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Muscle Injury after Repeated Bouts of Voluntary and Electrically Stimulated Exercise

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Abstract

Repeated bouts of eccentric exercise reduce the amount of exercise-induced muscle injury.

Purpose—This study sought to evaluate the importance of neural adaptations by comparing the repeated bout effect on muscle injury caused by voluntary and electrically stimulated eccentric exercise.

Methods—Sixteen subjects (nine men, seven women) were assigned into two groups; electrical stimulation (STIM) and voluntary (VOL). Each group performed 2 identical bouts of 80 eccentric contractions of the quadriceps femoris (QF) through a 90° arc at $\approx 45^\circ \cdot s^{-1}$, separated by 7 wk. T2-weighted magnetic resonance images of the QF were obtained before and 3 d after each exercise bout. Injury was assessed by determining changes in T2 relaxation time and muscle volume 3 d after exercise, and changes in isometric force and ratings of soreness for 28 d after exercise.

Results—The initial bout of exercise caused significant changes in T2 relaxation time, isometric force, and ratings of soreness in both STIM and VOL groups ($P < 0.05$). After the repeated bout, significantly smaller changes were noted in soreness ratings ($P < 0.05$), mean change in T2 ($P < 0.05$), and percentage of the QF demonstrating an increase in T2 ($P < 0.05$) compared with the initial bout in both exercise groups.

Conclusions—A repeated-bout effect was observed after electrically stimulated exercise, and the magnitude of the effect was similar to that observed with voluntary exercise. This suggests that the primary mechanism for the reduction in muscle injury after repeated exercise bouts is not related to changes in muscle recruitment and is potentially related to structural changes within the muscles.

Keywords

REPEATED-BOUNT EFFECT; T2 MRI; SORENESS; ECCENTRIC EXERCISE

Unaccustomed eccentric exercise often results in damage to the exercised muscle. This injury is accompanied by a loss in strength (8,29), a delayed onset muscle soreness (7,28,29), and an increase in serum levels of muscle-specific proteins such as creatine kinase (7,27,30,32). Exposure to a single bout of eccentric exercise has been shown to result in an adaptation that reduces muscle injury during subsequent bouts of similar eccentric exercise (7,11,26,31,34). This adaptation has been termed the “repeated-bout” or “protective” effect. The mechanism underlying this adaptation remains unclear, but McHugh et al. (24) suggest the adaptations are either neural (alterations in recruitment) or peripheral (changes within the muscle) in origin. Neural adaptations could limit injury through more efficient recruitment of motor units (31), increased synchrony of motor unit firing (14), and/or an increase in recruitment of slow-twitch muscle fibers (44). Supporting this idea, altered EMG activity has been observed during

repeated eccentric exercise bouts (6,44). Peripheral adaptations could limit injury through the removal of susceptible muscle fibers (2), the longitudinal addition of sarcomeres (20), and a stabilization of sarcomeres via up-regulation of cytoskeletal proteins (17).

Magnetic resonance imaging (MRI) has recently been used as a noninvasive method to identify exercise-induced muscle damage *in vivo* (3,11,38,40). After eccentric exercise, a delayed increase in the signal intensity of T2 (transverse) relaxation of skeletal muscle water, which peaks 2–6 d after exercise, has been observed (11). This delayed increase correlates with the changes in skeletal muscle ultrastructure in both humans (35) and animals (22) and follows a similar time course to markers of injury such as soreness, serum creatine kinase levels, swelling, and force loss (3,11,40). Because of its high spatial localization, MRI is thought to provide a quantitative index of the extent and pattern of muscle injury (37).

Eccentric exercise performed using electrical muscle stimulation (EMS) could provide insight into the role of neural adaptations in the repeated-bout effect because it evokes contractions while by-passing the central nervous system. Several animal studies have found reduced injury after repeated bouts of stimulated exercise (17,18,42,44) indicating that in animals, neural changes may play little role in reducing injury. Human data are lacking, with one study demonstrating a protective effect with EMS-evoked eccentric exercise (33). However, it is unclear as to whether subjects were fully recovered from the initial bout and whether a similar excitatory stimulus (current) was given in each bout. No previous study has compared the magnitude of the reduction in injury between electrically stimulated and voluntary contractions. Given that there appears to be evidence supporting both neural and peripheral adaptations comparing the size of the effect after EMS and voluntary exercise could provide insight into not only the type of adaptation that occurs but also whether neural and peripheral adaptations act in concert.

The purpose of this study is twofold: 1) determine whether muscle injury (assessed via MRI, strength, and soreness) was reduced after repeated bouts of electrically stimulated exercise and 2) compare the relative size of the repeated-bout effect, if it occurred, between electrically stimulated and voluntary exercise. The specific hypotheses tested were that 1) a similar amount of injury would occur after 2 bouts of EMS-evoked eccentric exercise, and 2) the magnitude of the repeated-bout effect would be less with EMS-evoked eccentric exercise compared with voluntary eccentric exercise. The rationale for this approach was that EMS would not allow any neural adaptations to influence the magnitude of muscle damage. In an attempt to provide a similar injury stimulus in each exercise bout, 7 wk separated each bout to allow full recovery of the injured muscles. Additionally, peak eccentric force and total eccentric work were matched between each exercise bout as was the stimulation current used during EMS.

METHODS

Subjects

Nineteen adult subjects (11 men, 8 women) volunteered to participate in this study. Subjects were screened for medical or orthopedic conditions that would preclude strenuous exercise or MRI of the knee extensors. All subjects were recreationally active by self-report, but none had performed resistance training with the lower body during the previous 12 months. All experimental procedures were approved by The Institutional Review Board of The University of Georgia and subjects provided written informed consent before participation.

Subjects were assigned to one of two experimental groups: 1) electrical stimulation (STIM; six men and four women) and 2) voluntary (VOL; five men and four women). The STIM group performed two bouts of eccentric exercise via surface electrical stimulation of the knee extensors. The VOL group performed two bouts of volitional eccentric exercise of the knee

extensors. Data from the initial exercise bout in the STIM group were also included in a separate study (Black and McCully, submitted).

General Protocol

The study was conducted over the course of 13 wk. Subjects were familiarized to the isometric strength testing procedure, the STIM protocol, and the one repetition maximum (1-RM) testing over the initial 2 wk. Baseline isometric strength and 1-RM were determined at the end of the familiarization period. After familiarization, each group performed eighty eccentric contractions of the knee extensors at the beginning of the third week. Contractions in the STIM group were performed using 100 Hz of EMS using a weight equal to 25% of their maximal voluntary isometric force, whereas subjects in the VOL group performed the contractions using 120% of their concentric 1-RM. Exercise was performed in the nondominant leg in the VOL, whereas exercise was randomly assigned to either the dominant or the nondominant leg in the STIM group. Isometric strength and muscle soreness were assessed immediately after exercise and 1, 2, 3, 4, 7, 10, 14, 21, and 28 d after exercise. T2 magnetic resonance (MR) images were collected before and on the third day after eccentric exercise. To examine the effect of a previous bout of eccentric exercise on a subsequent bout of eccentric exercise, subjects performed a second identical bout of eccentric exercise 7 wk after the initial bout. All procedures and tests were repeated exactly, and subjects were asked to refrain from exercise of any type between the two exercise bouts.

Assessment of Maximal Voluntary Isometric Force

Isometric knee extension was performed as described previously (3) on a custom-built chair with the leg secured via an inelastic strap to a rigid lever arm so that the knee was fixed at $\sim 70^\circ$ below horizontal. The moment arm was established by positioning a load cell (model 2000A; Rice Lake Weighing Systems, Rice Lake, WI) parallel to the line of pull and perpendicular to the lever arm. Torque was recorded from the load cell using a MacLab analog-to-digital converter (model ML 400; AD Instruments, Milford, MA) with a sample rate of 100 Hz. Values were transferred to a portable computer for storage and analysis (Apple Computer, Cupertino, CA). Subjects were familiarized to the strength testing during three to five sessions over a 2-wk period before the study with baseline strength established at the end of this 2-wk period. During each testing session, subjects performed a series of maximal voluntary isometric contractions (MVIC) with 2 min of rest between trials. Subjects were given verbal encouragement to maintain their effort for 3–5 s. For each effort, torque was measured from the plateau region of the torque tracing. Once two efforts differed by less than 5%, the larger of the two efforts was considered the subjects' MVIC and was recorded for further analysis. An intraclass correlation coefficient of 0.99 was found across the five testing days with a mean within subject's coefficient of variation of $3.2 \pm 2.2\%$ (mean $\pm$ SD) that ranged from 0.8% to 8.5% across the testing days (unpublished observations).

Eccentric Exercise Protocol

STIM group—Subjects performed unilateral, eccentric actions with the left or right quadriceps femoris (QF) on a seated knee extension machine (Magnum Fitness System; Badger Fitness, Milwaukee, WI). Stimulation electrodes (6.98×10.16 cm Uni-Patch Wabasha, MN) were placed on the skin over the distal vastus medialis (VM) and the proximal vastus lateralis of either the right or the left thigh. A commercial stimulator (TheraTouch model 4.7; Rich-Mar Corporation, Inola, OK) was used to evoke all contractions. Each bout consisted of eight sets of 10 repetitions with 3 min of rest provided between each set. To perform each eccentric repetition, subjects were seated in the knee extension apparatus with the thigh at 90° relative to the torso. Two researchers lifted the weight to the starting position (full knee extension). A third researcher then turned on the EMS, the leg was released, and the applied EMS smoothly

lowered the weight through a 90° arc (from 0° to 90° below horizontal) at approximately 45°·s⁻¹ such that each eccentric repetition lasted approximately 2 s. EMS was terminated once the weight reached the bottom of the arc. Mechanical stops on the knee extension machine were set to prevent the lever arm from moving past 90° below horizontal. During each set, when the weight could no longer be lowered in a smooth controlled manner by the applied current, the weight was lowered by ~5%, and the set continued until all 10 contractions were complete. The subsequent set was begun using this weight. Eccentric exercise proceeded in this manner until all eight sets were completed. EMS stimulation consisted of 450 μ s, biphasic square wave pulses, at a frequency of 100 Hz at a constant current. The current used during the exercise protocol was determined before the eccentric protocol and was sufficient to produce an isometric contraction of 25% of MVIC using the specially designed chair described previously.

VOL group—Concentric 1-RM of the nondominant knee extensors were determined on the seated knee extension machine. Subjects were required to move the weight from a knee angle of 90° below horizontal to full extension; a researcher then lowered the weight to ensure only concentric actions of the knee extensors were performed. Subjects were instructed on proper form, and a lift was not counted unless the weight was lifted through a full range of motion and proper form was maintained. Three minutes of rest was given between trials, and weight was increased in 5-lb increments until 1-RM was reached (usually two to four trials were necessary).

Eight sets of 10 eccentric knee extensions were then performed with the weight initially set to approximately 120% of the concentric 1-RM. Three minutes of rest was provided between sets. To perform each eccentric repetition, subjects were seated in the knee extension apparatus with the thigh at 90° relative to the torso. Two researchers lifted the weight to the starting position (full knee extension). The subjects then performed eccentric contractions to smoothly lower the weight through a 90° arc (from 0° to 90° below horizontal) to a 2-s count. During each set, when subjects could no longer lower the weight in a smooth controlled manner, the weight was lowered by ~5%, and the set continued until all 10 contractions were complete. The next set was begun using this weight. Exercise proceeded in this manner until all eight sets were complete. Seven weeks after completion of the initial exercise bout, the exercise protocol was repeated in the STIM and VOL groups.

Assessment of Muscle Injury

MVIC and ratings of muscle soreness were obtained before, immediately after, and on days 1, 2, 3, 4, 7, 10, 14, 21, and 28 after each bout eccentric exercise. MVIC was assessed as described previously. Muscle soreness was assessed using a 100-mm visual analog scale. The scale was anchored with “0” being a complete lack of soreness and with “100” being the worst soreness imaginable. Evidence suggests the VAS scale is both valid and reliable for determining pain responses to noxious stimuli (39). Subjects rated their soreness during a contraction of the QF. Subjects lifted a light weight (10–20% of concentric 1-RM, which was matched during all bouts) through a 90° concentric arc (from 90° to 0° below horizontal) and a 90° eccentric arc (from 0° to 90° below horizontal) at approximately 45°·s⁻¹. T2 MR images were collected 3 d (approximately 72 h) after the exercise bout. Imaging was performed 72 h postexercise because we felt this time point allowed sufficient time for T2 elevation and corresponds to the time course of changes in other markers of muscle injury such as swelling, soreness, and isometric force loss.

MRI and Analysis

Subjects were positioned feet first and supine in a 3.0-T whole body imager (Signa, General Electric, Milwaukee, WI). A 20-cm-diameter extremity coil (General Electric) was centered on an indelible-ink mark placed approximately 40% of the distance between the top of the

patella and the greater trochanter. A series of 8 to 12 T2-weighted, spin echo, transaxial images (TR/TE = 1600/30, 60) 10 mm thick and 5 mm apart were collected in a 20-cm field of view and a 256 × 256 matrix along a 20-cm region of the thigh. T2 images were calculated from the two echo times assuming a monoexponential decay.

Images were analyzed using WinVessel 2.01 (Meyer, Michigan State University, East Lansing, MI), essentially as described previously (3,38). After spatial calibration, a region of interest (ROI) was defined in each image by manually tracing the outline of the anatomical cross-section area of the QF. The mean T2 values from all slices were averaged to obtain a mean T2 of the QF muscle group. QF volume (cm³) in the imaged region was determined by summing the pixel count from each ROI and multiplying it by the area of a single pixel (FOV/matrix; 20 cm/256 pixels = 0.078 cm per pixel) then multiplying by slice thickness (1 cm) and spacing (0.5 cm). In this context, “volume” is used to refer to the volume of the section of muscle that fall within the region that was imaged. This region contains a large portion of the muscle belly but did not cover the entire length of the QF muscle group. Mean T2 values and volumes were also determined for the rectus femoris (RF), vastus lateralis (VL), vastus medialis (VM) and vastus intermedius (VI) in the same manner by tracing the outline of each individual muscle. Mean T2 values were determined from the preexercise (resting) images and the 3-d postexercise images. For the QF muscle group and each individual muscle, the mean change in T2 signal intensity ($\Delta T2$) was calculated by subtracting the pre-T2 from the post-T2. Change in muscle volume or swelling was calculated using the following equation: [(Postvolume - prevolume)/ prevolume] × 100 for the QF and for each individual muscle.

The volume of muscle demonstrating an increase in T2 signal intensity (VDI T2) was determined on a pixel-by-pixel basis, essentially as done previously (3,4,38). Pixels with a T2 between 20 and 35 ms were assumed to represent muscle in preexercise images, whereas pixels with a T2 greater than 35 ms were considered to represent noncontractile tissue such as fat (38). The mean and SD of the T2 values from pixels in the 20- to 35-ms range were calculated. Pixels falling within this range were summed across all images and “muscle” volume was calculated as described above. Additionally, pixels with a T2 greater than 35 ms were summed across all images. Pixels in matching postexercise images with a T2 greater than the mean plus 1 SD of muscle from the preexercise images (determined from the pixels falling in the 20- to 35-ms range in the preexercise images) were considered elevated. These pixels were summed across all images, and subsequently pixels from preexercise images with a T2 greater than 35 ms were subtracted. The remaining pixels were considered to represent muscle tissue that had an elevated T2 (i.e., VDI T2). This was done to correct for pixels containing noncontractile tissue such as fat, which have elevated T2 values compared to muscle, which would be found in both sets of images. VDI T2 was calculated as described above and expressed relative muscle volume from the preexercise images. The delayed increase in T2 relaxation time has been shown to correlate to histological assessment of muscle injury in both humans (35) and animals (22).

Statistical Analysis

Results are expressed as mean ± SD. All statistical tests were performed using SPSS version 14.0. Dependent sample *t*-tests were performed to compare differences in mean T2 signal intensity of the QF muscle group preexercise to postexercise after each exercise bout in both the EMS and VOL groups. A two-factor (bout × muscle) within-subjects ANOVA was conducted to examine differences in mean T2 intensity preexercise to postexercise for bout 1 and bout 2 in each of the individual muscle of the QF. Differences in VDI T2, change in mean T2, and change in volume (swelling) between the first and second bout were examined using dependent *t*-tests for the EMS and VOL groups. A two-factor bout (1 and 2) × muscle (RF, VM, VI, and VL) within-subjects ANOVA was performed to examine differences in the change

in mean T2 and swelling between bouts and muscles of the QF. A two-factor bout (1 and 2) $\times$ time (days posteccentric exercise) within-subjects ANOVA was performed to examine differences in voluntary force loss and ratings of soreness. Independent sample *t*-tests were performed to compare differences between the STIM and VOL group in VDI T2, Δ T2, absolute T2 intensity, and change in volume (for the entire QF and each individual muscle for each measure) as well as for peak soreness and peak change in MVIC after bout 1 and bout 2 of exercise. When a significant interaction was found, a one-way repeated-measures ANOVA was performed to test for simple effects and followed, when appropriate, by dependent-measures *t*-tests. Main effects were only interpreted in the absence of a significant interaction. A Bonferroni correction for multiple comparisons was used when evaluating differences in means for soreness, isometric force, and individual muscles of the QF both within and between exercise bouts. Significance was set *a priori* at $\alpha \leq 0.05$. Nine comparisons were made (for each day postexercise) when examining changes over time for soreness and isometric force; thus, the α level was set to 0.0055 (0.05/9). Adjusted *P* values (scaled to 0.05) are reported in the present study. All effect sizes were calculated as a Cohen's *D*, as the difference in means divided by the pooled SD of the means.

RESULTS

No adverse events occurred as a result of this study. Two male subjects in the STIM group did not perform the second exercise bout, and their data from bout 1 was not included in the data presented in this study. One chose not to continue, and in the other, EMS force had not returned to baseline levels at 7 wk. After completion of the study, data from one female subject from the STIM group were removed from data analysis. The subject demonstrated a marked increase in all T2 MRI-assessed measures of injury after bout 2 compared with bout 1 (102% for VDI T2, 75% for Δ T2, and 156% for muscle volume), whereas in contrast the subject's ratings of soreness (13%) and change in isometric force (47%) were reduced after bout 2 compared with bout 1, which were consistent with others in the STIM group. Data analysis was performed on 16 subjects (STIM: 4 men, 3 women; VOL: 5 men, 4 women) aged 26 (4) yr, with a height of 174 (11) cm and a mass of 67 (13) kg.

Eccentric exercise

Absolute weight lifted during the 80 eccentric contractions was matched between bout 1 and bout 2 in both the STIM and the VOL groups (Fig. 1A). Peak weight lifted was 59 (33) kg and 81 (27) kg for STIM and VOL groups, respectively. The weight lifted declined 53% ($P < 0.001$) across the 80 contractions in the STIM group, whereas the VOL group declined only 8% ($P = 0.2$). When expressed relative to each subject's MVIC-weight lifted was 57 (19%) and 110 (21%) of MVIC in the STIM and VOL groups, respectively (Fig. 1B). Stimulation amplitude (mA), EMS-evoked isometric torque (before each eccentric exercise bout), and MVIC are shown in Table 1 and did not differ between bout 1 and bout 2 in either group.

MRI

Representative T2 images before and 72 h postexercise images from bout 1 and bout 2 for each group are shown in Figure 2. Mean T2 signal intensities for the QF muscle group as well as each individual muscle of the QF (RF, VM, VI, and VL) are shown in Table 2. A significant group (STIM vs VOL) by bout (1 vs 2) interaction was found ($P = 0.003$) with T2 signal intensity. During the initial bout of eccentric exercise, the T2 intensity of the QF increased significantly in the STIM ($P = 0.001$) and VOL ($P = 0.007$) groups, respectively (Table 2). All four muscles of the quadriceps experienced an increase in T2 in the STIM group ($P < 0.05$ for each), whereas the RF, VM, and VI experienced an increase ($P < 0.05$) in the VOL group. After the second bout of eccentric exercise, the STIM group once again demonstrated a significant increase in T2 of the QF as a whole. However, although T2 signal intensity increased

in the RF, VM, VI, and VL, the increase only reached significance in the VL (Table 2). Conversely, no increases in T2 were found in the QF as a whole or in the four individual quad muscles after the second bout of voluntary exercise (Table 2). When absolute T2 intensity was compared between the STIM and the VOL groups after each exercise bout, the STIM group demonstrated a higher T2 intensity in the VM ($P = 0.04$) after bout 1 and in the VL ($P = 0.02$) after bout 2 (Table 2).

After the second bout of exercise, the volume of the QF demonstrating an increase in T2 (VDI T2) intensity declined significantly in both the STIM (41–28%; $P = 0.02$) and the VOL (16–6%; $P < 0.001$) groups (Fig. 3A and Table 3). The mean change in T2 intensity ($\Delta T2$) was reduced 51% ($P = 0.03$) and 75% ($P = 0.002$) in the STIM and VOL groups, respectively, after the second exercise bout (Fig. 3B and Table 3). VDI T2 and $\Delta T2$ were significantly higher in the STIM group compared with the VOL group after both bout 1 ($P = 0.001$ and $P = 0.003$ for VDI T2 and $\Delta T2$, respectively) and bout 2 ($P = 0.002$ and $P = 0.007$) of eccentric exercise (Table 3).

When $\Delta T2$ was examined on a muscle-by-muscle basis, a significant bout by muscle interaction was observed ($P = 0.005$) in the STIM group. After bout 1, $\Delta T2$ increased in all four muscles of the quad with the VM demonstrating the largest increase (Table 4). The only intermuscle difference in $\Delta T2$ after bout 1 was that the VM showed a larger increase than the VI (Table 4; $P = 0.001$). After bout 2, $\Delta T2$ was significantly reduced in the RF (53%; $P = 0.03$) and the VM (70%; $P = 0.02$) but not in the VI or the VL (Table 4). In the VOL group, a significant main effect for bout was found ($P = 0.04$) with $\Delta T2$ being reduced after bout 2 compared with bout 1 (Table 4). Additionally, a main effect for muscle was found ($P = 0.04$), with the RF demonstrating a significantly larger increase in $\Delta T2$ compared with the VL ($P = 0.024$; Table 4). When compared between the STIM and VOL groups after each exercise bout, the STIM group demonstrated a greater $\Delta T2$ in the RF ($P = 0.05$), VM ($P = 0.001$), and VL ($P = 0.02$) after bout 1 and in the VI ($P = 0.03$) and VL ($P = 0.005$) after bout 2 (Table 4).

Volume of the QF muscle group increased (relative to baseline) after each exercise bout in both the STIM and the VOL groups (Table 4). The change in volume was 30% and 26% less after the second exercise bout in the STIM and VOL groups, respectively, but the reduction did not reach statistical significance. A main effect for muscle ($P = 0.025$) was found in the STIM group when swelling was compared across the individual quad muscles with greater swelling in the RF compared with the VI ($P = 0.01$; Table 4). Swelling was not different between bouts or between individual quad muscles in the VOL group (Table 4). QF muscle volume increased to a greater extent in the STIM group compared with the VOL group after bout 1 ($P = 0.05$) and bout 2 ($P = 0.01$) of exercise (Table 3). On a muscle-by-muscle basis, greater swelling was observed in the STIM group in the VM ($P = 0.009$) after bout 1 of eccentric exercise and in the VM ($P = 0.04$) and VL (0.002) after bout 2 (Table 4).

When the magnitude of the change from bout 1 to bout 2 (indicative of the size of repeated-bout effect) was compared between the STIM and the VOL groups for VDI T2, $\Delta T2$, and swelling (for the entire QF and each individual quad muscle), a significant group $\times$ bout interaction was only found for $\Delta T2$ with STIM group demonstrating a larger magnitude of change in VM muscle ($P = 0.027$; all others $P \geq 0.167$) compared with the VOL group. This was the only T2 MRI measure of injury where the size of the repeated-bout effect was statistically different between the STIM and VOL groups.

Soreness

Ratings of muscle soreness over the initial week after each exercise bout can be seen in Figure 4A (VOL) and 4B (STIM). A significant main effect for day was found in the STIM group ($P < 0.001$; Fig. 4B) with days 1–3 postexercise demonstrating increased soreness compared

with prevalues ($P \leq 0.024$ for each day; Fig. 4B). A significant main effect for bout ($P = 0.022$) was also observed in the STIM group. In the VOL group, a significant bout by time (days postexercise) interaction was observed ($P = 0.001$), with soreness differing from preexercise on days 1–3 after bout 1 ($P \leq 0.05$ for each day; Fig. 4A) and on day 1 after bout 2 ($P = 0.028$ for each day; Fig. 4A). When individual days were compared between bout 1 and bout 2 in the VOL group, subjects reported less soreness on days 1–4 ($P \leq 0.03$; Fig. 4A) after the second exercise bout. Peak soreness was 32% ($P = 0.002$) and 47% ($P < 0.001$) lower after the second eccentric exercise bout compared with bout 1 in the STIM and VOL groups, respectively (Table 3). The group $\times$ bout interaction was not significant ($P = 0.569$) nor was the main effect for group ($P = 0.10$) when peak soreness was compared in the STIM and VOL groups across each exercise bout. However, a significant main effect was found for bout, with soreness being lower after the second bout ($P < 0.001$). Peak soreness was not statistically different between the STIM and VOL groups after each exercise bout ($P = 0.19$ and 0.06 for bout 1 and bout 2, respectively; Table 3).

Maximal voluntary isometric torque

When tested before each bout, maximal voluntary strength was not found to differ in either EMS or VOL group between bout 1 and bout 2 (Table 1). Changes in MVIC after an eccentric exercise can be seen in Figure 5. No significant differences in the magnitude of decline or in the time course of recovery of strength were seen between bout 1 and bout 2 in either STIM or VOL group. A significant main effect for time (days postinjury) was found in each group ($P < 0.001$). In the STIM, group strength was reduced from baseline levels immediately after exercise and on days 1–4 ($P < 0.03$ for each day). In the VOL group, strength was reduced immediately after and on days 1–3 after exercise ($P < 0.05$ for each day). The group $\times$ bout interaction was not significant ($P = 0.826$) when peak decline in isometric force was compared in the STIM and VOL groups for each exercise bout. The main effects for bout ($P = 0.06$) and group ($P = 0.514$) were also not statistically different. No differences were observed in peak decline of maximal strength between the STIM and the VOL group after bout 1 ($P = 0.59$) and bout 2 ($P = 0.51$) of exercise (Table 3).

DISCUSSION

The present study found that muscle injury was reduced to a similar extent after repeated bouts of voluntary and electrically stimulated eccentric exercise of the knee extensors. After a second identical bout of eccentric exercise, smaller changes were found in the MRI-measured T2 signal intensity, and ratings of muscle soreness were reduced in both exercise groups. Although the repeated-bout effect has been widely observed after voluntary exercise (see McHugh et al. for review) (24), less is known about its occurrence after stimulated exercise. Our results are consistent with data from animal (17,18,42,44) and human (33) studies that found reduced markers of muscle injury after repeated bouts of eccentric exercise using EMS. The present study differed from the previous human study in several important aspects. First, 7 wk of recovery were allowed between bouts in the present study compared with the 2 wk in the study of Nosaka et al. (33). Data from pilot work and the present study indicated that stimulated force had not fully recovered in the injured muscles 2 wk after the initial exercise bout (unpublished observations). Second, it is unclear as to whether the stimulation current was matched between bouts in the previous study (33). By allowing for complete force recovery and applying a similar EMS current, we were able to match peak eccentric force, total eccentric work, and likely activate a similar motor unit population during each exercise bout. By imposing similar stress to a similar fiber population, we were able to closely match the exercise bouts and thus test the influence of neural adaptations to the repeated-bout effect. Consequently, our findings of a similar magnitude of injury reduction after repeated bouts of electrically stimulated and voluntary eccentric exercise suggest the primary mechanism underlying the repeated-bout

effect is a structural adaptation within the muscle rather than a neural adaptation that alters muscle recruitment.

Our findings are consistent with the idea that muscle-specific adaptations such as the removal of susceptible muscle fibers (2,11), the longitudinal addition of sarcomeres (20), and/or an increase in cytoskeletal proteins (13) play a role in limiting injury during repeated exercise bouts. Increasing the number of sarcomeres in series could reduce the individual sarcomere extension during lengthening actions and shorten sarcomeres at any given muscle length, thus moving them away from the descending limb of the force—length relationship where they have been shown to be more susceptible to injury (25). Additionally, up-regulation of cytoskeletal proteins (such as titin, desmin, talin, vinculin) that compose the network that surrounds sarcomeres and help transmit tension across the myotendinous junction could strengthen and stabilize sarcomeres and protect them from future injury. Studies have shown that eccentric exercise can increase the levels of titin (19), desmin (10,19), talin (12), and vinculin (12). Increased titin expression has also been demonstrated in competitive athletes compared with nonathletic persons (23). Additionally, several recent studies (45,46) have indicated that alterations in cytoskeletal proteins in the days after an eccentric exercise are consistent with myofibillar remodeling and the addition of new sarcomeres. Remodeling and addition of new sarcomeres could limit injury not only by moving them away from the descending limb of the force—length relationship as discussed previously, but also by increasing the homogeneity of sarcomere lengths along an individual muscle fiber (21,41).

Voluntary eccentric exercise resulted in significantly less muscle injury compared with EMS-evoked eccentric exercise in the present study, despite the greater relative force levels during the voluntary protocol. A recent study by Crameri et al. (9) demonstrated similar differences in muscle damage between voluntary and electrically stimulated eccentric exercise, and taken together, these findings highlight the potential importance of the pattern of motor unit recruitment in muscle injury. EMS is thought to recruit muscle in a nonselective, spatially fixed, and temporally synchronous manner (i.e., the same motor units are repeatedly activated throughout the exercise bout) (15). Because of this, EMS likely activates a greater percentage of fast muscle fibers at any given load compared with voluntary actions. Fast fibers have been shown to be more susceptible to contraction-induced injury (13,43) than slow fibers. It has been proposed that their increased susceptibility is related to a lower contractile workload (43), an ability to generate higher forces (1), and/or ultrastructural differences such as reduced cytoskeletal proteins (13) and an increased heterogeneity of sarcomere lengths. Another possible explanation for the difference in injury is that voluntary recruitment may allow motor units to be recruited in a more efficient manner across an exercise bout as well as allowing recruitment of agonist muscles. Thus, voluntary actions could limit recruitment of fast-twitch fibers and allow a given level of force to be spread over a larger active area compared with EMS. Consistent with this idea, previous studies have suggested neural adaptations such as more efficient motor unit recruitment (31), increased synchrony of motor unit firing (14), and increased recruitment of slow twitch muscle fibers (44) could limit muscle injury. Additionally, high force per active area has been shown to be an important factor in initiating muscle injury (4,16). Despite the differences in magnitude of injury between the STIM and VOL protocols, our findings of a similar reduction in injury from bout 1 to bout 2 in both groups agree with previous findings (5,36), indicating protection occurs regardless of the amount of injury during the initial exercise bout.

The primary outcome measure in this study was the use of changes in T2 MRI signals as indices of muscle damage. We found similar results for changes in mean T2 values and in the percentage of muscle that showed significant increases in T2. Previous studies have shown that T2 changes reflect the magnitude and location of muscle damage (4,11,37,40). The advantage of the T2 changes in the assessment of muscle injury is its ability to localize injury

to specific muscles and to specific regions within a muscle or muscle group. In the present study, the mean T2 signal intensity increased in all four individual quadriceps muscles after both electrically stimulated and voluntary exercise. Indicating all four muscles were likely recruited/ activated and subsequently injured in both exercise modalities. When compared across both exercise bouts, a greater change was seen in RF compared with VL. This finding is consistent with that of Prior et al. (40) who also demonstrated a greater change in T2 intensity in the RF compared with the vasti muscles. A similar reduction in T2 signal intensity was seen across the RF, VM, VI, and VL after the second exercise bout. To our knowledge, these are the first data indicating a similar magnitude of adaptation across the knee extensor muscles. In the STIM group, a reduced change in T2 intensity was observed in the RF and VM but not in the VI and VL. The reason for this finding is not clear, but we believe it may be a consequence of the increased variability in the VI and VL and the small sample size ($n = 7$) tested rather than a difference in the adaptive stimulus applied to each muscle.

In conclusion, the present study showed that performance of a single bout of electrically stimulated eccentric exercise reduced the magnitude of muscle injury observed after an identical bout performed 7 wk later. The reduction in injury was similar to that observed after two bouts of voluntary eccentric exercise performed 7 wk apart. This finding indicates that the primary mechanism underlying the repeated-bout effect is not a change in muscle recruitment but rather is likely a muscle-specific adaptation potentially because of structural changes within the muscle. Additionally, stimulated eccentric exercise resulted in greater muscle injury at a comparatively lower force level than voluntary eccentric exercise. Suggesting differences in recruitment patterns, especially in regards to the number of fast and slow muscle fibers recruited at a given force level, may play a critical role in the difference in injury observed after the electrically stimulated and voluntary exercise.

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FIGURE 1.
Mean change in eccentric weight lifted during each protocol expressed as percent of each subject's maximal voluntary isometric force.

FIGURE 2.

Anatomically matched axial T2 MR images from the mid-thigh region of the QF from the EMS (A—D) and VOL (E—H) groups. A and C show preimages, whereas B and D show 72 h postimages from bout 1 and 2, respectively, in the STIM group. E and G show preimages, whereas F and H show 72 h postimages from bout 1 and bout 2, respectively, in the VOL group.

FIGURE 3. Percent of QF muscle volume considered injured (A), mean change in T2 (B), and change in QF muscle volume (C) from bout 1 (dark bars) and bout 2 (hatched bars) for the STIM and VOL groups. * Significant difference between values after bout 1 and bout 2 ($P < 0.05$).

FIGURE 4.

Ratings of muscle soreness over 10 d after an eccentric exercise in the VOL group (A) and STIM group (B) after bout 1 (*closed squares*) and bout 2 (*open squares*). * Significant difference between bout 1 and bout 2 ($P < 0.05$). † Denotes a significant difference from preexercise for both exercise bout.

FIGURE 5. Change in MVIC over 28 d after an eccentric exercise in the VOL group (A) and STIM group (B) after bout 1 (closed circles) and bout 2 (open circles). ** Significant main effect for day ($P < 0.05$) on days 1–3 and days 1–4 for the VOL and STIM groups, respectively.

TABLE 1

Subject characteristics and voluntary and EMS-evoked force levels.

Measure	STIM		VOL	
	Bout 1	Bout 2	Bout 1	Bout 2
Age (yr)	26 (3)		26 (5)	
Height (cm)	172 (12)		176 (12)	
Weight (kg)	65 (13)		69 (14)	
MVIC ($\text{N}\cdot\text{m}^{-1}$)	364 (130)	376 (143)	287 (125)	293 (157)
EMS isometric torque ($\text{N}\cdot\text{m}^{-1}$)	102 (57)	91 (38)		
EMS current (mA)	45 (6)	47 (9)		
Initial eccentric load (kg)	59 (33)	59 (33)	81 (27)	81 (27)

Vales are mean (SD).

STIM group: four men, three women.

VOL group: five men, four women.

TABLE 2
T2 values for the quadriceps femoris muscle group and each individual knee extensor muscle from preeccentric and 72 h posteccentric exercise.

Muscle	Bout 1		Bout 2	
	Pre-T2 (ms)	Post-T2 (ms)	Pre-T2 (ms)	Post-T2 (ms)
STIM				
QF	26.5 (2.6)	30.8 (4.9) *	27.4 (2.4)	30.2 (3.8) *
RF	28.4 (3.4)	33.5 (6.2) *	29.0 (3.0)	31.4 (5.1)
VM	26.0 (2.4)	32.1 (4.7) * [‡]	27.1 (3.3)	28.9 (3.9)
VI	25.9 (2.6)	29.2 (4.5) *	26.7 (2.1)	30.0 (4.5)
VL	26.6 (3.7)	29.5 (5.6) *	27.9 (2.5)	31.0 (3.9) * [‡]
VOL				
QF	26.6 (2.7)	27.8 (2.5) *	27.0 (2.4)	27.2 (2.4)
RF	28.3 (2.9)	30.4 (3.3) *	28.8 (3.0)	29.3 (2.5)
VM	26.4 (2.7)	27.8 (2.5) *	26.7 (2.4)	27.1 (2.6)
VI	25.9 (2.6)	27.1 (2.2) *	26.1 (2.1)	26.6 (2.3)
VL	25.9 (3.0)	26.6 (2.9)	26.1 (2.9)	26.3 (2.5)

Values are means (SD).

RF, rectus femoris; VM, vastus medialis; VI, vastus intermedius; VL, vastus lateralis.

* Significant difference from preeccentric to posteccentric ($P \leq 0.05$).

[‡] Significant difference between STIM and VOL ($P \leq 0.05$).

TABLE 3
Percent change and effect of the change in measures of muscle injury for the QF muscle group.

	Bout 1	Bout 2	Change (%)	Effect Size (SD)
STIM				
VDI T2 (%)	41 (17) [‡]	28 (16) ^{*,‡}	-32	-0.79
QF ΔT2 (ms)	4.5 (2.7) [‡]	2.8 (2.2) ^{*,‡}	-51	-0.69
QF swelling (%)	10 (8.0) [‡]	7 (3.0) [‡]	-30	-0.50
Peak force loss (%)	-31 (7)	-25 (8)	24	0.80
Peak soreness (mm)	60 (16)	41 (12) [*]	-32	-1.35
VOL				
VDI T2 (%)	16 (7)	6 (6) [*]	-62	-1.53
QF ΔT2 (ms)	1.2 (0.5)	0.3 (0.8) [*]	-75	-1.34
QF swelling (%)	3.9 (2.5)	2.9 (2.6)	-26	-0.39
Peak force loss (%)	-26 (19)	-19 (21)	28	0.37
Peak soreness (mm)	47(20)	25 (18) [*]	-47	-1.16

Values are mean (SD).

VDI T2, volume demonstrating an increased T2; ΔT2, change in mean T2 signal intensity.

* Significant difference from bout 1 ($P \leq 0.05$).

[‡] Significant difference between STIM and VOL ($P \leq 0.05$).

TABLE 4
Percent change and effect size of the change in T2 signal intensity and volume of the individual quadriceps muscles.

	Bout 1	Bout 2	Change (%)	Effect Size (SD)
STIM				
RF Δ T2 (ms)	5.1 (3.7) [‡]	2.4 (2.8) [*]	-53	-0.82
VM Δ T2 (ms)	6.1 (3.2) ^{‡‡}	1.8 (2.8) [*]	-70	-1.43
VI Δ T2 (ms)	3.3 (2.5)	3.4 (3.2) [‡]	+3	0.03
VL Δ T2 (ms)	2.9 (2.2) [‡]	3.0 (2.2) [‡]	+3	0.05
RF swelling (%)	14 (9) ^{‡‡}	12 (11)	-14	-0.20
VM swelling (%)	16 (8) [‡]	6 (3) [‡]	-63	-1.66
VI swelling (%)	6 (6)	6 (7)	0	0.00
VL swelling (%)	5 (5)	10 (3) [‡]	+100	1.21
VOL				
RF Δ T2 (ms)	2.1 (1.4) ^{‡‡}	0.6 (1.1) ^{**}	-71	-1.20
VM Δ T2 (ms)	1.4 (0.6)	0.4 (1.1) ^{**}	-71	-1.12
VI Δ T2 (ms)	1.2 (1.0)	0.5 (0.8) ^{**}	-58	-0.77
VL Δ T2 (ms)	0.7 (0.7)	0.2 (1.0) ^{**}	-71	-0.58
RF swelling (%)	4 (11)	3 (7)	-25	-0.08
VM swelling (%)	6 (4)	2 (4)	-74	-1.18
VI swelling (%)	5 (3)	4 (3)	-20	-0.33
VL swelling (%)	1 (7)	2 (4)	+69	0.16
Values are mean (SD).				

^{*} Significant difference from bout 1 ($P \leq 0.05$).

^{**} Significant main effect for bout ($P < 0.05$).

[‡] Significant difference between VM and the VI after bout 1 ($P = 0.001$).

^{‡‡} Significant main comparison between RF and the VI ($P = 0.01$; STIM) and between the RF and VL ($P = 0.024$; VOL).

^{‡‡‡} Significant difference between STIM and VOL ($P \leq 0.05$).