Effects of a 7-day eccentric training period on muscle damage and inflammation

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ABSTRACT

CHEN, T. C., and S. S. HSIEH. Effects of a 7-d repeated maximal isokinetic eccentric training period on muscle damage and inflammatory response. Med. Sci. Sports Exerc., Vol. 33, No. 10, 2001, pp. 1732–1738. Purpose: This study examined the effects of a 7-d repeated maximal isokinetic eccentric training period on the indicators of muscle damage and inflammatory response. Methods: Twenty-two college-age males were randomly assigned to eccentric training (ET) (N = 12) and control groups (CON) (N = 10). The initial exercise was 30 repetitions of maximal voluntary isokinetic eccentric contraction (ECC1) on nondominant elbow flexors with Cybex 6000 at 60°·s⁻¹ angular velocity. The ET group performed the same exercise for the following 6 consecutive days (referred to as ECC2 to ECC7) after ECC1. Upper arm circumference (CIR), range of motion (ROM), and maximal isometric force (MIF) were measured before, immediately after, and every 24 h for 7 consecutive days after ECC1. Plasma creatine kinase (CK), lactate dehydrogenase (LDH), glutamic oxaloacetate transaminase (GOT), leukocyte counts, and serum interleukin-1β and -6 (IL-1β, IL-6) levels were assessed before; at 2 h; and at 1, 3, 4, 6, and 7 d after ECC1. Muscle soreness was measured before and for 7 consecutive days after ECC1. Results: The ECC1 produced significant changes in most of the measures for both groups (P < 0.05), with the exception of leukocyte counts (P > 0.05). No indicators of increased damage (P > 0.05) were found from ECC2 to ECC7 for the ET group. Conclusion: Continuous intensive isokinetic eccentric training performed with damaged muscles did not exacerbate muscle damage and inflammation after ECC1. In addition, a muscular “adaptation effect” may occur as early as 24 h after ECC1, as shown by the ET group’s performance for 6 consecutive days after ECC1. Key Words: TOTAL WORK, CREATINE KINASE, INTERLEUKIN-1β, ADAPTATION EFFECT

Previous studies showed that a bout of unaccustomed eccentrically induced exercise results in muscle damage (8,9,16,22,29), the symptoms of which include loss of strength, reduction of range of motion (ROM), the development of muscle soreness, elevated creatine kinase (CK) activity, and limb swelling. When the same exercise is repeated a few days to several weeks after the first exercise, there is significantly less change in the indicators of muscle damage (i.e., CK, muscle soreness, ROM, upper arm circumference (CIR), and maximal isometric force (MIF)) when compared with the initial exercise (8,9,11,20,22,28,29). This phenomenon is referred to as the repeated bout effect (RBE) (9,22,29). Although some studies revealed that RBE might be present between 48 and 72 h after the initial exercise (8,23,28), exactly when and how this effect takes place is largely unknown.

Nosaka and Clarkson (22) and Chen and Hsieh (8) demonstrated that repeating the same bout of eccentric exercise with the elbow flexors of the nondominant arm on 3 d and 6 d after the first bout did not exacerbate damage or retard the recovery process. However, the results of these two studies (8,22) may not apply to real training situations, because most athletes cannot train once every 3 to 5 d. In fact, the general recommendation concerning training during the period of muscle damage is to ignore the sensations and train through the muscle soreness (2). Moreover, the eccentric component of muscle action could be minimized during early training, but this is impossible for athletes in most sports (30). Thus, in order to reflect real preseason training situations, participants must repeat the same exercise on each of the 6 d after ECC1, to determine whether doing so exacerbates muscle injury. Published research has not examined this question.

Recently, the cytokine response to strenuous eccentric-induced exercise has been examined in several studies (7,10,23). Cytokines play central roles in control of immune response, acute inflammatory response, and tissue repair process (26). In the acute inflammatory response, interleukin-1β and -6 (IL-1β, IL-6) have been most extensively studied (5,7,10,12,17,23). IL-6 was found to be increased a few hours after exercise, but it returned to preexercise levels later on (5,10). For IL-1β, increased blood levels a few hours after exercise were observed in some studies (7,12,17), but could not be confirmed by others (5,23,24). So far, there is no study to examine the effect of continuous isokinetic eccentric training on muscle damage and inflammatory response.

It is hypothesized that performing a 6-d repeated bout of eccentric training after the initial exercise would exacerbate inflammatory response and muscle injury at the early stage (within 48 h) of the repair process, and hinder recovery. Therefore, the purpose of the present study was to investigate the effects of a 7-d repeated bout of maximum,
isokinetic, eccentric training period on the indicators of muscle damage and inflammatory response.

**METHODS**

**Subjects.** Twenty-two college-age males volunteered for this study. The average physical characteristics of subjects were as follows: age, 19.3 ± 0.9 yr; height, 172.1 ± 5.1 cm; and weight, 66.2 ± 5.1 kg. A written consent was obtained from the subjects in accordance with the policy statement regarding the use of human subjects and informed consent of the journal *Medicine & Science in Sports & Exercise*. The subjects had not been involved in upper body weight training during the 6 months before the study, denied a history of recent upper extremity injury, and were free from soreness in the nondominant upper extremity. Subjects were randomly assigned to eccentric training (ET) (N = 12) and control groups (CON) (N = 10). Initially, 12 subjects were assigned to the CON group. However, two of the subjects appeared only twice during the experiment, so data analysis was performed on the remaining 10 CON subjects.

**Design and procedure.** Testing sessions were conducted over 8 d. On the first day, subjects of both groups performed a bout of maximal isokinetic voluntary eccentric exercise (ECC1) with the elbow flexors of their nondominant arm on the Cybex 6000 Extremity System (Cybex Lumex Inc., Ronkonkoma, NY) at 60°·s⁻¹ angular velocity. Calibration was performed before each testing session according to the Cybex 6000 Extremity System user’s guide. The subjects were stabilized during practice and testing via straps on the top of the nondominant upper arm, as well as on the abdomen and thighs.

The ECC1 consisted of three sets of 10 repetitions performed at a rate of one repetition every 2 s with 2 s of rest between eccentric actions, and 1 min between sets. Subjects were requested to maximally resist the actions in which the arm was forcibly extended from an elbow flexed (the elbow joint angle was approximately 50°) to an elbow extended position (the elbow joint angle was about 180°). The investigator brought each subject’s arm to the elbow flexed position after each eccentric action. In order to ensure that subjects put forth their maximum effort, we instructed them to shout loudly during each eccentric contraction; at the same time, we gave subjects verbal encouragement throughout the experiment (8). All subjects completed ECC1, but only the ET group performed the same ECC1 exercise for the following 6 d after ECC1 (referred to as ECC2 to ECC7).

**Measurements.** The following dependent variables were used to evaluate the degree of muscle damage and inflammatory response: total work, muscle soreness, CIR, MIF, active ROM, leukocyte counts, as well as plasma CK, LDH, GOT, serum IL-1β, and IL-6 concentrations. The criterion measures of CIR, MIF, and ROM were assessed before, immediately after ECC1, and every 24 h for 7 consecutive days after ECC1 for both groups. Muscle soreness was measured before and for 7 consecutive days after the initial exercise for both groups. Plasma CK, LDH, GOT activities, as well as serum IL-1β, IL-6, and leukocyte counts were tested before; at 2 h; and at 1, 3, 4, 6, and 7 d after ECC1 for both groups. Moreover, the measurement and recording of muscle soreness, total work, CIR, MIF, and ROM was performed by the same person throughout this study.

Change in arm circumference was used to measure the amount of swelling in the upper arm. Circumferential measurements were taken with a Gulick tape measure at four points (4, 6, 8, and 10 cm above the elbow joint) on the upper arm as the subject let the arm hang down by the side. These points were marked on the subject’s arm to ensure consistent placement of the tape measure. The accuracy of this measurement was shown to be within 2 mm (22).

Active elbow ROM, at a relaxed and at a flexed position, was measured by a goniometer, and ROM was calculated by subtracting flexed from relaxed angles (8,21,23). Muscle soreness was determined using a pressure scale (Imada Co. Ltd., Toyohashi, Japan), with the subject seated, and his arm resting on top of a desk. The measurement was then taken at the middle belly of the biceps brachialis. This measurement point was marked on the subject’s arm to ensure consistent placement for soreness measurements. Subjects rated the subjective soreness of their biceps when a pressure scale was gradually pressed down. The maximum value of the pressure scale for muscle soreness is 2.00 kg, and the observed pressure range (0.88–2.00 kg) was divided into a scale of 1 (not sore at all) to 10 (very, very sore). Three trials were taken, and the average of the three trials was used to calculate muscle soreness index for statistical analysis (8). The MIF was assessed with the subjects’ elbow joint angles at 90° on the Cybex 6000 Extremity System. Three 3-s repetitions were assessed with a 1-min rest between each contraction, and the average of three trials was used as the MIF score for statistical analysis (8).

**Blood Sampling and Analysis**

**Preparation of plasma.** Blood samples (10 mL) were drawn by venipuncture from the cubital fossa region of the arm. Each sample was immediately put into a blood Vacutainer containing EDTA; centrifuged for 10 min to obtain plasma; and tested for white blood cell count (WBC), neutrophils, monocytes, lymphocytes, CK, LDH, and GOT within 6 h after being collected.

**Preparation of serum.** Venous blood was drawn into 10-mL tubes and allowed to clot for 30 min at 37°C. Then the tubes were centrifuged and the serum was withdrawn. All samples were stored at −80°C until analysis.

**Analysis.** Changes in CK, LDH, and GOT were assessed spectrophotometrically using a Genstar chemistry analyzer (Electro-Nucleonics, Inc., Fairfield, NJ) at 340 nm using a quantitative, kinetic determination method (Sigma Diagnostics, St. Louis, MO). Samples were analyzed in duplicate, and the mean of both measures was used for subsequent statistical analysis.

Leukocyte counts were determined using a Cell-Dyn 3000 (Abbott Laboratories, Mountain View, CA), and
analyzed by laser-based flow cytometry. All specimens were analyzed within 6 h of collection. The baseline values for WBC, neutrophils, monocytes, and lymphocytes were $4,000–10,000 \cdot \mu L^{-1}$, $1800–7500 \cdot \mu L^{-1}$, $80–800 \cdot \mu L^{-1}$, and $800–4500 \cdot \mu L^{-1}$, respectively.

Serum concentrations of IL-1$\beta$ and IL-6 were determined by enzyme-like immunosorbent assay (ELISA) using test kits (Endogen Inc., Woburn, MA). Testing for IL-1$\beta$ and IL-6 was done by ELISA according to the instructions of the manufacturers. The average levels of IL-1$\beta$ and IL-6 found in normal serum were 0.0 and less than 43.0 pg·mL$^{-1}$, respectively. All measurements were performed in duplicate. The coefficient of variation (intra-assay variation) was 7.8% and 7.0% for the assays of IL-1$\beta$ and IL-6, respectively.

Statistical analysis. Dependent variables were analyzed with a mixed design (repeated variables mixed with independent variables) of two-way analysis of variance (ANOVA). A repeated measures ANOVA was used to assess change in total work for the ET group for all bouts. If significance was indicated, a Scheffe’s post hoc test was used to determine when the significance occurred. The independent Student’s t-test was used to analyze difference between the ET and CON groups for total work at ECC1. The Pearson product-moment correlation coefficient ($r$) was used to determine the relationship between the decreasing percentage of MIF on each ECC and total work. Reliability of the muscle soreness, CIR, and ROM measures was also determined by the Pearson product-moment correlation coefficient ($r$) between the values taken 1 d before and immediately before the ECC1. Values of $P < 0.05$ were considered to be statistically significant.

RESULTS

There were no significant differences between the ET and CON groups, and no interactions in any of the indicators ($P > 0.05$) (Figs. 1–8), except for total work ($P < 0.05$). Although subjects in the ET group could not produce the same amount of total work in ECC2 to ECC7 (as compared with ECC1), these subjects saw no significant changes in MIF, ROM, muscle soreness, CIR, IL-1$\beta$, IL-6, leukocyte counts, and blood enzymes.

The values of the criterion measures taken at 1 d before the ECC1 and immediately before the ECC1 were not significantly different ($P > 0.05$). The reliability coefficients ($r$) for muscle soreness index, CIR-4 cm, CIR-6 cm, CIR-8 cm, CIR-10 cm, and ROM were 1.00, 0.91, 0.96, 0.97, 0.97, and 0.96, respectively. Hence the reliability of the criterion measures were judged to be good.

Total work. No significant difference ($P > 0.05$) between the ET and CON groups on total work of the ECC1 was found. For the ET group, the total work values of ECC2 to ECC7 were only 69%, 65%, 57%, 70%, 72%, and 73% respectively of the ECC1 total work (Fig. 9).

Muscle function. The MIF dropped significantly, to about 42% of the preexercise value immediately after ECC1, and then started to regain some strength. However, it still was not completely restored 7 d after ECC1 for either group (Fig. 1). The MIF values immediately after each ECC (ECC1 to ECC7) were only 58%, 45%, 54%, 54%, 55%, 56%, and 50%, respectively, of the pre-ECC1 value for the

FIGURE 1—Maximal isometric force (means ± SD) for ET and CON before (pre), immediately after (post), and for 7 d after ECC1. All time points are significantly different from pre-ECC1 value. *$P < 0.05$ for ET and CON.

FIGURE 2—Range of motion (ROM) (means ± SD) for ET and CON before (pre), immediately after (post), and for 7 d after ECC1. All time points for each group are significantly different from pre-ECC1 values. *$P < 0.05$ for ET and CON.

FIGURE 3—Muscle soreness (means ± SD) for ET and CON before (pre) ECC1 and for 7 d after ECC1. Soreness scale ranged from 1 (not sore at all) to 10 (very, very sore). Significantly different time points for $P < 0.05$ (*) occurred at 1, 2, and 3 d from pre-ECC1 values for ET and CON.
ET group (Fig. 10). However, the percentage by which the MIF value decreased immediately after each ECC for the ET group was positively correlated with total work performed (r = 0.80, P < 0.05). After ECC1, there was also a significant decrease in ROM for both groups. No evidence of recovery was seen for the next 3 d, but ROM gradually recovered after this (Fig. 2).

**Muscle soreness.** For both groups, muscle soreness developed 1 d (P < 0.05) after ECC1, and was sustained through 3 d after ECC1, then gradually diminished (Fig. 3). However, the soreness level was almost back to baseline on 7 d for both groups.

**Circumference.** Changes in CIR at all four positions were not significantly different between the two groups. As shown in Figure 4, CIR at 8 cm above the elbow joint gradually increased from immediately after ECC1 to 5 d after ECC1, then the swelling started to subside for both groups. Subsequent bouts of eccentric training did not increase CIR more than the values after ECC1 for the ET group, as compared with the CON group.

**Biochemical markers.** There was considerable inter-subject variability in the enzyme responses. The levels of CK, LDH, GOT, IL-1β, and IL-6 showed significant elevations after ECC1, and the time course of changes in these biochemical markers was similar for both groups. The activities of CK, LDH, and GOT reached their peak at 4 d after ECC1 for both groups, and no further increase after ECC2 to ECC7 was found for the ET group (Figs. 5–7). On the contrary, there were small increases in IL-1β (Fig. 8) and IL-6 (data not shown) after ECC1 for both groups. These two indicators were not elevated further from ECC2 to ECC7 for the ET group. However, only IL-1β was found to exceed the normal level, and the peak for IL-1β (0.43 pg·mL⁻¹) occurred at 2 h after ECC1 for both groups.

In the assays for the WBC, neutrophils, monocytes, and lymphocytes, the control samples were at normal levels for both groups. In other words, these markers were not significantly elevated, and did not exceed normal levels before, at 2 h after ECC1, or at any time after ECC1 (these data are not shown in the text). In addition, the changes in cytokine were not associated with changes in leukocyte counts.
DISCUSSION

The results of this investigation indicated that repeated bouts of the ECC performed on each of the following 6 d after ECC1 did not affect recovery from ECC1 for the ET group. Muscle soreness developed after ECC1 for both groups, but there was no further soreness produced from ECC2 to ECC7 for the ET group (Fig. 3). MIF dropped to approximately 42% of the preexercise value immediately after ECC1, and then gradually recovered over the next 7 d for both groups (Fig. 1). The pattern of recovery in ROM (Fig. 2) and CIR (Fig. 4) was also similar for both the ET and CON groups. These changes were similar to those found in previous studies, when subjects performed the same bout of exercise on day 3 and day 6 after the first exercise (8,22), or only on day 2 after the initial exercise (28). These results suggest that ECC1 caused substantial damage to skeletal muscle, but the same ECC performed 1 to 6 d after ECC1 were not deleterious to the indicators of muscle function and damage (e.g., ROM, MIF, CIR, muscle soreness).

Previous studies showed that eccentric exercise of the active muscles can cause signs of inflammation that include the development of muscle soreness, swelling, and weakness (3). Inflammation can significantly retard further training, and may persist from 1 to 3 wk (2,3,28). Thus, it was thought that training or exercise of damaged muscle would exacerbate inflammation, or affect the recovery process (22). Yet in this study, muscle soreness and CIR, indirect markers of acute inflammatory response, did not appear aggravated from ECC2 to ECC7 (Figs. 3 and 4), even though the repair process was not completed by the beginning of ECC2 to ECC7. In addition, Nosaka and Clarkson (22) found that the data of echointensity in ultrasound images did not increase further before or after either the second bout of exercise (on day 3 after the first bout), or the third bout (on day 6 after the first bout).

Performing repeated bouts of maximal voluntary eccentric exercise using the same arm 3 and 6 d after ECC1 does not produce the same amount of total work (8); this is consistent with the results of the present study. When ECC2 to ECC7 were performed at 1 to 6 d after ECC1 by the ET group, the ET subjects were only able to reach 57% to 73% of the first amount of total work (Fig. 9). Although Clarkson et al.(9) and Croisier et al. (10) speculated that muscle soreness prevents subjects from voluntarily producing maximal force, it seems that ECC2 to ECC7 may not be stressful enough to produce additional injury to muscle. However, Newham et al. (18) demonstrated that a loss in voluntary strength has nothing to do with muscle soreness or loss of central drive, as similar strength loss can be observed when the muscle is stimulated at a high frequency. Thus, the exercises in all seven bouts would be considered of their maximal intensity, even if at a lower absolute magnitude during muscle damage for the ET group.

Eccentric exercise-induced increases in plasma activities of enzymes found in skeletal muscle have been attributed to skeletal muscle damage (2,19,21). In the present study, there was a large delayed release of plasma CK, LDH, and GOT into the circulation after ECC1 for both groups (Figs. 5–7).
After ECC2 to ECC7, there was no further increase in circulating CK, LDH, or GOT, suggesting that the muscle had become resistant to the destructive process, or that no further muscle damage had occurred (8,11,22).

Another possible explanation for the results would be that the muscles became more resistant to subsequent injury after ECC1, by producing a springy structure (19), by changes in specific neural control (14), and by increasing the strength of the cell membrane or surrounding connective tissue (16). It is also possible that part of the explanation lies in the removal of the stress-susceptible fibers after the initial exercise (3). If the weak fibers were still healthy and functional, then they should be disrupted by the repeated bout of eccentric exercise, and damage would be evident (16). However, this study showed no further damage to muscle fibers (MIF, total work, CK, LDH, GOT) even though ECC2 to ECC7 were repeated before full recovery occurred. Thus, a training adaptation may have been caused by derecruitment of motor units with damaged fibers and increased activity in healthy motor units (16,22).

Ader et al. (1) showed that the central nervous system (CNS) can significantly adapt its output after the initial bout of exercise, especially when the muscles are greatly damaged. Adaptation occurs soon after ECC1, while the muscle is still damaged or recovering from the insult (11). Accordingly, Nosaka and Clarkson (22) and McHugh et al. (16) suggested that the neural adaptation would have a “protective” effect, to set a limit for excessive force generation, or to better distribute the contractile stress over a larger number of active fibers.

On the other hand, IL-1β and IL-6 saw small increases after ECC1 for both groups, and there were no further increases in IL-1β (Fig. 8) or IL-6 after ECC2 to ECC7 for the ET group. It is interesting that only IL-1β serum levels were above normal value, whereas most values were 0 pg·mL⁻¹, with some extreme levels reaching 0.44 to 3.06 pg·mL⁻¹. This is in agreement with the results of recent human studies derived from eccentric-damaging exercise protocols (6,12,17). These responses were similar in kind to the so-called acute phase response to infection and sepsis (6,7,12). However, the acute phase responses observed after ECC1 were much smaller than those associated with infection or sepsis (6,7,12). Because IL-1β stimulates muscle proteolysis in vitro (4), increased IL-1β may represent muscle damage (7,27) and an adaptive response that modulates muscle protein turnover during recovery from ECC1 (7,12).

However, it should be noted that one limitation of this study is that we measured cytokines systemically in the peripheral blood, rather than locally in the damaged skeletal muscle. Therefore, the comparisons should be viewed with caution, because our measurements may not represent what is happening in the muscle.

Previous studies showed that IL-6 is one of the major substances released during acute inflammation (26). However, we found that the serum IL-6 concentration before and during the period of experimentation (before ECC1 to after ECC7) was less than 8.05 pg·mL⁻¹. By comparison, levels of 94.4 pg·mL⁻¹, greater than 10,000 pg·mL⁻¹, and 25 ng·mL⁻¹ were observed after a marathon race (24), in septic patients (15), and after endotoxin infusion (13), respectively. The discrepancy between the present study and previous studies (5,10,24) can probably be ascribed to the differences in eccentric exercise protocols, in muscle groups involved, in the intensity and duration of the exercise, in assays, as well as in statistics assessment.

In the current study, IL-1β levels increased significantly 2 h after ECC1, whereas leukocyte counts remained low before ECC1 and between ECC1 and ECC7. The higher IL-1β levels were not associated with the leukocyte counts. It is suspected that other neural and hormonal factors are more likely to be the cause. Pober and Cotran (25) suggested that cytokines may be released by virtually all immunologically relevant cells when they have been activated by antigen-presenting cells, or by neural or hormonal stimulation.

Although a limited amount of study has focused on repeating a bout of eccentric exercise 24 h later, a number of investigators have studied repeated bouts spaced at longer intervals, such as 2 d (28), 3 and 6 d (8,22), 5 or 14 d (11), 2 and 4 wk (19), and 6 or 10 wk apart (20). All studies found that changes in the indirect markers of muscle damage (MIF, CK, ROM) or inflammation (CIR, soreness, WBC, neutrophils, monocytes) were significantly less after the second bout compared with changes after the initial exercise. Apparently, an adaptation took place in response to the initial injury and subsequent recovery, which then acted to protect the active muscles (11). It is clear that the adaptation lasts for a considerable amount of time; however, it is unknown how soon after the first exercise this adaptation occurs. In the present study, if the ECC2 to ECC7 had resulted in an earlier resolution of the indicators of muscle damage and inflammation, we could have speculated that an adaptation had occurred; this was not the case. However, the fact that muscle damage and inflammatory response measures were not aggravated after the ET group performed 6 consecutive days of repeated bouts of exercise after ECC1 suggests that the “protective effect” would be produced as early as 24 h after ECC1.

In conclusion, a repeated 6-d strenuous isokinetic voluntary eccentric exercise with damaged muscle did not exacerbate muscle damage and inflammatory response after initial exercise. A muscular “adaptation effect” may occur as early as 24 h after the initial exercise. Moreover, there was a significant reduction in the amount of total work on the subsequent days for eccentric training subjects. The information of this study may prove a useful reference for coaches and athletes during muscle damage.

The authors would like to thank Sara Reals and Jean Curran for their assistance in review of this manuscript.

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