How long does the protective effect on eccentric exercise-induced muscle damage last?

KAZUNORI NOSAKA, KEI SAKAMOTO, MIKE NEWTON, and PAUL SACCO

Exercise and Sports Science, Graduate School of Integrated Science, Yokohama City University, Yokohama, JAPAN; School of Biomedical and Sports Science, Edith Cowan University, Western Australia, AUSTRALIA

ABSTRACT

NOSAKA, K., K. SAKAMOTO, M. NEWTON, and P. SACCO. How long does the protective effect on eccentric exercise-induced muscle damage last? *Med. Sci. Sports Exerc.*, Vol. 33, No. 9, 2001, pp. 1490–1495. **Purpose:** One bout of eccentric exercise produces an adaptation that reduces muscle damage in subsequent bouts. Because it is not known how long this adaptation lasts, the present study investigated the maximal length of the attenuated changes in muscle damage indicators after high-force eccentric exercise. **Methods:** Male students ($N = 35$) were placed into three groups and performed two bouts of eccentric exercise of the nondominant elbow flexors separated by either 6 ($N = 14$), 9 ($N = 11$), or 12 ($N = 10$) months. Maximal isometric force (MIF), range of motion (ROM), upper arm circumference (CIR), muscle soreness (SOR), and plasma creatine kinase activity (CK) were measured before and for 5 d after exercise. Magnetic resonance (MR) images of the transverse and longitudinal scans of the upper arm were taken 4 d after exercise. Changes in the criterion measures were compared between the first and second bouts and between groups by a two-way repeated measures ANOVA. **Results:** A faster recovery in MIF was evident after a second bout performed at 6 or 9 months, and reduced SOR as well as smaller increases in CIR, CK, and T2 relaxation time of MR images also occurred after the second exercise bout at 6 months compared with initial responses. No significant differences between the bouts were found for ROM, and the12-month group did not show any repeated bout effect. **Conclusion:** These results show that the repeated bout effect for most of the criterion measures lasts at least 6 months but is lost between 9 and 12 months. **Key Words:** MAXIMAL ISOMETRIC FORCE, PLASMA CK ACTIVITY, MUSCLE SORENESS, SWELLING, T2 RELAXATION TIME

A novel bout of eccentric exercise induces skeletal muscle damage, but repeating the same exercise within several weeks results in significantly less damage (5,6,15). This phenomenon, often referred to as the "repeated bout effect," is characterized by a faster recovery of strength, a smaller restriction in range of motion about a joint, reduced swelling and muscle soreness, smaller increases in muscle proteins in the blood, fewer abnormalities on magnetic resonance (MR) or ultrasound images, and blunted immune responses after repeated exercise bouts (5,6,8,15,17,21).

Although some characteristics of the repeated bout effect have been well described (15), the mechanism by which the neuromuscular system adapts to confer protection is not clear. To better understand the mechanism of the repeated bout effect, it is important to determine how long the effect lasts. Numerous studies have investigated the time course of the repeated bout effect up to 9 wk (5,6,15). However, there are little data on the extent of protection with exercise intervals of longer duration. Byrnes et al. (3) reported less muscle soreness and smaller increases in serum creatine kinase (CK) and myoglobin when a second bout of downhill running was repeated up to 6 wk, but not 9 wk, after the first bout. Our preliminary studies (19,20) found that increases in plasma CK levels and changes in MR images were smaller after a second eccentric exercise bout that was performed 6 months after the first bout; however, further study using a larger sample size is needed to confirm these findings.

Therefore, the purpose of this study was to investigate whether attenuation of changes in indicators of muscle damage occurs when a second exercise bout of the elbow flexors is performed at 6 months, 9 months, or 12 months after the first bout of damaging exercise.

METHODS

Subjects. Subjects comprised 35 male students who were nonathletes and had not been involved in any regular resistance training. Subjects gave written informed consent consistent with the policy statement regarding the use of human subjects by the American College of Sports Medicine. Their mean ± SD (range) age, height, body mass, and lean body mass (LBM, determined by Body Composition Analyzer, TBF-300GS, Tanita Co., Tokyo, Japan) was 19.9 ± 1.5 (18–23) yr, 170.8 ± 5.2 (162–185) cm, 61.5 ± 7.0 (47.0–87.0) kg, and 50.3 ± 3.7 (39.2–72.7) kg, respectively, at the beginning of the study. Subjects were randomly placed into one of three groups, 6-month ($N = 14$), 9-month ($N = 11$), and 12-month ($N = 10$), based on the length of time between the first and second exercise bouts. All

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physical characteristics (age, height, body mass, and LBM) were similar between the groups ($P > 0.4–0.9$), and no significant ($P > 0.7–0.9$) differences in height, body mass, or LBM occurred over the course of the experiment. Subjects did not perform any resistance training of the upper body between the first and second bouts and were requested not to change their life style (diet, exercise, etc.) during the experimental period. Subjects were requested to avoid any vigorous physical activities or unaccustomed exercise, other than that required in the study, for 2 wk before each eccentric exercise bout and during the experimental periods. All subjects were free from any musculoskeletal disorders and were asked to abstain from any medicine and dietary supplements during the experimental periods.

Exercise. Subjects performed two bouts of 24 maximal eccentric actions of the elbow flexors separated by either 6, 9, or 12 months with the nondominant arm on a modified arm curl bench (5,19). During the eccentric actions, subjects sat on the bench, and the arm was positioned in front of the body on a padded support adjusted to 45° (0.79 rad) of shoulder flexion, and the forearm was kept supinated with the wrist placed against the lever arm. After 1 s of maximal isometric contraction, the forearm was forcibly extended from an elbow half-flexed (90°, 1.57 rad) to an elbow extended (180°, 3.14 rad) position in 3 s. Subjects were verbally encouraged to generate maximal isometric force at the flexed position and to maximally resist during the eccentric phase of the motion. This action was repeated every 15 s for 24 contractions. Elbow flexor force was measured by a load transducer (9E01-L43, NEC San-ei, Tokyo, Japan) installed in a specially designed wrist attachment and monitored and recorded by a digital indicator (F360A, Unipulse, Saitama, Japan) and a computer (Macintosh Performer 5410, Apple Computer, Inc., Cupertino, CA). The peak force during each eccentric action was recorded from the digital indicator at a sampling rate of 100 Hz, and the work during each eccentric action was calculated as the integrated force for 3 s using a software program (LabVIEW, National Instruments, Austin, TX). Subjects were instructed to perform the eccentric exercise with maximal effort for both bouts.

Criterion measures. Several indirect markers of muscle damage that have been used in previous studies (5,19,20) were measured before (pre), immediately after (post), and at 24-h intervals for 5 d after the exercise (D1-D5). The test-retest reliability for the measurements was examined in our previous studies (18–20) and was shown to be consistent.

Maximal isometric force (MIF) during a 3-s contraction was determined at an elbow joint angle of 90° (1.57 rad) with the shoulder maintained in 90° (1.57 rad) flexion. Measurement was taken twice (1 min between the measurements) using a load cell (Model 1269, Takei Scientific Instrument Co. Ltd., Tokyo, Japan) positioned in between two cables between the wrist and the frame of the measurement device. The load cell was interfaced with the same computer system used for the force measurement during eccentric exercise protocol, and MIF was determined by the average peak force for 1 s during the 3-s contraction. The mean value of the two measurements was used for the analyses. Changes in MIF from the preexercise value were calculated, and recovery of MIF was defined as the ratio between the amount of change in MIF from immediately pre- to post-exercise and the amount of change from immediately post to 1–5 d (D1–D5) postexercise. For example, the recovery of MIF at 5 d postexercise was given by the formula: (D5-post)/(pre-post) × 100.

Relaxed (RANG) and flexed elbow joint angle (FANG) were measured twice for each measurement by a goniometer, and the angle subtracting FANG from RANG was used as range of motion (ROM) of the elbow joint. Relative changes in RANG, FANG, and ROM from the preexercise values were obtained, and the average change over D1–D5 was calculated.

Upper arm circumference (CIR) was assessed at 3, 5, 7, 9, and 11 cm from the elbow joint by a tape measure with the arm hanging relaxed by the side of the body, and the mean value of the five measurements was used for further analyses. Relative changes in CIR from the preexercise value were obtained, and the average amount of change from D1 to D5 was obtained.

Muscle soreness (SOR) during palpation of the upper arm and during flexion and extension of the forearm passively by the examiner was evaluated by a visual analog scale (VAS) that consisted of a 50-mm line with “no pain” on one end and “extremely sore” on the other. During palpation, the forearm was placed on an armrest of a chair at an elbow joint angle of approximately 90°. Because SOR peaked at 1–3 d after exercise, the mean SOR value of D1–D3 was calculated.

Approximately 5 mL of blood was drawn from the antecubital vein at all measurement time points except immediately after exercise and centrifuged for 10 min to obtain plasma. The plasma samples were stored at −20°C until analysis for CK activity. Plasma CK activity was determined spectrophotometrically by the VP-Super (Dinabott Co. Ltd., Tokyo, Japan) using a test kit (Dinabott Co. Ltd., Tokyo, Japan). The normal reference ranges for male adults with this method was 45–135 $IU \cdot L^{-1}$ according to the manual of the test kit.

MR images of the upper arm were obtained from subjects in the 6-month ($N = 7$) and 12-month ($N = 6$) groups at 4 d after exercise, based on the finding of our previous study that swelling peaked 4–5 d after exercise and changes in MR images were most prominent 3–6 d post exercise (19). Transverse images of 12 sections with 8-mm thickness and 2-mm intersection gaps were taken by using a 0.5-T 21.3 MHz superconducting magnet (SMT-50X, Shimadzu Co. Ltd., Kyoto, Japan). On an MR image taken approximately 6 cm above the elbow joint, a circular region of interest (area: 200 mm^2) was chosen at the center of a transverse area of the biceps brachii, brachialis, and triceps brachii to determine T2 relaxation time (18,19). T2 relaxation time has been shown to reflect the magnitude of muscle damage (8,18).

Statistical analysis. Changes in all criterion measures over time were compared between the first bout and second

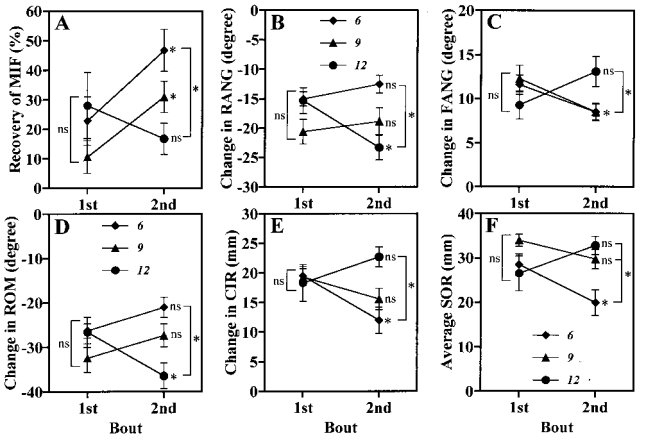

FIGURE 1—Comparison between the first (1st) and second exercise bout (2nd), which was performed 6 months (6), 9 months (9), or 12 months (12) after the first bout. A, Recovery of maximal isometric force (MIF) from immediately post to 5 d post exercise; B, the average amount of change in relaxed elbow joint angle (RANG); C, change in flexed elbow joint angle (FANG); D, change in range of motion (ROM); E, change in upper arm circumference (CIR) at 1–5 d postexercise; and F, the average muscle soreness (SOR) value of 1–3 d postexercise. Values shown are mean ± SEM. Comparisons between the groups for the first bout and between bouts for each group are shown. * $P < 0.05$, ** $P < 0.01$, ns: not significant.

bout using a two-way repeated-measures ANOVA for the three groups (6-month, 9-month, and 12-month). Comparisons were also made for the differences between the groups for the first bout and second bout. When the ANOVA produced a significant main effect, a Tukey's honestly significant difference *post hoc* test was used to detect differences in the measures between the bouts as well as between the groups at different time points. Statistical significance was set at $P < 0.05$. The values shown are mean ± SEM.

RESULTS

There were no significant differences in peak force or total work between the first and second exercise bouts or between groups. Changes in all criterion measures after the first exercise bout were of a similar magnitude between groups, and all criterion measures changed significantly after both exercise bouts.

Maximal isometric force. Mean MIF dropped to 47.0 ± 1.5% of the preexercise value (force deficit of 53%) immediately after the first exercise bout and recovered to 56.7 ± 3.0% of the preexercise value (remaining force deficit of 43.3%) at 5 d after the exercise ($N = 35$, all subjects). There was no significant difference in preexercise MIF between the bouts for the 6-month (165.5 ± 6.7 N vs 169.9 ± 5.5 N), 9-month (162.8 ± 4.4 N vs 168.3 ± 5.0 N), and 12-month groups (161.7 ± 5.2 N vs 164.2 ± 5.5 N). The force deficit immediately after the second exercise bout was not significantly different from that after the first bout (53.0%) for any of the groups (6-month: 51.2 ± 2.0%, 9-month: 60.4 ± 1.6%, 12-month: 59.0 ± 2.8%). The recovery of MIF at 5 d postexercise was significantly ($P < 0.05$) larger in the second bout compared with the first bout for the 6-month (1st: 22.9 ± 8.2% vs 2nd: 46.8 ± 7.1%) and 9-month (1st: 10.6 ± 5.5% vs 2nd: 31.0 ± 5.3%) groups; however, no significant difference between the bouts was shown for the12-month groups (1st: 28.1 ± 11.2% vs 2nd: 16.8 ± 5.3%) (Fig. 1A). MIF recovery after the second bout was significantly ($P < 0.05$) larger for the 6-month group compared with the 12-month group (Fig. 1A).

Elbow joint angles and range of motion. ROM decreased by approximately 30° from immediately post to 3 d postexercise but was still some 20° less than the preexercise level 5 d after exercise for both bouts. The average changes in RANG, FANG, and ROM at 1–5 d postexercise from the preexercise level are shown in Figure 1B–D. The average change in RANG from 1–5 d after exercise was not significantly different between the bouts for the 6- and 9-month groups; however, a significantly ($P < 0.05$) larger change occurred after the second compared with the first bout in the 12-month group (Fig. 1B). The average amount of change in FANG was significantly ($P < 0.05$) smaller (approximately 5°) after the second bout compared with the first bout for the 6- and 9-month groups; however, there were no significant difference between the bouts for the 12-month group. Also, no significant differences between bouts occurred for the change in ROM for the 6- and 9-month groups, but the 12-month group showed a significantly ($P < 0.05$) larger decrease in ROM after the second bout compared with the first bout (Fig. 1D). Significant ($P < 0.05$) differences in RANG, FANG, and ROM were found between the 6- and 12-month groups (Fig. 1B–D) and in FANG between the 9- and 12-month groups (Fig. 1C) for the second bout.

Upper arm circumference. CIR increased approximately 8 mm immediately postexercise and increased further at 2 d postexercise. By 5 d postexercise, CIR had increased by some 25 mm (Fig. 1E). A significantly ($P < 0.01$) smaller increase in CIR was found after the second bout (13.9 ± 2.5 mm) compared with the first bout (24.5 ± 2.3 mm) for the 6-month group; however, no significant difference between bouts was evident for the 9-month (1st: 23.2 ± 2.3 mm vs 2nd: 18.4 ± 2.2 mm) and 12-month (1st: 18.9 ± 3.3 mm vs 2nd: 20.8 ± 2.1 mm) groups (Fig. 1E). The amount of increase in CIR after the second bout was significantly ($P < 0.05$) larger for the 12-month group compared with the 6-month group (Fig. 1E).

Muscle soreness. Muscle soreness developed 1 d after exercise, peaked at 2–3 d after exercise then gradually attenuated. The average muscle soreness at 1–3 d postexercise when extending the elbow joint is shown in Fig. 1F. Compared with the first bout (28.5 ± 2.4 mm), soreness was significantly ($P < 0.01$) lower after the second bout (19.9 ± 2.9 mm) for the 6-month group. In contrast, no differences between the bouts were observed for the 9- (1st: 34.0 ± 1.4 vs 2nd: 29.7 ± 2.2 mm) and 12-month (1 st: 26.5 ± 3.9 vs 2nd: 32.8 ± 2.1 mm) groups. The 6-month group showed a significantly ($P < 0.01$) lower muscle soreness after the second bout compared with the 9-month and 12-month groups. The changes in muscle soreness when palpating the elbow flexors and flexing the elbow joint followed a similar pattern as those of the soreness with elbow extension.

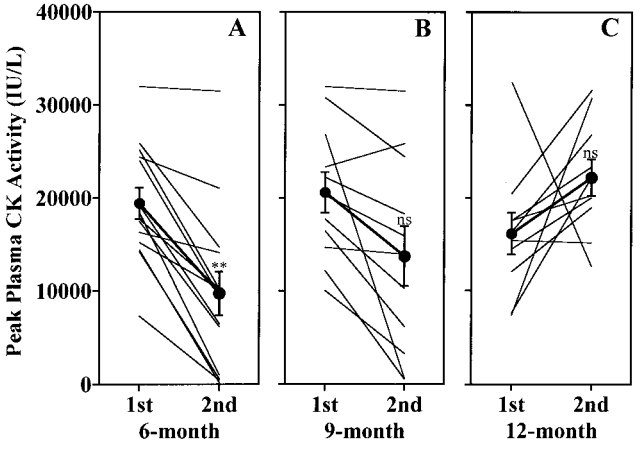

FIGURE 2—Peak plasma CK activity for the first (1st) and second exercise bout (2nd) for each subject in the 6-month (A), 9-month (B), and 12-month (C) groups. The mean (± SEM) values of the subjects are also shown. ** $P < 0.01$; ns, not significant.

Plasma CK activity. CK activity increased significantly ($P < 0.01$) after the first bout, and peaked at 3–5 d post exercise (19,403 ± 1,677,20,590 ± 2,184, and 16,131 ± 2,252 IU·L^{-1}), respectively, for the 6-, 9-, and 12-month groups). The increase was significantly ($P < 0.01$) smaller after the second bout compared with the first bout for the 6-month group (9724 ± 2354 IU·L^{-1}) but not for the 9-month (13,704 ± 3,197 IU·L^{-1}) and 12-month (22,184 ± 1,955 IU·L^{-1}) groups (Fig. 2). After the second bout, the 12-month group showed significantly ($P < 0.01$) larger increases than the 6-month group. There was a large variability in the peak CK values among the subjects for the first bout (7,427–32,490 IU·L^{-1}) and the second bout (6-month: 188–32,135 IU·L^{-1}, 9-month: 760–31,630 IU·L^{-1}, 12-month: 12,850–31,810 IU·L^{-1}). As shown in Figure 2, all subjects in the 6-month group and 10 of 11 subjects in the 9-month group showed a lower increase after the second bout compared with the first bout. In the 12-month group, however, only one subject showed a smaller increase after the second bout compared with the first bout, the rest showing either similar or larger increases after the second bout.

MR images. Figure 3 depicts the mean values of T2 relaxation times in the region of interest for the biceps brachii, brachialis, and triceps brachii at 4 d after the first and second bouts for the 6- and 12-month groups. T2 relaxation times in the brachialis and biceps brachii were significantly ($P < 0.01$) higher than those in the triceps brachii after the first bout for the 6- and 12-month groups. After the second bout, the 6-month group showed significantly ($P < 0.01$) lower T2 values in the brachialis (55.2 ± 10.3 vs 36.0 ± 7.8 ms) and biceps brachii (44.1 ± 12.1 vs 32.4 ± 8.4 ms) compared with that after the first bout. In contrast, no significant differences in T2 relaxation time between the first and the second bouts were found for the 12-month group.

DISCUSSION

The present study found that a faster recovery of strength (Fig. 1A), reduced muscle swelling (Fig. 1E) and soreness (Fig. 1F), smaller increases in plasma CK activity (Fig. 2), and fewer abnormalities on MR images (Fig. 3) were evident when the interval between eccentric exercise bouts was 6 months, but not 12 months. There were no significant differences in the RANG (Fig. 1B), FANG (Fig. 1C), and ROM (Fig. 1D) changes between bouts for the 6-month group. The 9-month group showed no significant difference between bouts for all criterion measures except the MIF recovery rate (Fig. 1A) and FANG (Fig. 1C). In the 9-month group, the tendency for most of criterion measures was to fall between those of the 6-month and 12-month groups (Figs. 1 and 2). The 12-month group showed not only no protective for all measures but also significantly larger changes in RANG (Fig. 1B), FANG (Fig. 1C), ROM (Fig. 1D), and CIR (Fig. 1E) after the second bout compared with the first bout.

These findings confirm those of previous studies (5,19,20), which reported that the repeated bout effect lasted up to 6 months. Foley et al. (8) recently reported that increases in T2 relaxation time were significantly lower and earlier after the second bout of eccentric exercise performed 8 wk after the first bout. The lower increases in T2 relaxation time were still evident for 6 months in the present study (Fig. 3). Byrnes et al. (3) showed that a prophylactic effect on muscle protein release and generation of muscle soreness lasted up to 6 wk but not 9 wk. This difference may be a reflection of the different methodologies (downhill running, leg muscles) used and/or the extent of muscle damage found in their study (i.e., peak CK: less than 1000 IU·L^{-1}). It is important to note that there was a large variability in the extent of muscle damage and response to the same exercise protocol among subjects (Fig. 2). If the degree of muscle damage is a factor in determining the duration of the protective effect, one might have expected

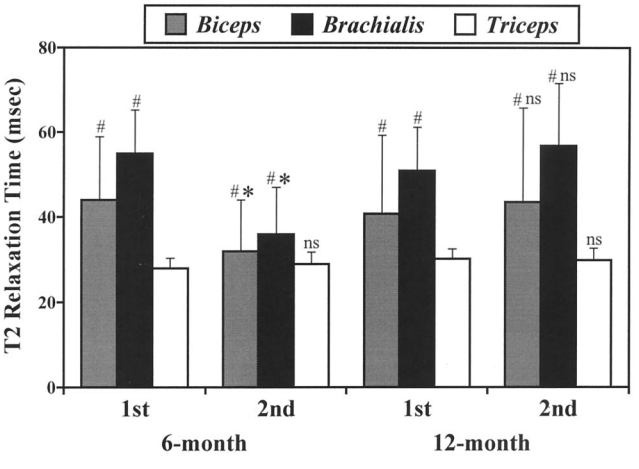

FIGURE 3—T2 relaxation time of the biceps brachii, brachialis, and triceps brachii at 4 d after the first (1st) and the second (2nd) exercise bout performed 6 months (6-month) or 12 months later (12-month). Mean values of T2 relaxation time of each muscle are shown (mean ± SEM) for 7 subjects in the 6-month and 6 subjects in the 12-month group. Comparisons between the first and second bouts are shown on the bars on the second bout graph (* $P < 0.05$; ns, not significant). A significant difference between the triceps brachii (used as a reference) and biceps brachii and brachialis is indicated by # ($P < 0.05$).

that those individuals who showed the greatest muscle damage in the first bout would show a greater and longer-lasting protective effect. However, this does not seem to be the case, because those subjects who showed the highest CK release after the first bout did not necessarily have a lower CK release in the second bout (Fig. 2). Furthermore, the extent of variability between subjects in response to exercise was similar between the bouts. A large intersubject variability in plasma CK response and changes in other indicators of muscle damage after eccentric exercise is well recognized (5,18); however, the explanation for this phenomenon is not clear. Differences in muscle fiber type between subjects may be associated with the magnitude of CK response or the susceptibility to muscle damage; however, no evidence to support this is available to date.

It should be noted that the degree of protection conferred when the interbout interval was 6 months was weaker than when the second bout of eccentric exercise was performed within 10 wk (20). Our previous study (20) showed that when the second bout was repeated at 6 wk after the first eccentric exercise bout, MIF at 5 d postexercise approximated the preexercise level, changes in RANG, FANG, and ROM were relatively small, the amount of increase in CIR was negligible, and plasma CK activity did not increase. The protective effect appears to be greatest when the second bout is performed within 2 wk of the first bout (4,7,14). These findings, taken together with those of other studies support the concept that the magnitude of the repeated bout effect decreases as the time interval between bouts increases and all protection is lost between 9 and 12 months after the first eccentric exercise bout.

Others have suggested that the mechanism underlying the repeated bout effect at shorter time intervals (i.e., less than 4 wk) could be explained by a reduction in force generating capacity during the second bout (9,11). Because force during eccentric exercise is considered to be one of the critical factors in determining the degree of subsequent muscle damage (1), reduced force generation during eccentric actions would occur when the second bout is performed with the muscles in a weakened condition. However, in our study, we found no significant differences in preexercise MIF, work or force during exercise, and the isometric force deficit immediately postexercise between the bouts. This suggests that the faster recovery of MIF seen in the 6-month and 9-month groups and that attenuation muscle damage and inflammation seen in the 6-month group (Figs. 1–3) cannot be explained by less force generation during eccentric actions as has been proposed by previous studies (9,11,23).

McHugh et al. (15) recently reviewed the possible neural, connective tissue, and cellular adaptations that have been cited as possible mechanisms for the repeated bout effect. For adaptations occurring within the muscle itself, protein synthesis needs to occur after the initial eccentric exercise bout. If so, what proteins are associated with the repeated bout effect that remain for 6–9 months without additional stimulation? Changes in cytoskeletal or membrane proteins may be associated with the protective effect as suggested by McHugh et al. (15). Limited information is available for the protein turnover rate of the human skeletal muscle, but Goldspink (10) has documented that the protein turnover rate is 7–15 d for rat skeletal muscles. It seems reasonable to assume that the protein turnover rate of human skeletal muscle proteins is longer than that of small animals, because such physiological processes are proportional to body mass to the power 0.25 (12). Lundholm et al. (13) estimated from the rate of tyrosine release *in vitro* that the half-life of human skeletal muscle was 20 d. If this is also the case for the *in vivo* condition, it seems unlikely that muscle proteins synthesized after the initial exercise bout would remain for 6 months, although it could be that some muscle proteins have longer turnover rates. It has been proposed by Morgan (16) that the protective effect is the result of incorporation of additional sarcomeres into the exercised muscle fibers. It would be interesting to examine whether the increased numbers of sarcomeres remain for more than 6 months without any additional eccentric exercise.

Because changes in nervous system associated with long-term memory can be maintained for many months (2), one might speculate that neural adaptations are associated with the repeated bout effect. However, it is important to note that all adaptation was lost by 12 months. An increase in motor unit activation and/or a shift to slow-twitch fiber activation (23) may explain the protective effect; however, the length of time that any changes in motor unit activation can be maintained remains to be elucidated. There is a similarity between the repeated bout effect and acquired immunity in which B cells play an important role. It is known that memory B cells can confer long-lasting immunity against subsequent exposures to a pathogen (22); however, it is not clear whether this long-lasting B cell immunity plays a role in the protective effect. It is possible that the initial eccentric exercise bout can promote immunological memory to provide protection against severe muscle damage in the second bout of the same eccentric exercise. However, it cannot explain the limited length (6–9 months) of the protection found in the present study. Pizza et al. (21) reported that a lower state of circulating neutrophil and monocyte activation in the second bout of eccentric exercise was associated with to the faster recovery of muscle function, reduced swelling and muscle soreness, and smaller increases in plasma CK activity. However, it remains to be determined whether a blunted immune response results in reduced muscle damage or whether reduced muscle damage leads to a blunted immune response.

In conclusion, the present study showed that the repeated bout effect produced by 24 maximal eccentric actions of the elbow flexors lasted 6–9 months; however, the length varied between subjects and among markers of muscle damage. The maximal length of time the repeated bout effect is present should be considered when designing studies in which similar exercise protocols are used. In human studies, obvious difficulties exist in determining the mechanisms underlying adaptations associated with the repeated bout effect and how they change over time. Further studies should be directed toward investigating the neurological, biochemical,

and immunological changes associated with exercise-induced muscle damage, which may provide the basis for the adaptations associated with the repeated bout effect.

Address for correspondence: Kazunori Nosaka, Exercise and Sports Science, Graduate School of Integrated Science, Yokohama City University, 22-2, Seto, Kanazawa-ku, Yokohama, 236-0027, Japan; E-mail: nosaka@yokohma-cu.ac.jp.

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