Cellular adaptation to repeated eccentric exercise-induced muscle damage

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Stupka, N., M. A. Tarnopolsky, N. J. Yardley, and S. M. Phillips. Cellular adaptation to repeated eccentric exercise-induced muscle damage. J Appl Physiol 91: 1669–1678, 2001.—Eccentrically biased exercise results in skeletal muscle damage and stimulates adaptations in muscle, whereby indexes of damage are attenuated when the exercise is repeated. We hypothesized that changes in ultrastructural damage, inflammatory cell infiltration, and markers of proteolysis in skeletal muscle would occur about as a result of repeated eccentric exercise and that gender may affect this adaptive response. Untrained male (n = 8) and female (n = 8) subjects performed two bouts (bout 1 and bout 2), separated by 5.5 wk, of 36 repetitions of unilateral, eccentric leg press and 100 repetitions of unilateral, eccentric knee extension exercises (at 120% of their concentric single repetition maximum), the subjects’ contralateral nonexercised leg served as a control (rest). Biopsies were taken from the vastus lateralis from each leg 24 h postexercise. After bout 2, the postexercise force deficit and the rise in serum creatine kinase (CK) activity were attenuated. Women had lower muscle macrophages were elevated in men and women after bout 1 and bout 2 (P < 0.05). Muscle protein content of women was significantly greater in women 24 h after bout 2 vs. rest and bout 1 (P < 0.05), but there were no gender differences in the relative magnitude of the force deficit. Muscle Z-disk streaming, quantified by using light microscopy, was elevated vs. rest only after bout 1 (P < 0.05), with no gender difference. Muscle neutrophil counts were significantly greater in women 24 h after bout 2 vs. rest and bout 1 (P < 0.05) but were unchanged in men. Muscle macrophages were elevated in men and women after bout 1 and bout 2 (P < 0.05). Muscle protein content of the regulatory calpain subunit remained unchanged whereas ubiquitin-conjugated protein content was increased after both bouts (P < 0.05), with a greater increase after bout 2. We conclude that adaptations to eccentric exercise are associated with attenuated serum CK activity and, potentially, an increase in the activity of the ubiquitin proteosome proteolytic pathway.

Z-disk streaming; inflammatory cells; proteolysis; gender-based difference

The primary purpose of this study was to investigate the effect of repeated eccentric exercise on cellular adaptations in indexes of damage and the intracellular pathways responsible for proteolysis (i.e., calcium-activated neutral proteases such as calpain and the ATP-dependent ubiquitin pathway) and also to examine the postexercise inflammatory response as a means of quantifying the potential for extracellular (i.e., lysosomal) proteolysis. We hypothesized that changes in cellular damage, the inflammatory response, and proteolytic pathways would be characteristic of the adaptation to repeated performance of eccentric contractions. Given the apparent reduction in contraction-induced damage that occurs with repeated bouts (12, 18) of eccentric exercise, we hypothesized that we would observe a reduction in the exercise-induced initiation of the both the intracellular and extracellular (inflammatory) proteolytic pathways. A further rationale for studying the adaptive responses of these parameters was that the

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Materials and Methods

Subjects. Healthy, nonsmoking male (n = 8) and female (n = 8) participants volunteered to take part in the study and gave written, informed consent to all procedures before participating. The study was approved by the McMaster University Research Ethics Board. None of the subjects had participated in a regular, structured exercise program for at least 6 mo before participating in the study. Five of eight female subjects were taking oral contraceptives; however, all subjects were tested as close as possible to the midfollicular phase of the menstrual cycle (7–11 days after menses; mean = 9 ± 3 days).

Testing protocol. Two weeks before beginning the study, subjects reported to the testing lab for a familiarization session in which they were acquainted with the isokinetic dynamometer (Biodex Medical Systems, Shirley, NY) and other testing equipment. Subjects had their single repetition maximum (1 RM; the maximum weight that can be lifted in one repetition) determined while performing a unilateral (i.e., using only one of their legs) leg press and unilateral knee extension. Subjects’ 1 RM was first estimated from the values and procedures described by Mayhew et al. (21). Once a predicted 1 RM was determined, the actual 1 RM was determined, usually with a single effort. A second measure of 1 RM was also included before the second bout of eccentric contractions (6–7 wk after the initial 1 RM test), but there was no significant difference found from the initial 1 RM (change = +8 ± 9%, P = 0.91).

On the testing day, subjects reported to the laboratory, where they had a baseline venous blood sample taken before engaging in 10 min of light (75 W) cycle ergometry. After completing the cycling, subjects were seated in the isokinetic dynamometer so that the peak isokinetic torque of the knee extensors could be determined. Subjects were given three opportunities to achieve their peak torque; the amount of rest between each effort was not held constant but was at least 20 s. The maximum torque of the three efforts, for both legs, was taken as the subject’s maximum torque. Concentric torque at 30 and 180°/s and eccentric torque at 30°/s were determined.

After the preliminary baseline warm-up session, the subjects performed a series of resistance exercises designed to elicit muscle damage of the knee extensor muscle group. Subjects performed all exercises using the weakest leg while the contralateral leg acted as nonexercised control (rest). The first set of resistance exercises used a standard leg press machine (Nautilus) and required that the subjects lower a mass equivalent to 120% of their predetermined unilateral concentric 1 RM. To perform an eccentric muscle action, subjects were seated with the entire leg at 90° relative to the torso and knees flexed to ~90° while they had the weight lifted for them by the investigators, so that the leg was at ~15° off flexion, after which they lowered the weight through an arc of ~75° (i.e., back to ~90°). Each subject performed 36 (5 sets × 12 repetitions per set) eccentric muscle actions using the leg press machine with 3 min rest between each set. Subjects were required to lower the weight at a fixed cadence (1 s to raise the weight and then 2 s to lower the weight) verbally given by an investigator. Subjects then performed 100 eccentric muscle actions (10 sets × 10 repetitions per set) using the same leg on a standard knee extension machine (Nautilus). Again subjects were required to lower a weight, lifted for them by the investigators, equivalent to 120% of their predetermined unilateral 1 RM. Subjects sat on the apparatus with the thigh at 90° relative to the torso and the knee at 90° relative to the thigh. Subjects performed flexion of the knee through an arc of ~75°. The starting position for this maneuver required the subjects to remain seated while holding the knee at ~15° from horizontal and to lower the weight to a point that resulted in the knee being flexed at ~90°. The same lifting cadence was maintained to assure a relatively constant lowering velocity, and subjects were verbally encouraged to maintain their effort throughout the range of motion. If subjects were deviating from the required lifting cadence (as a result of fatigue), then a brief (30 s) rest was allowed during the set, so that the subject could complete the set while still giving an appropriate effort. A maximum of two rests per set was allowed. Subjects also had 3 min of rest between each set. Each subject experienced some degree of fatigue during the weight lowering, but all subjects were able to complete the protocol. We acknowledge that it is possible that the subjects could have lowered the weight at slightly different velocities when they were becoming fatigued; however, to control for this we maintained a constant timing during the lowering motion. It appeared that all subjects maintained a high level of effort, in that they maintained a constant rate at which the load was lowered. After cessation of the resistance exercise, subjects rested for 30 min before maximum concentric isokinetic peak torque at velocities of 30 and 180°/s and eccentric torque at 30°/s were determined by using the isokinetic dynamometer, as described above.

Subjects reported back to the testing center 24 h after performance of the resistance exercise to have their concentric (30°/s, 180°/s) and eccentric isokinetic (30°/s) peak torque determined, as described above. Subjects also had a venous blood sample drawn. At this time muscle biopsies were taken from the vastus lateralis of both the nonexercised (rest) and exercised legs (bout 1) for the determination of muscle damage, Western blotting, and histochemical characteristics. Briefly, subjects’ skin was anesthetized with 2% lidocaine, and a small (~4–5 mm) incision was made ~20–25 cm proximal to the knee on the lateral aspect of the lateral thigh. A 5-mm Bergströms biopsy needle custom modified for manual suction was used for muscle sampling. The muscle biopsy (~70–120 mg) was blotted with a gauze pad to remove any
excess blood and was dissected free of any visible adipose or connective tissue. A small (~5–10 mg) longitudinal section of each muscle biopsy was immediately placed into 2% glutaraldehyde for determination of muscle damage and was stored at 4°C until processing. Another portion (~50 mg) of the muscle biopsy was placed in liquid N2 and stored at -80°C for analysis of protein content by Western blotting. A further piece of muscle, determined by visual analysis to have intact fibers, was oriented in cross section in embedding medium (optical cutting temperature) and quick frozen in isopentane cooled by liquid N2 and stored at -50°C before histochemical analysis. Preliminary histochemical, immunohistochemical, and Western blotting analysis (n = 5 subjects) revealed no significant differences between resting samples for any of the parameters examined (data not shown). Hence, the rest muscle samples taken from the nonexercised leg after the first (bout 1) and second (bout 2) were assumed to be equivalent.

Subsequently, subjects reported back to the testing center at 48 h, 96 h, and 7 days after the performance of the resistance exercise bout, during which time they had a venous blood sample taken and had their concentric (30°/s, 180°/s) and eccentric (30°/s) peak isokinetic torque determined.

To examine the effect of a previous bout of eccentric resistance exercise on a subsequent bout of eccentric exercise (bout 2), subjects returned to the testing center 5–6 wk after performing the initial eccentric resistance exercise bout to repeat the entire protocol, including strength testing, blood samples, and muscle biopsies. Between exercise bouts, subjects refrained from doing any strenuous physical activity.

**Blood.** Venous blood samples were collected into vacutainers (Becton Dickinson), allowed to clot, and then centrifuged at 10,000 RPM (at 4°C) for ~10 min. The serum was stored at -20°C until analysis. Serum was assayed spectrophotometrically for CK by use of a commercially available kit (Sigma Diagnostics). All samples were run in triplicate, and the coefficient of variation was <10% for each sample. Crude muscle homogenate was stored in aliquots at -80°C until analysis, and care was taken to minimize the number of freeze-thaw cycles. Proteins were separated on a 10% SDS-polyacrylamide separating gel and a 4% SDS-polyacrylamide stacking gel; 40 μg of protein was run in each lane. Rest, bout 1, and bout 2 samples for a male and female subject were always run on the same gel, along with a broad-range molecular weight standard (161-0319, Bio-Rad). The gels were run with the power supply set at 100 V for 1 h at room temperature.

Gels stained for the 30-kDa regulatory subunit of calpain were transferred to polyvinylidene difluoride membranes (Bio-Rad) for 1 h, at room temperature, with the power supply set at 100 V. Gels stained for ubiquitin-conjugated proteins were transferred overnight, on ice, at 25 V. The polyvinylidene difluoride membranes were blocked with 9% gelatin dissolved in Tris-buffered saline with 0.1% Tween (TTBS; 500 mM NaCl, 20 mM Tris HCl, pH 7.5) for 1.5 h.

The 30-kDa regulatory subunit of calpain antibody (7530, Santa Cruz Biotechnologies, Santa Cruz, CA) was diluted to 1:500 in TTBS, and the ubiquitin antibody (UBIQm, Nova-cesta Laboratories, New Castle Upon Tyne, UK) dilution was 1:50 in TTBS. Blots were incubated with the primary antibody overnight. Blots for calpain were incubated for 1.5 h with a secondary donkey anti-goat antibody with a conjugated biotinylated-streptavidin alkaline phosphatase enzyme (2020, Santa Cruz), which was diluted 1:3,000 in TTBS. Blots for ubiquitin were incubated for 1.5 h with a secondary donkey anti-mouse antibody with a biotinylated-streptavidin alkaline phosphatase-conjugate substrate kit with 5-bromo-4-chloro-3-indolyl phosphate and nitroblue tetrazolium (170-6432, Bio-Rad).

Blots were digitized by using a scanner, and a computerized image analysis system was used to determine the band...
density (arbitrary units) and molecular weight using known molecular weight standards. Raw digitized values were used in all statistical analyses. For quantification of ubiquitin, all bands that showed a consistent and measurable positive signal for ubiquitin were digitized, and the results were analyzed as the sum of all ubiquitin-containing bands (see Fig. 6A). Each blot was run in an identical fashion with three lanes containing muscle from the three conditions (rest, bout 1, and bout 2), and both a male and female subject were run at the same time on each blot. Results are presented normalized to rest to give a clear idea of condition-dependent (rest, bout 1, and/or bout 2) changes in protein expression within a subject after exercise; the density value of the digitally determined values for bout 1 and bout 2 are expressed relative to rest. Hence, in this analysis, all rest band densities are shown as unity.

Statistics. All data were analyzed by use of repeated-measures ANOVA procedures with appropriate levels, when applicable (gender: male or female; condition: rest, bout 1, and bout 2; and time: 30 min, 24 h, 48 h, 96 h, and 7 days), and degrees of freedom. Data that did not conform to a normal distribution were log transformed (serum CK), and the log-transformed data were analyzed by ANCOVA to account for baseline differences between genders. A Newman-Keuls post hoc test was used to locate pairwise significant differences, when appropriate. In analyses in which no gender-based difference (neither a main effect nor a significant interaction) was observed, then the data were collapsed across gender and only condition- and/or time-specific effects were analyzed. Observed power for the statistical comparisons was also computed by using $\alpha = 0.05$, for some variables, for both main effects and interactions. The level of significance was set to $P < 0.05$. All data in texts, graphs, and tables are presented as means $\pm$ SD.

RESULTS

Subject characteristics. The subjects’ descriptive characteristics are presented in Table 1. The men were significantly taller and heavier and had greater 1-RM values for leg press and knee extension exercise ($P < 0.05$).

Force data. A main effect for gender ($P < 0.05$) was noted when absolute torque was analyzed, as one would predict. However, we analyzed the torque values as relative changes (normalized to preexercise values) and found no significant gender-by-time interaction ($P = 0.56$); hence, we have collapsed the data across gender and present only the relative differences with respect to time (see Fig. 1). In addition, there were no significant differences between trials (bout 1 and bout 2) in absolute peak torque before the eccentric protocol for any contraction speed (30 or 180/s) or type (eccentric or concentric). Men: before bout 1 = 239 $\pm$ 38, 171 $\pm$ 35, and 285 $\pm$ 56 N·m, 30/s concentric, 180/s concentric, and 30/s eccentric, respectively; before bout 2 = 242 $\pm$ 37, 179 $\pm$ 38, and 293 $\pm$ 64 N·m, 30/s concentric, 180/s concentric, and 30/s eccentric, respectively. Women: before bout 1 = 157 $\pm$ 22, 113 $\pm$ 21, and 189 $\pm$ 23 N·m 30/s concentric, 180/s concentric, and 30/s eccentric, respectively; before bout 2 = 164 $\pm$ 21, 120 $\pm$ 21, and 197 $\pm$ 29 N·m 30/s concentric, 180/s concentric, and 30/s eccentric, respectively). There was a significant time-by-bout interaction ($P < 0.05$) observed for concentric torque at both speeds and also for eccentric torque (see Fig. 1). As expected, the eccentric

Table 1. Subject characteristics

<table>
<thead>
<tr>
<th></th>
<th>Women</th>
<th>Men</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, yr</td>
<td>23.2 $\pm$ 2.0</td>
<td>22.2 $\pm$ 2.0</td>
</tr>
<tr>
<td>Height, cm</td>
<td>168.4 $\pm$ 4.9$^*$</td>
<td>178.3 $\pm$ 8.1</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>65.1 $\pm$ 7.9$^*$</td>
<td>82.5 $\pm$ 10.3</td>
</tr>
<tr>
<td>Leg press 1 RM, kg</td>
<td>69.7 $\pm$ 14.8$^*$</td>
<td>112.5 $\pm$ 18.3</td>
</tr>
<tr>
<td>Knee extension 1 RM, kg</td>
<td>21.9 $\pm$ 4.6$^*$</td>
<td>36.6 $\pm$ 5.3</td>
</tr>
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Values are means $\pm$ SD ($n = 8$). 1 RM, one-repetition maximum.

$^*$Significantly different from men ($P < 0.05$).
cally biased contraction protocol resulted in a significant immediate loss in force-generating capacity for all velocities and contraction types (bout 1 = −31 ± 9% and bout 2 = −32 ± 5%, 30°/s concentric; P < 0.05) in isokinetic force-generating capacity after both bouts. This fatigue was prolonged and lasted until 96 h postexercise (−17 ± 8%, 30°/s concentric; P = 0.05) after bout 1. By 7 days postexercise, however, force was restored to baseline levels after bout 1. After bout 2, force had returned to baseline levels at 48 h (−11 ± 9%, 30°/s concentric; Fig. 1). Similar results for the maximal isokinetic torque measured at 30°/s concentric were seen when torque was measured at 180°/s concentric and at 30°/s eccentric. As Fig. 1A shows, however, maximal isokinetic torque changed with a slightly different time course for 30°/s concentric vs. both 180°/s concentric (Fig. 1B) and 30°/s eccentric (Fig. 1C). Force at 96 h postexercise was not significantly different from preexercise values (−13 ± 13%, 180°/s concentric; −14 ± 15%, 30°/s eccentric). Similarly, to that seen for 30°/s concentric, force recovered more rapidly after bout 2, returning to baseline levels at 48 h for both 180°/s concentric and 30°/s eccentric (−13 ± 9%, 180°/s concentric; −14 ± 19%, 30°/s eccentric). Post hoc power analysis revealed an observed power (α = 0.05) for a main (bout) effect of 0.92 (30°/s concentric) to 0.86 (30°/s eccentric), a time effect of 0.92 (30°/s concentric) to 0.87 (30°/s eccentric) and a time-by-bout interaction 0.82 (30°/s concentric) to 0.78 (30°/s eccentric).

**Serum CK activity.** In analysis using ANCOVA to account for baseline differences, there was a significant gender-by-time-by-bout interaction (P < 0.05) for serum CK. After bout 1, serum CK activity increased significantly over baseline values at 48 h [men = +857 ± 920% vs. preexercise (Pre), range = 333–2075 U/l; women = +524 ± 182% vs. Pre, range = 139–566 U/l; P < 0.05], 96 h [men = +939 ± 930% vs. Pre, range = 302–2,110 U/l; women = +1,006 ± 1,003%, range = 229–1,376 U/l; P < 0.05], and at 7 days postexercise, but only for the men [men = +314 ± 432% vs. Pre, range = 159–909 U/l; P < 0.05). After the second exercise bout, serum CK activity was different from resting values at both 48 h (men = +528 ± 388% vs. Pre, range = 243–635 U/l; women = +461 ± 248% vs. Pre, range = 139–290 U/l; P < 0.05) and 96 h postexercise (men = +428 ± 393% vs. Pre, range = 137–1,078 U/l; women = +754 ± 445% vs. Pre, range = 172–545 U/l; P < 0.05). Serum CK activity was lower 48 h, 96 h, and 7 days postexercise after the second exercise bout compared with the first in both men and women (P < 0.05; see Fig. 2). When CK values were normalized relative to body weight, a gender-by-time-by-bout interaction was also still evident; hence, statistically speaking, very similar results were observed (results not shown). Post hoc power analysis revealed an observed power (α = 0.05) for a main (bout) effect of 0.88, for a time effect of 0.92, and for a gender effect of 0.82.

**Muscle damage.** There was no difference, either a main effect or an interaction, between genders in the extent of Z-disk streaming at any time point. Figure 3 presents data collapsed across gender. There was more Z-disk streaming in the exercised leg compared with the control leg after bout 1 (P < 0.05). After bout 2, the amount of Z-disk streaming tended to be lower vs. bout 1, but this difference did not reach statistical significance (see Fig. 3). However, there was also no significant difference in the amount of Z-disk streaming in the exercised leg vs. rest after bout 2. Post hoc power analysis revealed an observed power (α = 0.05) for a main (exercise) effect of 0.64 and an exercise-by-gender interaction of 0.39.

**Immunohistochemistry (neutrophil and macrophage infiltration).** The number of neutrophils per square millimeter of tissue showed a significant interaction between gender and condition (P < 0.05). The number of neutrophils per square millimeter of tissue did not increase significantly over baseline values in men, 24 h after bout 1 nor bout 2, despite an almost fourfold increase over rest after each bout. In women, the number of neutrophils per square millimeter of tissue was

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**Fig. 2.** Serum creatine kinase (CK) activity (log scale; see MATERIALS AND METHODS for description of statistical procedures). *Significantly different vs. Pre bout 1 (P < 0.05); +significantly different vs. Pre bout 2, but different from bout 1 (P < 0.05); §significantly different vs. same time point from bout 1 (P < 0.05). Values are means ± SD (n = 8).

**Fig. 3.** Extensive Z-disk streaming (see MATERIALS AND METHODS for definition) per square millimeter of tissue. Data were collapsed across gender. *Significantly different vs. Rest (P < 0.05). Values are means ± SD (n = 16).
significantly greater after bout 2 compared with bout 1 and rest ($P < 0.05$). Furthermore, women had greater neutrophil counts than men after bout 2 ($P < 0.05$; see Fig. 4). The number of macrophages per square millimeter of tissue was greater (almost twofold) in women after bout 2 compared with bout 1 ($P = 0.11$), whereas the number of macrophages was similarly elevated above rest values after both bouts of exercise in men (see Fig. 5). Post hoc power analysis revealed an observed power ($\alpha = 0.05$) for a main (bout) effect of 0.87 [myeloperoxidase-positive (MPO+) cells] and 0.83 (CD68+ cells) and an exercise-by-gender interaction of 0.56 (MPO+ cells) and 0.55 (CD68+ cells).

**Ubiquitin-conjugated proteins.** Ubiquitin-conjugated protein content showed a main effect for time ($P < 0.05$), being elevated over rest values after bout 2 in both men and women ($P < 0.05$; see Fig. 6B). Post hoc power analysis revealed an observed power ($\alpha = 0.05$) for a main (bout) effect of 0.72 and an exercise-by-gender interaction of 0.51.

**The 30-kDa regulatory calpain subunit.** The regulatory calpain subunit protein content did not change in response to exercise (see Fig. 7B). There was no gender-related difference in calpain expression at rest nor after exercise (see Fig. 7C). Post hoc power analysis revealed an observed power ($\alpha = 0.05$) for a main (exercise) effect of 0.56 and an exercise-by-gender interaction of 0.22.

**DISCUSSION**

The results of the present study indicate that a single bout of eccentric exercise (bout 1) induced adaptations in skeletal muscle that resulted in a reduced force deficit and attenuated CK release after a second bout (bout 2) and changes in the inflammatory response along with increases in ubiquitin-conjugated protein content. Despite the apparent “protective” effect of bout 1 on CK release and the force deficit, after bout 2 there was no difference between the amount of Z-disk streaming observed after bout 2 vs. bout 1. Gender differences in the response of serum CK activity, muscle inflammatory cell infiltration, and ubiquitin-conjugated protein content are more pronounced after bout 2. These results highlight the importance of understanding the complex interplay between exercise and gender on muscle adaptation and recovery.
the force loss seen at 30°/s were obtained from eccentric previous results (18), quantitatively similar results for to ascertain why this was the case. In agreement with modes to induce damage (18); however, we are unable smaller muscles (biceps; Ref. 7) and different exercise smaller than that seen in other studies that have used torque (30°/s) was depressed at all time points up to attenuation or the contraction type (eccentric or concentric; regardless of the velocity (30°/s or 180°/s) of the contrac-
tion). Attenuation of the exercise-induced force deficit after repeated eccentric exercise has been well documented (7, 12, 18); however, the mechan-
ism mediating this adaptation remains to be elucidated but is almost certainly related to whatever adap-
tations occur to reduce the degree of sarcomeric disruption, and likely the disruption of the excitation-
contraction coupling process, resulting from exercise (12, 25, 33, 34).

Decreased ultrastructural disruption and changes in inflammatory response may have contributed to decreased serum CK activity after the second exercise bout. It has been suggested that the blunted CK response is due, at least in part, to an enhanced rate of clearance (19), and this may have contributed to the present results. Although we observed an attenuated rise in CK after bout 2, there was still a significant elevation above resting (Pre) values (Fig. 2). This finding is in contrast to numerous previous studies (see Refs. 7, 8, and 11 for reviews) in which no rise in CK has been observed after a second bout of eccentrically biased exercise. It is possible that differences in the eccentric exercise protocol and/or muscle groups used contributed to our findings. Nonetheless, serum CK activity is, at best, a very indirect and nonspecific index of damage and does not correlate with other indexes of muscle damage (23); hence, it is essential to evaluate adaptations to repeated eccentric exercise by using a variety of indexes of damage. Similar to the present results, serum CK activity at rest and during training has been previously reported to be lower in women compared with men (1, 3). In male rats, administration of 17β-estradiol can attenuate increases in plasma CK activity after a 2-h treadmill run (3). A blunted CK response in women may be due to the antioxidant properties of 17β-estradiol (2, 32). Others have noted a lower CK release in women compared with men in response to strenuous training (10). It should, however, be emphasized that not all studies have shown a benefi-
cial effect of estradiol administration and that, at least in mice, estradiol can actually increase the sus-
ceptibility to injury (35).

The amount of ultrastructural damage, as character-
ized by Z-disk streaming, was elevated vs. rest after bout 1 only. After bout 2, Z-disk streaming was not statistically different from rest values. However, the lack of a significant difference in the magnitude of Z-disk streaming in the exercised leg after bout 1 vs. bout 2 weakened the strength of this finding. It has been questioned whether the degree of damage seen in a single biopsy is representative of the muscle as a whole (34), which is a valid question. We have prelimi-
ary data that indicate the variability between biopsy sites (within the same leg) in Z-disk streaming can be considerable (coefficient of variation >40%; L. J. Beaton and S. M. Phillips, unpublished observations). It is certain that this variability, in combination with a small sample size, contributed to the present results.
and possibly to our inability to detect a significant reduction in Z-disk streaming after bout 2, which is a hallmark of the adaptive response to repeated eccentric contractions (12). Our observations of Z-disk streaming are in agreement with earlier research showing a relatively large reduction in myofibrillar disruption after repeated, eccentric quadriceps exercise, but which was reported not be significantly different because of the small sample size used in the previous research (18). The precise mechanism mediating a reduction in sarcomeric disruption and Z-disk streaming after repeated eccentric exercise is not known but may be related to a reduction in sarcomere length, which would reduce strain (12). Alternatively, a neurally mediated change in muscle recruitment patterns could account for the reduction in damage (33, 34). However, it is possible that a reduction in Z-disk streaming could reflect an attenuation of calpain activity because the Z-band-associated proteins are preferred substrates of this proteolytic pathway (4, 5). We did not see any change in calpain content (Fig. 7), which may not be surprising given the relatively late time points at which we obtained biopsies (calpain activity has been shown to be elevated early after eccentric exercise and then return to baseline by 24 h; e.g., Ref. 4); however, it would be interesting to see whether calpain (µ and m) activities are affected by repetition of eccentric exercise, possibly because of a reduced cytosolic calcium concentration, although such data may be difficult to obtain in humans.

We have previously shown that the extent of Z-disk streaming was similar between genders 48 h postexercise (30). Together with the findings from the present study, a lack of any gender difference in Z-band streaming may seem contradictory to reports of greater discontinuous dystrophin staining and desmin desolution in male rodents compared with female rodents 6 h and 48 h after a bout of downhill running (20). However, species differences, along with a greater oxidative stress experienced during running (20) vs. more resistance-based exercise (present study), may contribute to the disparity between the present and previous studies (20). We were unable to detect discontinuous dystrophin staining nor significant desmin loss in any muscle samples from the present or previous (30) study (unpublished observations).

Exercise-induced muscle damage stimulates an acute-phase inflammatory response, which includes infiltration into skeletal muscle by neutrophils and macrophages (13). Neutrophil infiltration follows a relatively rapid time course, peaking relatively early in the postexercise period (14); therefore, it is possible that in men neutrophils may have already returned to baseline levels by 24 h postexercise or were simply not stimulated to infiltrate the muscle. The increase in infiltrating neutrophils seen in women after the second exercise bout may be due to a change in the timing of the inflammatory response. In support of this notion, peak ER-BMDM1 leukocyte (a macrophage subset) infiltration is delayed in female mice compared with male mice after an acute bout of exercise (28). Different time courses of inflammatory cell responses have also been observed in circulating inflammatory cells after repeated eccentric exercise (27). These results (27, 28) lend some support to the idea that part of the adaptive response to repeated eccentric exercise is mediated by a different time course of inflammatory cell activation.

Muscle macrophage counts were elevated after exercise bouts 1 and 2 in both men and women. This was expected, because the time course for macrophage infiltration is slower than that for neutrophils. The number of macrophages per square millimeter of tissue in women tended to double after the second exercise bout compared with the first, whereas in men macrophage counts were similarly elevated after both bouts. Although the changes in macrophage cell infiltration were not significant, it does lend support to our hypothesis that adaptations to repeated eccentric exercise are characterized, in part, by changes in the time course of inflammatory cell infiltration (27). To fully investigate this hypothesis, however, a time course study would be needed.

Ubiquitin-conjugated protein content was elevated over resting values 24 h after the second exercise bout, but not the first. Ubiquitin binds covalently to damaged or abnormally folded proteins and targets them for degradation by the 20S proteosome via the ATP-dependent ubiquitin proteosome pathway (9). Increased free ubiquitin and ubiquitin-conjugated protein expression have been detected in humans 48 h after an acute bout of eccentric elbow flexor exercise (31). It is possible that 24 h after the initial exercise bout may not have been enough time to detect increases in ubiquitin-conjugated protein content. Moreover, there may not be a tight relationship between ubiquitinated protein content and proteolysis via the proteosomal pathway (31).

After the second exercise bout, we had not expected to see an increase in ubiquitin-conjugated protein content. Elevated ubiquitin-conjugated proteins after the second exercise bout suggests that in response to repeated eccentric exercise the kinetics of ubiquitin proteosome pathway may be changed. An alternative hypothesis is that after repeated eccentric exercise a more “regulated” form of intracellular proteolysis occurs, mediated via the ATP-dependent ubiquitin proteosome pathway as opposed to a relatively non-directed inflammatory cell-mediated extracellular proteolysis. Increased ubiquitin-conjugated protein content at 24 h may indicate a more efficient removal of damaged contractile and cytoskeletal proteins and thus may promote regeneration and remodeling (9). Some support for this contention comes from the recent observation that chronic low-frequency stimulation, a powerful stimulus for damage and remodeling in rabbit skeletal muscle, also results in increased proteosome activity (24). The relationship between muscle damage, protein degradation, and the enzyme pathways responsible for this degradation requires further investigation.

The 30-kDa regulatory subunit of calpain protein content did not increase in response to exercise, and there was no gender difference. Twenty-four hours
postexercise may, however, be too late to detect increases in calpain protein content, because in rats calpain activity is increased shortly after the cessation of exercise (4) and returns to preexercise values within 24 h (4). Calpain activity postexercise has not, to our knowledge, been investigated in humans. It has been hypothesized that protein degradation, including removal of Z-disks (17), is initiated by calpain, but subsequent proteolysis is dependent on other proteolytic enzyme pathways, chiefly the ATP-dependent ubiquitin proteasome pathway (5).

We did have as a secondary purpose of this study an exploration of some gender-dependent effects with respect to contraction-induced muscle damage and the possible repeated bout-induced reduction in muscle damage. This purpose was derived from literature reports of gender-based differences in postexercise CK response (8), inflammatory response (28, 30), and ultrastructural disruptions (20). Hence, it would appear prudent, on the basis of the preceding reports (8, 20, 28, 30), to conduct a structured examination of possible sex-based differences in contraction-induced damage and the subsequent proteolytic responses. Although we did observe some significant gender-based differences in some outcomes (CK, see Fig. 2; MPO+ cells, see Fig. 4), we were somewhat underpowered (in terms of sample size) to assess effects in other parameters (see RESULTS). However, given the scarcity of data that have examined proteolytic pathway activation after contraction-induced damage in humans, particularly of both genders (9, 24, 31), we believe that our study represents an important advance in our understanding in this area. Future studies will have to examine in greater detail the time-dependent adaptations in the similar parameters that we have examined here (i.e., a greater number and frequency of muscle biopsies) and will undoubtedly have to consider gender as a relevant variable when selecting subjects.

The cellular pathways affected in the adaptation to eccentric exercise-induced muscle damage have not been well characterized in humans. This preliminary study extends previous work by examining the effects of a repeated bout of exercise on ultrastructural damage, inflammatory cell infiltration, and calpain and ubiquitin-conjugated protein content. Serum CK activity and force deficit were attenuated after repeated eccentric exercise. The amount of Z-disk streaming we observed after bout 2 was not statistically different from that at rest. In addition, the increase in neutrophil infiltration in women after the second exercise bout suggests that adaptations to eccentric exercise-induced muscle damage may include changes in the time course of inflammatory cell infiltration and protein degradation. The force deficit and the ultrastructural disruption after exercise were similar between men and women. Thus, all gender-related differences in exercise-induced muscle damage were observed in the secondary responses to eccentric contraction-induced injury, including serum CK activity, inflammatory cell infiltration, and activation of protein degradation pathways.

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REFERENCES


