HIGHLIGHTED TOPIC | Eccentric Exercise

A contralateral repeated bout effect attenuates induction of NF-κB DNA binding following eccentric exercise

Ling Xin,1 Robert D. Hyldahl,2 Stuart R. Chipkin,1 and Priscilla M. Clarkson1,3

1Department of Kinesiology, University of Massachusetts Amherst, Amherst, Massachusetts; and 2Department of Exercise Sciences, Brigham Young University, Provo, Utah

Submitted 30 January 2013; accepted in final form 7 August 2013

Xin L, Hyldahl RD, Chipkin SR, Clarkson PM. A contralateral repeated bout effect attenuates induction of NF-κB DNA binding following eccentric exercise. J Appl Physiol 116: 1473–1480, 2014. First published August 15, 2013; doi:10.1152/japplphysiol.00133.2013.—We investigated the existence of contralateral repeated bout effect and tested if the attenuation of nuclear factor-kappa B (NF-κB; an important regulator of muscle inflammation) induction following eccentric exercise is a potential mechanism. Thirty-one healthy men performed two bouts of knee extension eccentric exercise, initially with one leg and then with the opposite leg 4 wk later. Vastus lateralis muscle biopsies of both exercised and control legs were taken 3 h postexercise. Knee extension isometric and isokinetic strength (60°/s and 180°/s) were measured at baseline, pre-exercise, immediately postexercise, and 1/day for 5 days postexercise. Serum creatine kinase (CK) activity and muscle soreness were assessed at baseline and 1/day for 5 days postexercise. NF-κB (p65) DNA-binding activity was measured in the muscle biopsies. Isometric strength loss was lower in bout 2 than in bout 1 at 24, 72, and 96 h postexercise (P < 0.05). Isokinetic strength (60°/s and 180°/s) was reduced less in bout 2 than in bout 1 at 72 h postexercise (P < 0.01). There were no significant differences between bouts for postexercise CK activity or muscle soreness. p65 DNA-binding activity was increased following eccentric exercise (compared with the control leg) in bout 1 (122.9% ± 2.6%; P < 0.001) and bout 2 (109.1% ± 3.0%; P < 0.05). Compared with bout 1, the increase in NF-κB DNA-binding activity postexercise was attenuated after bout 2 (P = 0.0008). Repeated eccentric exercise results in a contralateral repeated bout effect, which could be due to the attenuated increase in NF-κB activity postexercise.

eccentric exercise; muscle damage; inflammatory response; NF-κB

UNACCUSTOMED EXERCISES, especially those involving eccentric (muscle lengthening) actions, induce transient muscle damage (7, 18, 31). Exercise-induced muscle damage is typically manifested by cellular damage, muscle soreness, prolonged strength loss, and increased blood levels of intramuscular proteins such as creatine kinase (CK) and myoglobin (7). This exercise causes initial mechanical damage to the muscle followed by an acute inflammatory response (41) leading to secondary damage. As part of the inflammatory response, neutrophils are quickly recruited from the circulation into the damaged muscle areas (33). Neutrophils release reactive oxygen and nitrogen species and pro-inflammatory cytokines (4, 5), which then activate transcription factors such as nuclear factor-kappa B (NF-κB) by a series of events (23). As a result, NF-κB binds to specific genomic regulatory regions and drives the expression of target gene products, many of which are proinflammatory proteins such as cyclooxygenase-2 (COX-2), monocyte chemoattractant protein-1 (MCP-1), and interleukin-6 (IL-6) (32). These proteins increase inflammation that is believed to play a primary role in the secondary muscle damage after strenuous eccentric exercise (5).

Muscle damaging exercise typically results in an adaptation response. For example, a repeated bout of eccentrically biased exercise produces less damage in a muscle that has been exposed to a similar exercise bout fewer than 6 mo before, a phenomenon known as the “repeated bout effect” (RBE) (7, 30). One of the proposed mechanisms for RBE is attenuated inflammatory response postexercise (27). In support of this potential mechanism, Pizza et al. (35) reported a reduction in some blood leukocyte receptors after a repeated bout of eccentric exercise of elbow flexors. In a later study, the same group demonstrated that blood neutrophil numbers were significantly lower at 3, 6, and 9 h postexercise after a second bout of eccentric exercise of elbow flexors (34). In addition, Smith et al. (38) observed a reduction in IL-6 and MCP-1 and an elevation in anti-inflammatory IL-10 during the initial 12-h period after the second bout of downhill running compared with the first bout.

To our knowledge, only three studies have sought to determine if the RBE also exists in the contralateral muscle group after a single bout of eccentric exercise (8, 16, 39). In two of the three studies, subjects were assigned to either an ipsilateral or a contralateral group. Subjects in the ipsilateral group performed the second bout of eccentric exercise with the same arm (elbow flexors) 2 wk later; subjects in the contralateral group performed two bouts using the opposite arm in the second bout. Both studies observed significant reductions in strength loss after the second exercise bout for both ipsilateral and contralateral groups, although the magnitude of change was lower in the contralateral muscle compared with the ipsilateral muscle (16, 39). The third study (8) reported no evidence of contralateral RBE in leg muscles. However, the amount of strength loss induced by the first bout in the third study was only 10% compared with 25% and 30% in the two studies described above. Therefore, it is still uncertain if a contralateral RBE also exists in leg muscles, because the failure to observe a contralateral RBE in the third study may be attributable to the insufficient muscle damage stimulus.

Given the paucity of studies exploring the contralateral RBE, it is not surprising that the mechanisms underlying this process have not yet been elucidated. Using electromyography...
(EMG) analysis, Starbuck and Eston (39) concluded that the observed contralateral RBE is due to neural adaptation whereby motor unit recruitment is optimized during the second exercise bout, leading to less initial damage. EMG is usually used to investigate the motor unit activation in the muscle during the actual exercise bout and thus is primarily associated with the initial muscle damage. Therefore, neural adaptation can primarily explain the reduced initial muscle damage during the repeated exercise bout. Because the contralateral RBE includes a reduction in the anticipated muscle damage symptoms up to 10 days or even longer after the exercise bout, other mechanisms are likely to be involved.

Our laboratory recently provided direct evidence of an increase in the activation of the transcription factor NF-κB (1.6-fold change) at 3 h after eccentric exercise in humans (18). Because NF-κB plays an important role in the secondary damage response after eccentric exercise (5, 23), we hypothesized that attenuation of NF-κB activation may be involved in the contralateral RBE. To test this hypothesis, the present study examined muscle function, blood CK activity, muscle soreness, and NF-κB DNA-binding activity after the initial bout with the ipsilateral leg and after the second bout 4 wk later with the contralateral leg. The goals of this study were to determine if the contralateral RBE exists in leg muscles and to identify possible molecular mechanisms for observed effects. We hypothesized that contralateral RBE also exists in leg muscles, and NF-κB activity would be attenuated in the contralateral leg.

MATERIALS AND METHODS

Study design. The study period consisted of 15 testing visits during which subjects performed two bouts of eccentric exercise spaced 4 wk apart (Fig. 1). In bout 1, subjects exercised one leg (knee extensors); in bout 2, subjects repeated the exercise bout with the knee extensors of the contralateral leg. This study was originally intended to examine the effects of two botanical supplements with anti-inflammatory and antioxidant properties on the muscle response to strenuous exercise. Subjects were randomly assigned in a double-blind manner to receive the effects of two botanical supplements with anti-inflammatory and muscle soreness, and NF-κB DNA-binding activity after the initial bout with the ipsilateral leg and after the second bout 4 wk later with the contralateral leg. The goals of this study were to determine if the contralateral RBE exists in leg muscles and to identify possible molecular mechanisms for observed effects. We hypothesized that contralateral RBE also exists in leg muscles, and NF-κB activity would be attenuated in the contralateral leg after the second exercise bout.

Study design. The study period consisted of 15 testing visits during which subjects performed two bouts of eccentric exercise spaced 4 wk apart (Fig. 1). In bout 1, subjects exercised one leg (knee extensors); in bout 2, subjects repeated the exercise bout with the knee extensors of the contralateral leg. This study was originally intended to examine the effects of two botanical supplements with anti-inflammatory and antioxidant properties on the muscle response to strenuous exercise. Subjects were randomly assigned in a double-blind manner to receive two botanical supplements (supplement 1: rhodiola + rose hips + astaxanthin; supplement 2: ashwagandha + grape seed + prickly pear) for 35 days. However, repeated-measures analysis of variance (ANOVA) detected no significant differences between the supplement and placebo groups for CK, muscle soreness, or strength ($P > 0.05$). Therefore, we pooled the data from all subjects to further examine the contralateral RBE.

Subjects. Thirty-one healthy men (age $20.7 \pm 0.5$ yr, height $178.5 \pm 1.3$ cm, weight $81.1 \pm 3.1$ kg; mean $\pm$ SD) completed the study. All subjects were recruited from the local community and signed the informed consent form approved by the Institutional Review Board (IRB) of the University of Massachusetts Amherst. Subjects were sedentary using the standard activity level of less than six metabolic equivalent tasks (METs), and they had not participated in resistance training of the legs within the previous 6 mo. Subjects were excluded if they were unwilling to refrain from taking dietary supplements or nonsteroidal anti-inflammatory drugs during the course of the study (except the botanical supplements or the placebo) and if they were smokers. No subjects had skeletal, muscular, or neuromuscular dysfunction or any other known medical conditions that could prevent them from completing the study exercise requirements.

Study visits. The study design is illustrated in Fig. 1. On visit 0, subjects gave written informed consent. There were seven visits for both bout 1 and bout 2 periods. The leg tested (exercised) in bout 1 was determined by alternating from one leg to the other as subjects were recruited. Therefore, there was an equal number of right and left legs tested (exercised) during both bouts in the study. During visit 1, subjects had baseline muscle strength tests on the randomized leg and a fasting ($>8$ h) blood draw for CK analysis. Visit 2 occurred 24 h after visit 1; subjects came to the lab after an overnight fast ($<12$ h). Upon arrival for visit 2, subjects consumed a standardized breakfast of $0.4$ kcal ($55\%$ carbohydrate, $30\%$ fat, and $15\%$ protein). Subjects were then administered a baseline muscle soreness evaluation and a pre-exercise strength measure, then exercised one leg (knee extensors) followed immediately by a postexercise strength measure. A muscle biopsy of both eccentric exercised (ECC) and control (CON) legs (vastus lateralis muscle) was taken at 3 h postexercise. On each day of the following 5 days (visits 3–7), subjects were assessed for soreness, strength, and serum CK. During the bout 2 period, subjects repeated the regimen of bout 1 except that the contralateral knee extensors were exercised.

Muscle strength. Measures of isometric and isokinetic strength of the knee extensor muscles were assessed on the Biodex System 4 isokinetic dynamometer (Biodex Medical Systems, Shirley, NY). Subjects were tested in the seated position with the lateral femoral epicondyle aligned with the axis of rotation of the dynamometer. Full knee extension ($0^\circ$) was entered as a reference value into the computerized dynamometer system. Three (3 s) trials at 70° of knee flexion with 1 min rest between trials were assessed for isometric strength. Isometric peak torque values were defined as the average of the highest obtainable value among the three trials. Since several studies (10, 11, 13) have shown a slower recovery of concentric strength at faster angular velocity compared with the restoration of isometric or concentric strength at slower angular velocity after damaging exercise, we measured both isometric and isokinetic strength at different speeds for a more comprehensive analysis of strength loss. Therefore, after a 5-min rest after the isometric strength test, subjects performed three consecutive isokinetic strength measures at speeds of 60 and 180°/s with a 2-min rest between each set of three repetitions. Isokinetic extension peak torque values were defined as the average of the highest value among the three trials. The intra-assay coefficient of variation (CV) of isometric, isokinetic strength at 60 and 180°/s was 4.2, 3.1, and 4.9%, respectively, suggesting the repeatability of the strength measures.

Creatine kinase activity. Blood collected from the antecubital vein was allowed to clot for $\sim 15$ min. Serum was then obtained by centrifugation for 15 min at 4,400 rpm. Serum samples were analyzed...
for creatine kinase (CK) activity using standard clinical procedures (Abbott Laboratories, Worcester, MA). The intra-assay CV was 3.9%.

Muscle soreness. Soreness/pain was evaluated using a visual analog scale (VAS), a 100-mm horizontal line with 0 mm on the left indicating “no pain” and 100 mm on the right indicating “unbearable pain.” After performing two full squats against the subject’s own body weight, the subject placed a vertical line through the 100-mm line corresponding to the peak level of pain experienced during the squats. The distance from the left end of the scale to the mark was regarded as the soreness level. All subjects provided pre-exercise (baseline) soreness evaluation. Subjects were only cleared to participate if the baseline VAS score was determined to be >10 mm in each leg.

Eccentric exercise. Subjects were seated on the dynamometer chair and completed 10 sets of 10 eccentric repetitions at a speed of 30°/s with a 10-s rest between repetitions and a 1-min rest between sets. The start position of the eccentric exercise was 35° of knee flexion. During each eccentric contraction, the subject was verbally encouraged to maximally extend or “kick” his leg against the dynamometer, which moved at 30°/s from 35° to the subject’s maximal flexion angle in the normal seated position. At the end of each eccentric contraction, the tester moved the subject’s leg back to the 35° start position. The work performed during each set was measured, and the total work accomplished during the exercise bout was calculated by adding up the work of the 10 sets.

Muscle biopsy. A percutaneous needle muscle biopsy was obtained from both nonexercised and eccentric-exercised legs at 3 h after the eccentric exercise. Two biopsies on the same leg were performed at least 3 cm apart to minimize the confounding effect from biopsy procedure. The muscle biopsies were performed under local anesthesia using 2% lidocaine. A small incision (~1-3 cm) was made in the skin and fascia. A Bergstrom 5-6 mm biopsy needle was then inserted into the muscle, and a small core of tissue (~50–200 mg) was removed and snap-frozen in liquid nitrogen. The wound was closed using two or three sutures, and the leg was wrapped in a compression bandage. An ice bag was applied to the biopsy area while the subject rested for ~15 min. The collected tissue was stored at ~80°C until analysis.

ELISA-based NF-κB (p65) DNA-binding activity. Nuclear extract isolation was performed using a protocol that we described previously (18). Briefly, nuclear extracts were prepared by homogenizing muscle biopsy samples (>20 mg) in a low-salt lysis buffer. Homogenized tissue was then subjected to two cycles of freeze/thaw using an ethanol/dry ice freeze bath and a 37°C water bath. Samples were then vortexed and centrifuged at 3,000 rpm for 3 min at 4°C to separate the supernatant (cytosolic extracts) from the nuclear pellet. The nuclear pellet was resuspended in high-salt buffer. The samples were then vortexed and centrifuged at 3,000 rpm for 3 min at 4°C. The supernatant, which contained the nuclear protein fraction, was stored at ~80°C. A standard bicinchoninic acid assay (Pierce, Rockford, IL) was used to quantify nuclear proteins. NF-κB DNA-binding activity was determined using nuclear extracts and an ELISA-based TransAM NF-κB p65 assay kit (Active Motif, Carlsbad, CA) according to the manufacturer’s instructions as described previously (18). Briefly, 12 μg of nuclear extract was added to wells coated with a consensus binding sequence (5'-GGGACTTTCC-3') for NF-κB and incubated for 1 h at room temperature. Wells were then washed, and a primary antibody against p65 subunit was added and left to incubate for 1 h. Next, all wells were treated with a secondary antibody conjugated to horseradish peroxidase (HRP). A subsequent colorimetric reaction was initiated with the addition of a developing colorimetry solution for 5–7 min followed by the application of a stop solution. The absorbance of the plate was then read at 450 nm on a microplate reader (FLUOstar Optima, BMG Labtech, Offenburg, Germany). Wild-type and mutated consensus oligonucleotides were used as competitors for NF-κB binding to ensure specificity of the reaction. All samples were run in duplicate, and the average value was used for data analysis. The intra-assay CV was 5.1%.

Data analyses. A paired t-test was used to analyze the total work during the exercise that the subjects completed in bout 1 and 2. With baseline and pre-exercise muscle strength data, Pearson correlation analysis and a paired t-test was performed to test the test-retest reliability of the muscle strength measurements. Muscle strength, CK, and soreness data were analyzed via a repeated-measures ANOVA to obtain the main effects of time (exercise), bout (bout 1 vs. bout 2), and the interaction term. CK data were not normally distributed and were log transformed prior to performing the ANOVA. The NF-κB DNA-binding activity data were expressed in arbitrary absorbance units (450 nm) and analyzed via ANOVA to obtain the main effects of exercise (ECC vs. CON), bout (bout 1 vs. bout 2), and the interaction term. When appropriate, Tukey’s post hoc analysis was performed. All statistical tests were conducted using a SAS statistical software package (V9.2; SAS Institute, Cary, NC) with significance set at P < 0.05.

RESULTS

Total exercising range of motion and work completed per bout. The total exercising range of motion was 76.4 ± 6.5° and 78.3 ± 6.8° for bout 1 and bout 2, respectively. The exercising range of motion difference (1.9 ± 6.4°) between the two bouts was not statistically significant (P = 0.26). The amount of total work performed by subjects during the eccentric exercise for bout 1 and bout 2 was 16,123.0 ± 4,088.9 and 16,528.5 ± 4,335.9 J, respectively. The difference in the amount of total work performed between the two bouts was 405.5 ± 2,815.7 J, and this difference was not statistically significant (P = 0.44). The consistency of exercising range of motion and total work performed between bouts is evidence that the data are not significantly confounded by the exercise protocol design.

Muscle strength. Muscle strength was measured at baseline, pre-exercise (Pre), immediately after exercise (Post), and every 24 h for 120 h following two bouts of eccentric exercise. Table 1 displays the baseline and pre-exercise strength data. All Pearson correlation coefficients (r) of baseline vs. pre-exercise values were highly significant (P < 0.005). Paired t-test results indicated that there was no significant difference between baseline and pre-exercise values for isometric strength and isokinetic strength at 60°/s. For isokinetic strength at 180°/s, the bout 1 baseline value was significantly lower than other values (bout 1 pre-exercise, bout 2 baseline and pre-exercise values), perhaps due to the familiarization process. Overall, the muscle strength data were judged to be reliable. For all muscle strength variables, the pre-exercise values were included, whereas baseline values were excluded from statistical analysis.

Table 1. Baseline and preexercise values of isometric (at 70° of knee flexion) and isokinetic (at 60°/s and 180°/s) knee extension peak torque (N·m)

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>Pre-Exercise</th>
<th>Difference Between Baseline and Pre-Exercise</th>
</tr>
</thead>
<tbody>
<tr>
<td>Isometric</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Boat 1</td>
<td>230.0 ± 47.7</td>
<td>233.1 ± 58.3</td>
<td>3.1 ± 23.6</td>
</tr>
<tr>
<td>Boat 2</td>
<td>235.3 ± 48.5</td>
<td>229.8 ± 44.8</td>
<td>5.1 ± 23.2</td>
</tr>
<tr>
<td>Isokinetic (60°/s)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Boat 1</td>
<td>188.2 ± 44.2</td>
<td>197.0 ± 44.0</td>
<td>8.9 ± 24.9</td>
</tr>
<tr>
<td>Boat 2</td>
<td>188.2 ± 37.3</td>
<td>187.5 ± 36.9</td>
<td>0.7 ± 11.6</td>
</tr>
<tr>
<td>Isokinetic (180°/s)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Boat 1</td>
<td>124.5 ± 36.3</td>
<td>133.3 ± 37.5</td>
<td>8.8 ± 20.3</td>
</tr>
<tr>
<td>Boat 2</td>
<td>134.1 ± 32.7</td>
<td>137.6 ± 31.1</td>
<td>4.1 ± 11.7</td>
</tr>
</tbody>
</table>

Data are means ± SD (n = 30). *Significantly different from bout 1 pre-exercise, bout 2 baseline, and pre-exercise values.
because the pre-exercise test was closer to the subsequent measurement time points, and thus minimizing the confounding effect from the familiarization process.

Figure 2A shows the percent change in isometric peak torque at 70° of knee flexion after two bouts of exercise. There were significant bout ($F_{1,29} = 21.23$, $P < 0.0001$), time ($F_{6,174} = 22.64$, $P < 0.0001$), and interaction ($F_{6,170} = 2.78$, $P = 0.013$) effects. The maximal isometric torque loss was observed at 24 h postexercise after both exercise bouts, decreasing by $42.1 \pm 4.3$ and $28.4 \pm 4.1\%$ in bout 1 and bout 2, respectively. Tukey's post hoc tests showed that isometric torque was significantly lower in bout 1 than in bout 2 at 24 h ($P = 0.034$), 72 h ($P = 0.002$), and 96 h ($P = 0.035$) postexercise. The isometric torque returned to pre-exercise levels at 96 h after exercise in bout 1 and 72 h after bout 2, suggesting a faster force recovery after bout 2. Figure 2B illustrates isokinetic knee extension peak torque at 60°/s. The analysis demonstrated significant bout ($F_{1,29} = 6.56$, $P = 0.016$), time ($F_{6,174} = 37.19$, $P < 0.0001$), and interaction ($F_{6,170} = 3.30$, $P = 0.004$) effects. The isokinetic torque at 60°/s returned to pre-exercise level at 120 h after exercise bout 1 and 72 h after bout 2, suggesting more rapid force recovery after bout 2. Tukey's post hoc tests showed that isokinetic torque at 60°/s was significantly ($P = 0.006$) less reduced in bout 2 (average 9.4% loss) than bout 1 (average 26.9% loss) at 72 h postexercise. Data for isokinetic peak torque at 180°/s are depicted in Fig. 2C. There were significant bout ($F_{1,29} = 13.36$, $P = 0.001$) and time ($F_{6,174} = 21.98$, $P < 0.0001$) effects but no significant interaction ($F_{6,170} = 1.77$, $P = 0.108$). Tukey's post hoc tests showed that isokinetic torque at 180°/s was significantly ($P < 0.01$) less reduced in bout 2 (average 8.9% loss) than bout 1 (average 20.9% loss) at 72 h postexercise.

Serum CK activity. There was a significant increase in CK activity over time ($F_{5,145} = 48.58$, $P < 0.0001$) after both bout 1 and bout 2 (Fig. 3). There were no significant differences in the CK increase between bout 1 and bout 2 ($F_{1,29} = 0.73$, $P = 0.398$), and there was no significant interaction ($F_{5,138} = 0.88$, $P = 0.496$). Of the 30 subjects who had complete samples for analysis, three were deemed to be outliers because CK levels increased more than three times the standard deviation (peak values of 5,992, 14,318, and 20,078 U/L, respectively). Excluding the outlier values did not change any of the results listed above to significant level.

Muscle soreness. Muscle soreness was evaluated pre-exercise (Pre) and every 24 h for 120 h following the two bouts of eccentric exercise (Fig. 4). There was a significant increase in soreness over time ($F_{5,150} = 88.73$, $P < 0.0001$). Muscle soreness peaked at 24 h after exercise and returned to pre-exercise levels at 96 h postexercise, independent of bout. There were no significant bout ($F_{1,30} = 1.13$, $P = 0.296$) or interaction ($F_{5,144} = 1.06$, $P = 0.387$) effects.

NF-κB (p65) DNA-binding activity. For the majority of subjects there was adequate tissue from the muscle biopsy samples to perform the NF-κB DNA-binding activity for both bout 1 and bout 2 ($n = 26$). There were no significant differences between the subcohort and whole cohort ($n = 31$) for age, height, weight, CK,
Fig. 4. Muscle soreness following two bouts of maximal eccentric exercise in opposite legs; n = 31 for each time point. Values are presented as means ± SD. *Significant change compared with pre-exercise values (P < 0.05).

The overall objectives of the current study are to examine the existence of contralateral RBE in leg muscles and determine the possible involvement of NF-kB in this phenomenon. Our results demonstrated that there was significantly less muscle strength loss in the contralateral leg after the second exercise bout, suggesting the existence of a contralateral RBE in our exercise model. Furthermore, we found an attenuated increase in NF-kB DNA-binding activity in the contralateral leg in bout 2 (ECC relative to CON, 109.1 ± 3.0%) compared with bout 1 (ECC relative to CON, 122.9 ± 2.6%).

DISCUSSION

The most commonly measured indirect markers for exercise-induced muscle damage are prolonged muscle strength loss, delayed onset muscle soreness, and blood CK levels (7). The eccentric exercise protocol used in this study effectively induced muscle damage as indicated by the significant time main effect (P < 0.0001) for all the measured indirect markers of muscle damage (strength loss, soreness, CK). These findings are in agreement with previous studies (3, 17, 19).

Strength response to the contralateral repeated bout of eccentric exercise. Muscle strength has been considered the best indirect marker for exercise-induced muscle damage because it is relatively accurate, reliable, and indicative of muscle function (44). Because muscle recovery may be also dependent on the type of movement (isometric or isokinetic) and/or angular velocity of muscle action, we measured both isometric and isokinetic strength of the quadriceps at two angular velocities (60 and 180°/s). For all the muscle strength variables, there was significant lower strength loss and faster strength recovery in the contralateral leg after bout 2. These results suggest that contralateral RBE exists in leg muscles regarding muscle strength loss in our exercise model.

To date, only two studies (16, 39) reported on the contralateral RBE, both of which were conducted on arm muscles (elbow flexors). Our results support these two studies and extend them to leg muscles. To the best of our knowledge, only one study (8) was conducted with the primary purpose to examine the existence of contralateral RBE in leg muscles. However, the authors in that study found no significant difference in isometric strength loss after both exercise bouts. The failure of this study (8) to observe contralateral RBE in leg muscles may be due to the relatively modest muscle damage stimulus (~10% muscle loss after exercise), which may have been insufficient to induce detectable levels of adaptation. In contrast, there was an ~40% loss in muscle strength after bout 1 in the current study. Although the primary goal was not to examine contralateral RBE, a study conducted by McHugh and Pasiakos (28) also provided data suggesting that contralateral RBE is not evident in leg muscles. It should be noted that in McHugh and Pasiakos’s study, the exercising range of motion was limited to 40° (either from 30 to 70° or from 70 to 110°), which may have been insufficient to induce a significant contralateral RBE. Furthermore, our data also indicate that the contralateral RBE is evident at least 4 wk after the initial bout; previous studies reported that the contralateral RBE was found after two wk. Taken together, the strength data presented in this study provide additional support for the existence of a contralateral RBE.

CK response to the contralateral repeated bout of eccentric exercise. We observed a similar increase in serum CK after both exercise bouts, suggesting no contralateral RBE for CK. This result does not concur with findings of Howatson and Van Someren (16), who reported that the CK increase was attenuated in the contralateral arm 96 h after a second exercise bout. One possible reason why we did not observe a contralateral RBE for CK in the current study may be due to the high
intersubject variability. High intersubject CK variability postexercise is observed frequently (6). Indeed, Connolly et al. (8), who were the first group that investigated the contralateral RBE, discarded their CK data from analysis for this reason. In the current study, some subjects had very high postexercise CK levels (e.g., 20,078 U/l), whereas others displayed only a relatively modest increase (e.g., 199 U/l). Even after we excluded the data from the three outliers, the standard deviation of CK was still large and represented almost one-half of the mean values for most time points postexercise in bout 1 and even higher in bout 2.

Soreness response to the contralateral repeated bout of eccentric exercise. In the three studies that primarily investigated the contralateral RBE (8, 16, 39), muscle soreness/pain was attenuated in the contralateral limb after the second exercise bout. In contrast, our data failed to demonstrate a significant difference in muscle soreness between bout 1 and bout 2. Similar to the three published studies, we used theVAS to evaluate muscle soreness. The peak average muscle soreness after bout 1 observed in our study was close to the magnitude of soreness/pain after bout 1 in the three previous studies (8, 16, 39). A major difference between the current study and the three earlier reports is that we included muscle biopsies in our study. The measurement of muscle soreness via the VAS is subjective and therefore susceptible to any factor that could affect the subjects’ ability to perceive and evaluate the soreness level accurately. The discomfort from the muscle biopsy may be a confounding factor in the measurement of muscle soreness in our study, hindering our ability to detect a RBE. The possible confounding effect of the biopsy is supported by our observation that soreness peaked at 24 h postexercise, whereas other studies consistently show that soreness typically peaks at ~48 h postexercise.

NF-κB DNA-binding activity response to the contralateral repeated bout of eccentric exercise. We observed that NF-κB DNA-binding activity was significantly higher in the ECC leg compared with CON leg in both bout 1 and bout 2, suggesting that NF-κB was activated postexercise in the activation of NF-κB following exercise has been consistently demonstrated in rodent models (14, 20, 26) as well as human studies using peripheral blood lymphocytes (12, 21). However, the literature regarding the effect of exercise on NF-κB activity in human skeletal muscle is still limited, and the results from different studies are equivocal (9, 18, 40, 43). Therefore, the observed increase of NF-κB DNA-binding activity posteccentric exercise in the current study reinforces acute exercise as a stimulus for NF-κB activation.

An acute inflammatory response after strenuous eccentric exercise has been suggested to contribute to secondary muscle damage and delay the regenerative processes (37). Accumulating evidence suggests that NF-κB is a critical transcriptional regulator in the acute inflammatory response following exercise (5, 23). NF-κB is a ubiquitously expressed transcription factor and regulates the expression of ~150 genes, many of which encode pro-inflammatory mediators such as inducible nitric oxide synthase (iNOS), IL-6, IL-8, IL-1β, and MCP-1 (32). Concomitant NF-κB activation and upregulation of pro-inflammatory genes following exercise has been reported in both rodent (5, 25, 26) and human studies (18, 21, 43). Inasmuch as pro-inflammatory cytokines can increase protein degradation (24, 41, 42), it is conceivable that activated NF-κB expression following strenuous exercise may contribute to secondary muscle damage via upregulating proinflammatory genes.

Given the importance of NF-κB in the acute inflammatory response that contributes to secondary muscle damage after eccentric exercise, we speculate that the diminished activation of NF-κB in the contralateral leg in bout 2 may contribute to the observed contralateral RBE in the current study. Although there are no data to demonstrate a cause-effect link between attenuated activation of NF-κB and the contralateral RBE, there is indirect evidence to support that inhibition of NF-κB activation is associated with reduced muscle damage. Genetic ablation of NF-κB activation in mice improved muscle regeneration and limited infiltration of inflammatory cells into damaged muscle (1, 29). Similarly, administration of antioxidant such as Honokiol (5) or melatonin (2) in rats reduced exercise-induced muscle damage parallel with attenuated NF-κB activation and downregulation of proinflammatory genes regulated by NF-κB. Furthermore, 6 or 8 wk of a human eccentric exercise training program attenuated exercise-induced NF-κB activation and reduced muscle damage (12, 21). Collectively, these data suggest that the attenuated NF-κB may contribute to less muscle strength loss in the contralateral leg in bout 2.

It should be pointed out that the contralateral leg was not exposed to exercise in the initial bout and thus there was no direct stimulus for molecular and cellular adaptation such as reduced NF-κB activation in the contralateral leg. Therefore, the attenuated NF-κB may not be a driving or independent mechanism for the contralateral RBE in the current study. Instead, NF-κB may be an effecter of an upstream mechanistic pathway that could be transferred to the nonexercising contralateral leg muscles. Neural adaptation is most probably the candidate mechanism upstream of NF-κB, because previous studies (15, 39) demonstrated that neural adaptation is critical for the contralateral RBE. In humans, performing unilateral lengthening actions of left wrist flexor resulted in an increase in the corticospinal excitability and almost abolished the intracortical and interhemispheric inhibition for the contralateral relaxed right wrist flexor muscle (15). In addition, EMG data (39) showed that there was an increased recruitment of slow-twitch motor units in the contralateral muscle during the repeated bout of eccentric exercise. Because fast-twitch fibers have been shown to be more susceptible to eccentric contraction-induced damage (11, 22), the shift from fast-twitch to slow-twitch motor unit recruitment can result in less initial muscle damage during and immediately after the exercise bout. As a result, a smaller inflammatory response is initiated, which may include the attenuated NF-κB activation. From this perspective, the attenuated NF-κB activation is just a consequence not the cause of the contralateral RBE. Regardless if attenuated NF-κB is an independent or effecter mechanism, NF-κB may be an important mediator or synergist in the observed contralateral RBE.

There are several limitations to this study, and thus caution should be taken while interpreting findings observed in this study. First, although we did not find significant differences between the supplement and placebo groups for all the measured variables, the supplements may have had a small non-significant confounding effect. Second, we did not recruit an ipsilateral control group to avoid confounding effects of the well-documented ipsilateral RBE while examining the effects
of the supplements on muscle recovery post-damaging exercise. Third, the subjects performed the two exercise bouts with 4 wk apart so that it is long enough to observe the possible effects of the supplements. However, the contralateral RBE might be more profound if the second exercise bout was performed sooner after the first bout, and that might be part of the reason why we did not detect a contralateral RBE regarding CK or soreness. Finally, muscle biopsies could also be a confounding factor as aforementioned. Future studies can probably recruit a control group of subjects who will only have muscle biopsies without exercising at all.

Summary

The current study demonstrated that contralateral RBE exists in knee extensors after maximal eccentric exercise and may have implications in clinical and rehabilitation settings. For example, remobilization of a weakened or injured limb produces muscle damage (36) that can impede rehabilitation. Consequently, the contralateral RBE in legs can be taken advantage of when designing a rehabilitation plan. If one leg of a patient was immobilized because of an injury or a disease, eccentric exercise of the healthy leg before rehabilitation begins for the immobilized limb may provide protection against exercise-induced muscle damage for the immobilized leg during rehabilitation exercise, thereby facilitating recovery from injury. Moreover, a contralateral RBE must be considered in studies that make use of alternate limbs for the investigation of interventions to reduce exercise-induced muscle damage, and it may be more appropriate to use a between-subjects design rather than a within-subjects design. More importantly, our findings for the first time suggest that NF-κB may be involved in the contralateral RBE and point to a mechanistic basis for the contralateral RBE. The identified involvement of NF-κB in the contralateral RBE may be important in the development of interventions to enhance contralateral RBE when setting up rehabilitation exercise for a unilaterally immobilized limb and in identifying targets of future therapies to facilitate recovery from injury.

ACKNOWLEDGMENTS

The authors thank Justin Keadle, Karen Riska, Brittany Rahmberg (University of Massachusetts Amherst, MA) and Valerie Greger and Karen Shaver (Interleukin Genetics for support of this study. We also thank Dr. Lawrence M. Schwartz, Dr. Maria Urso, and Dr. Monica Hubal for their careful review of the manuscript.

REFERENCES


