the activation of motor units provides the final output pathway of the motor system, these changes are likely to have significant implications for the performance of submaximal contractions in damaged muscles. Furthermore, we have previously reported an increase in motor unit synchronization in biceps brachii for ≥24 h after eccentric exercise (Dartnall et al. 2008). This finding—combined with the decreased motor unit recruitment threshold observed in the present study—may act to distribute the load over a greater number of motor units (and therefore muscle fibers) during voluntary contractions in damaged muscles and help to increase the resistance to muscle damage that occurs with a subsequent bout of eccentric exercise (for a review see McHugh 2003). Whether this altered motor unit activity plays a role in the reduced muscle damage from repeated bouts of eccentric exercise remains to be determined.
GRANTS
This research, supported by the National Health and Medical Research Council of Australia, forms part of the PhD studies of T. J. Dartnall who was supported by a University of Adelaide Postgraduate Research Scholarship.

REFERENCES
