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Abstract/Résumé

A single bout of eccentric exercise confers a long-lasting protective effect against subsequent bouts of the same exercise. This study investigated how the protective effect was lessened when the interval between the initial and secondary exercise bouts was increased from 4 to 12 weeks. Thirty young men performed two bouts of 12 maximal eccentric actions of the elbow flexors of the nondominant arm separated by either 4 (n = 9), 8 (n = 10), or 12 (n = 11) weeks. Maximal isometric strength, flexed and relaxed elbow joint angles, range of motion, upper arm circumference, muscle soreness, plasma creatine kinase (CK), and myoglobin (Mb) were measured before, immediately after, and for 4 days after exercise. Changes in criterion measures were compared between bouts for each group and among groups by two-way repeated-measures ANOVA. There were no significant differences among groups in the changes in all measures following the first bout. Significantly ($p < 0.05$) smaller responses in all measures were observed after the second bout as compared with first bout for the 4 and 8 weeks, but only in strength, muscle soreness, CK, and Mb for the 12 weeks. It was concluded that some aspects of the protective effect were attenuated after 8 weeks, and the factors responsible for the effect vary among the measures.

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Une seule séance d'exercices pliométriques donne une protection de longue durée contre les effets des séances subséquentes d'un même exercice. Cette étude analyse l'atténuation de l'effet protecteur quand l'intervalle entre la première séance et les séances subséquentes augmente de 4 à 12 semaines. Treize hommes participent à deux séances de 12 actions pliométriques maximales des fléchisseurs du coude du bras non dominant; ces séances sont espacées de 4 (n = 9), 8 (n = 10), ou 12 (n = 11) semaines. Les variables suivantes sont évaluées avant, immédiatement après, et durant les 4 jours suivant la séance: tension isométrique maximale, angles articulaires du coude relâché et fléchi, amplitude de mouvement, circonférence du bras, douleur musculaire, créatine kinase plasmatique (CK), et myoglobine (Mb). Une analyse de variance à deux facteurs avec mesures répétées est utilisée pour établir les différences significatives à l'intérieur d'un même groupe d'une séance à l'autre et entre les groupes. Les variables mesurées chez les groupes à l'étude ne présentent pas de différence significative après une séance. À la 4^{e} et à la 8^{e} semaine, toutes les mesures prises à la 2^{e} séance sont significativement plus faibles ($p < 0,05$) que celles prises à la 1re séance; à la 12^{e} semaine, seules les mesures de tension musculaire, de douleur musculaire, de CK, et de Mb sont plus faibles. En conclusion, des facettes de l'effet protecteur sont modifiées à la 8^{e} semaine et les facteurs responsables varient d'une mesure à l'autre.

Introduction

Unaccustomed eccentric exercise induces skeletal muscle damage but also confers a protective effect against the damage induced by the same eccentric exercise (Clarkson et al., 1992; McHugh, 2003; McHugh et al., 1999). This phenomenon, often termed "repeated bout effect," is characterised by faster recovery of muscle function, reduced swelling and muscle soreness, and smaller increases in muscle proteins (e.g., creatine kinase [CK], myoglobin) in the blood following the second exercise bout compared to the initial bout (Clarkson and Tremblay, 1988; Newham et al., 1987; Nosaka et al., 1991). The repeated-bout effect has been also demonstrated by attenuated immune responses (Pizza et al., 1996) and fewer abnormalities in ultrasound and/or magnetic resonance images (Foley et al., 1999; Nosaka and Clarkson, 1996). The mechanism responsible for this protective effect has not been fully elucidated, but several characteristics of this phenomenon have been described: (a) the adaptation is produced by a single bout of eccentric exercise; (b) the adaptation is specific to the exercise mode and muscles involved in the exercise; (c) the adaptation requires only a few maximal eccentric actions inducing minor damage; (d) the adaptation is already evident without complete recovery; and (e) the adaptation lasts several weeks to several months (McHugh, 2003; McHugh et al., 1999).

Previous studies have shown that the protective effect lasts more than 6 months but less than 1 year following the initial bout of maximal eccentric exercise of the elbow flexors (Nosaka and Clarkson, 1996; Nosaka et al., 2001b). It is important to note that the protective effect observed in the 6-month interval does not appear to be as strong as that shown in a shorter interval between bouts such as less than 10 weeks. When the second bout was performed within a week of the initial exercise, in an early recovery phase, no adverse effects on markers of damage were observed, although tentative decreases in muscle function occurred immediately after exercise (Chen, 2003; Ebbeling and Clarkson, 1990).

In contrast, when the second eccentric exercise was performed 2 weeks after the first bout, prior to full recovery of muscle function, prolonged decreases in muscle function and development of muscle soreness occurred, but no increases in CK activity were observed (Clarkson and Tremblay, 1988; Newham et al., 1987; Nosaka et al., 2001a). It seems that the repeated-bout effect is conferred soon after the initial bout, but the effect demonstrated in the recovery phase (i.e., less than 2 weeks) differs from the effect observed after full recovery (i.e., more than 4 weeks). The time required for full recovery is dependent on the magnitude of muscle damage, but it appears that more than 4 weeks are needed for the case of maximal eccentric exercise of the elbow flexors (Clarkson et al., 1992).

Nosaka et al. (1991) reported that changes in indirect markers of muscle damage following exercise were suppressed more when the interbout interval was set at 6 weeks compared to 10 weeks. It seems that the magnitude of the protective effect decreases when the interval between two exercise bouts is increased, and that there is a threshold somewhere between 4 and 12 weeks for the protective effect to start attenuating. It is also important to clarify the difference in the duration of the protective effect among markers of muscle damage, because a more specific protective effect may be required when one wants to avoid muscle soreness, or to have a benefit of enhanced recovery of muscle function, for example. However, these aspects of the protective effect have not been comprehensively examined. Also we thought that understanding the time course for the loss of the protective effect among several indirect markers of muscle damage would shed some light on the underlying mechanism of the protective bout effect.

Therefore the purpose of this study was to examine the time course of attenuation of the protective bout effect by manipulating the interval between the eccentric exercise bouts of the elbow flexors from 4 to 12 weeks.

Methods

Thirty male students, who were nonathletes and had not been involved in a regular resistance training program for the past 12 months, gave informed consent to take part in this study. The study was in conformity with the policy statement of the Declaration of Helsinki and the local human research ethics committee. To minimize intersubject variability in the criterion measures shown below, only male subjects were used. Their mean ± *SD* (range) physical characteristics were as follows: age 20.4 ± 1.9 (18–27) yrs, height 171.4 ± 4.7 (163–182) cm, body mass 60.8 ± 5.6 (52.9–77.9) kg. They were randomly placed into one of three groups: 4-w (n = 9), 8-w (n = 10), or 12-w (n = 11), with no significant differences between groups. Subjects were instructed not to perform any upper body resistance training during the study period, and to report any changes in lifestyle (e.g., diet, exercise). All were free from any musculoskeletal disorders of the upper extremities, and all refrained from any medications or dietary supplements at least 7 days before and 4 days after the eccentric exercise protocol.

EXERCISE

The young men performed two bouts of 12 maximal eccentric actions of the elbow flexors with the nondominant arm on a modified arm curl machine (Nosaka and

Clarkson, 1996). This number of muscle actions was chosen as it provided a sufficient stimulus to induce significant muscle damage but, based on our pilot experiment, was mild enough so that muscle function would recover completely within 4 weeks. The two eccentric exercise bouts were separated by either 4 (4-w), 8 (8-w), or 12 (12-w) weeks. During eccentric contractions the arm was positioned in front of the body on a padded support adjusted to 45° (0.79 rad) shoulder flexion, and the forearm was kept supinated with the wrist placed against the lever arm. The subject's elbow joint was forcibly extended after 1 sec of maximal isometric contraction from an elbow flexed (90°, 1.57 rad) to an elbow extended (180º, 3.14 rad) position in 3 sec (0.52 rad·s^{-1}). Subjects were verbally encouraged to generate maximal isometric force at the starting position and to maximally resist against the elbow extending action throughout the range of motion. This action was repeated every 15 sec for 12 times.

The force during the eccentric exercise was measured by a load transducer (9E01-L43, NEC San-ei, Tokyo) installed in a specially designed wrist attachment and was monitored and recorded by a digital indicator (F360A, UNIPULSE, Saitama, Japan) and a computer (Macintosh Performa 5410, Apple Computer, Inc., Cupertino, CA). The peak force of each eccentric action was recorded from the digital indicator, and the work in each eccentric action was calculated as the integrated force for 3 sec using a software program (LabVIEW, National Instruments, Austin, TX) on the computer. Subjects were instructed to perform the eccentric exercise with maximal effort for both bouts.

CRITERION MEASURES

Several indirect markers of muscle damage used in previous studies (Clarkson et al., 1992; Nosaka et al., 2001a; 2001b) were measured before (pre), immediately (i.e., within 5 min) after (post), and at approximately 24-hr intervals for 4 days after each exercise bout (D1–D4). Although it takes more than 4 days for the markers to return to baseline, the repeated-bout effect can be clearly demonstrated in this period. Thus the measurement time points were thought to be appropriate for examining the repeated-bout effect.

Maximal isometric strength was measured twice (1 min between the measurements) for 3 sec each by a transducer (Model 100, Takei Scientific Instrument Co. Ltd., Tokyo) connected to a digital recorder at an elbow joint of 90° (1.57 rad), and the mean value was used for the analyses. Although it has been reported that an angle for generating maximal strength shifts to a longer muscle length after eccentric exercise (Proske and Morgan, 2001), the present study measured the strength at a fixed elbow joint angle of 90°.

Three elbow joint angles—flexed, relaxed, and stretched—were measured at least twice with a plastic goniometer. Flexed elbow joint angle was defined as the angle when subjects tried to fully flex the elbow joint to touch the shoulder with the palm. The joint angle when subjects let their arm hang relaxed by their side was defined as relaxed. And extending the elbow joint as much as possible was defined as the stretched elbow joint angle. The angle subtracting the flexed from the stretched angle was deemed to be the elbow joint's range of motion (ROM). Four markers (lateral epicondyle of the humerus, acromion process, and midpoint

of the styloid process of the ulna and radius) were made on the skin to obtain consistent measurements during the experiment.

Upper arm circumference was assessed at 3, 5, 7, 9, and 11 cm from the elbow joint by a tape measure while the arm hung down by the side, and the mean value of the five measurements was used for analysis. The marks were maintained using a semi-permanent ink marker during the experimental period. Muscle soreness during palpation on the upper arm and extension of the elbow joint was evaluated by a visual analogue scale (VAS) that had a 50-mm line anchored by "no pain" on one end and "extremely sore" on the other.

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 pending analysis for creatine kinase (CK) activity and myoglobin (Mb) concentration. Plasma CK activity was determined spectrophotometrically by the VP-Super (Dinabott Co. Ltd., Tokyo) using a test kit (Dinabott Co. Ltd.). Plasma Mb concentration was measured by a biochemical analyser (Model TBA-30A, Toshiba Co. Ltd., Tokyo) using a test kit (Denka-Seiken Co. Ltd., Tokyo). The normal reference ranges for men using this method are 45–135 $IU \cdot L^{-1}$ and 21–76 $\mu g \cdot L^{-1}$ for CK and Mb, respectively, according to the method sheet in the test kit.

STATISTICAL ANALYSIS

The null hypothesis tested in this study was that no significant differences would be found between 4, 8, and 12 weeks for the repeated-bout effect. To test this hypothesis we conducted three analyses. First we compared changes in all criterion measures over time (pre, post, D1–D4) between the first and second bouts using two-way repeated-measures ANOVA for each group (4-w, 8-w, and 12-w; 2 Bout × 6 Time interaction). Since no significant differences were found between bouts in the preexercise values for any of the criterion measures, some of the data were normalised either by calculating percent changes from the baseline or the amount of change from the baseline. Second, the three groups were compared by repeated-measures ANOVA for each bout (first, second; 3 Group × 6 Time interaction). Finally, to confirm the second analysis, we conducted comparisons between two groups (4-w vs. 8-w, 4-w vs. 12-w, and 8-w vs. 12-w) for the second bout using two-way repeated-measures ANOVA (2 Group × 6 Time interaction). When a significant main effect was found, a Sheffé post hoc test was used to specify the differences. Statistical significance was set at $p < 0.05$. All data are presented as mean ± *SEM* unless otherwise stated.

Results

No significant differences were found in height and body mass between the first and second bouts for all groups. Preexercise values for all criterion measures were not significantly different between groups before the first exercise bouts. No significant differences in preexercise values between bouts were evident for any measures. When the first and second bouts for each group were compared, the 4-w and 8-w groups showed significantly smaller changes in all measures following the

second bout vs. the initial bout, but this was not the case for the 12-w group (Figures 1–5). Changes in flexed (Figure 1b) and relaxed elbow joint angles (Figure 2a), ROM (Figure 2b), upper arm circumference (Figure 3), and muscle soreness upon extension (Figure 4b) were not significantly different between bouts for the 12-w group. All measures changed significantly from baseline following the first eccentric exercise bout for all 3 groups, and the changes were not significantly different among groups. However, some significant differences between groups were evident following the second bout (Figures 1–5).

FORCE DURING ECCENTRIC EXERCISE AND MAXIMAL ISOMETRIC STRENGTH

Changes in peak force during eccentric exercise were not significantly different between the first and second bouts for all groups. No significant differences were observed in the changes in peak torque during exercise among groups for both exercise bouts. Peak force decreased approximately 30%, from 233.5 ± 12.8 N to 157.2 ± 10.8 N over the 12 maximal eccentric actions. Total work performed during the exercise (4,226 ± 388.3 N to 4,342 ± 412.8 N) expressed by the sum of the area under the force curve of each eccentric action was not significantly different between bouts nor among groups.

All groups showed similar maximal isometric strength values immediately before exercise for the first (4-w: 163.9 ± 8.2 N; 8-w: 161.1 ± 5.8 N; 12-w: 162.1 ± 6.9 N) and second bouts (4-w: 156.4 ± 7.0 N; 8-w: 164.2 ± 7.3 N; 12-w: 161.3 ± 10.5 N). The relative changes in strength from the preexercise value (100%) for the first and second bouts are shown in Figure 1a. Maximal isometric strength dropped to approximately 55% of preexercise value immediately after and recovered to 65% at 4 days after the first exercise bout. The drop in strength immediately after the second exercise bout did not differ significantly from that after the first bout for any of the groups (4-w: 53%; 8-w: 56%; 12-w: 58%). Maximal isometric strength did not recover to preexercise levels at 4 days after the second exercise bout for any of the groups (4-w: 87%; 8-w: 84%; 12-w: 73%), but the recovery was significantly faster for all groups compared to that seen following the first bout (Figure 1a). Compared to the 4-w and 8-w groups, the recovery of strength was significantly smaller for the 12-w group.

FLEXED AND RELAXED ELBOW JOINT ANGLE, RANGE OF MOTION (ROM)

As shown in Figure 1b, flexed elbow joint angle increased approximately 12° from pre to immediately after the first exercise bout, and was still elevated at 4 days after exercise (7°). After the second bout the 4-w and 8-w groups showed significantly faster recovery of the flexed elbow joint angle; however, this was not observed for the 12-w group. Changes in flexed elbow joint angle following the second bout were significantly different between the 4-w and 12-w groups, but not between the 4-w and 8-w groups or the 8-w and 12-w groups.

Relaxed elbow joint angle decreased approximately 16° immediately after the first bout, with the nadir at 2–3 days postexercise (Figure 2a). The amount of decrease in relaxed elbow joint angle from pre- to immediately postexercise was significantly smaller after the second bout compared to the first bout for the 4-w and 8-w groups. Both groups showed significantly smaller changes in the angle

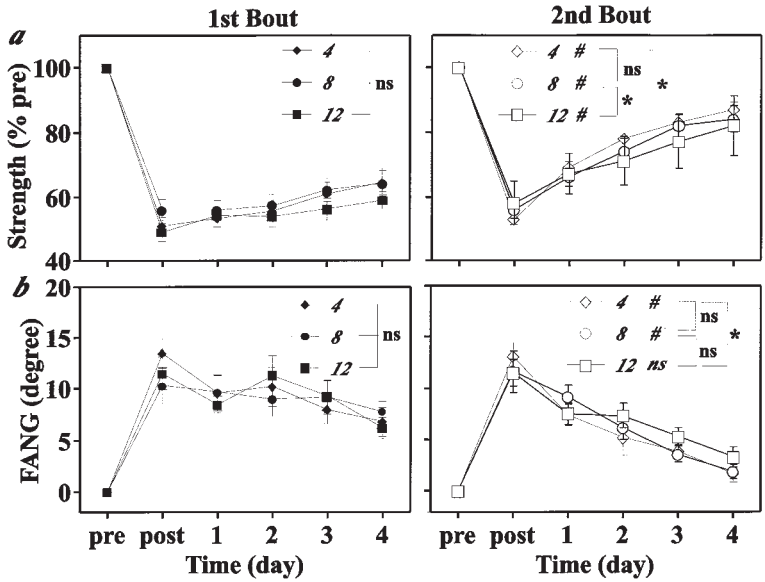

Figure 1. Changes in maximal isometric strength (*a*) and flexed elbow joint angle (FANG: *b*) from preexercise value (pre: 100% for strength and 0 for FANG), immediately after (post), and 4 days (1–4) after the first and second exercise bouts for the 4-wk (4), 8-wk (8), and 12-wk (12) groups. Values are mean ± *SEM*. The 2nd bout graph (right) shows comparisons between bouts for each group: # = significant difference ($p < 0.05$) from the first bout; ns = no significant difference between bouts. The results of comparisons among groups for first (left) and second bout (right) are also shown. *$p < 0.05$.

after the second bout compared to the first bout, but the 12-w group showed no differences between bouts. When comparing the groups for the second bout, no difference was found between the 4-w and 8-w groups; however, other comparisons (4-w vs. 12-w; 8-w vs. 12-w) were shown to be significant.

Changes in ROM were similar to the relaxed elbow joint angle, and the only difference between the two was that the decrease in ROM was 8–12° larger than that in the relaxed elbow joint angle (Figure 2b). The amount of decrease in ROM from pre- to immediately postexercise was significantly smaller after the second bout for the 4-w (–16°) and 8-w (–19°) groups compared to the first bout (–25°). However, this was not the case for the 12-w group, which showed a similar decrease in ROM for both exercise bouts. Compared to the 12-w group, the 4-w and 8-w groups showed significantly smaller decreases in ROM after the second bout, but no significant difference between the 4-w and 8-w groups was evident.

UPPER ARM CIRCUMFERENCE AND MUSCLE SORENESS

Upper arm circumference increased significantly after the first exercise bout, and the largest increase (approx. 17 mm from baseline) was observed at 4 days after the first exercise bout (Figure 3). Compared to the first exercise bout, the amount

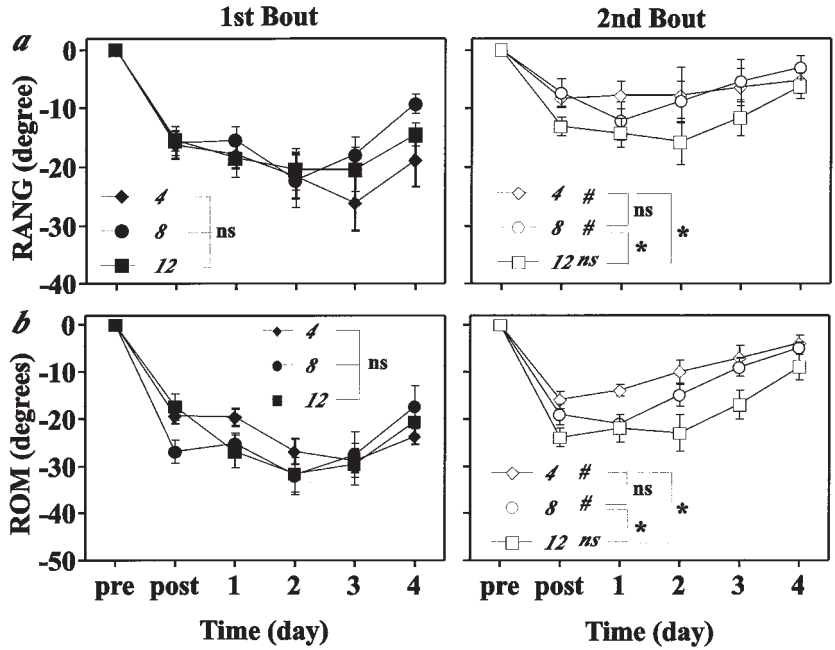

Figure 2. Changes in relaxed elbow joint angle (RANG: *a*) and range of motion (ROM: *b*) from preexercise value (pre: 0), immediately after (post), and 4 days (1–4) after the first and second exercise bouts for the 4-wk (4), 8-wk (8), and 12-wk (12) groups. Values are mean ± *SEM*. The 2nd bout graph (right) shows comparisons between bouts for each group: # = significant difference ($p < 0.05$) from the first bout; ns = no significant difference between bouts. The results of comparisons among groups for first (left) and second bout (right) are also shown. *$p < 0.05$.

of increase in circumference after the second bout was significantly smaller for the 4-w and 8-w groups, but the 12-w group showed no significant difference between bouts. The differences between the 4-w and 12-w groups and between the 8-w and 12-w groups were significant; however, no significant difference was seen between the 4-w and 8-w groups for the second bout.

Muscle soreness upon palpation and extension developed 1 day after exercise and peaked between 1 and 3 days after both exercise bouts (Figure 4). However, the magnitude of soreness was significantly reduced after the second bout with the exception of extension soreness for the 12-w group. Compared to the first bout (palpation 31 mm, extension 34 mm), peak soreness was significantly smaller after the second bout for all groups for palpation (4-w: 19 mm; 8-w: 18 mm; 12-w: 17 mm) and for the 4-w (19 mm) and 8-w (15 mm) groups for the extension; however, no difference was found between bouts for extension soreness (26 mm) in the 12-w group. The differences in extension soreness after the second bout between the 12-w group and the 4-w or 8-w groups were significant, but no significant difference was found between the 4-w and 8-w groups (Figure 4).

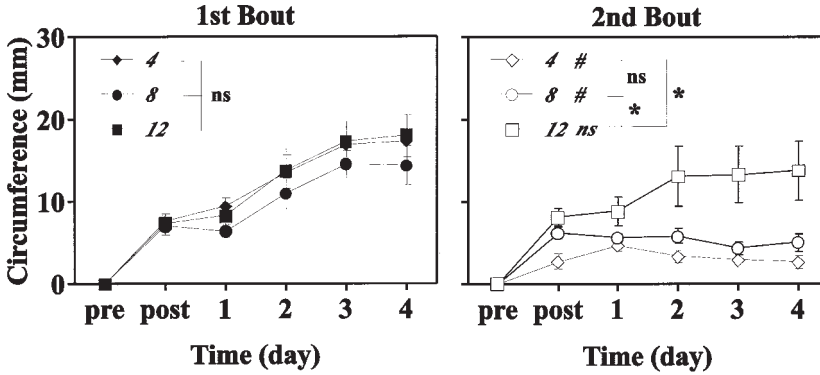

Figure 3. Changes in upper arm circumference from preexercise value (pre: 0), immediately after (post), and 4 days (1–4) after the first and second exercise bouts for the 4-wk (4), 8-wk (8), and 12-wk (12) groups. Values are mean ± *SEM*. The 2nd bout graph (right) shows comparisons between bouts for each group: # = significant difference ($p < 0.05$) from the first bout; ns = no significant difference between bouts. The results of comparisons among groups for first (left) and second bout (right) are also shown. *$p < 0.05$.

PLASMA CK AND AST ACTIVITY AND MB CONCENTRATION

Figure 5 demonstrates changes plasma CK activity and Mb concentration. Plasma CK activity peaked 3–4 days after exercise, and Mb peaked 2–3 days after exercise for the first bout. Changes in both CK and Mb were significantly smaller following the second than the first bout for all groups, but the magnitude of the difference between bouts for the 12-w group was not as large as that of the other two groups (Figure 5). After the second bout, no significant increases were observed in CK and Mb for the 4-w and 8-w groups, but the 12-w group showed significant increases with peaks appearing at the same time as the first bout. A significant difference between the 12-w group and the 4-w or 8-w groups was evident for CK and Mb following the second bout, but no significant difference was observed between the 4-w and 8-w groups.

Discussion

The protective effect conferred by a single bout of eccentric exercise has been shown by many studies (McHugh, 2003), but the model and protocol used to examine the effect vary between studies. Although several studies have reported the protective effect by using the model of eccentric exercise of the elbow flexors, only two (Foley et al., 1999; Nosaka et al., 1991) set the interval between bouts at 4 to 12 weeks. The present study confirms the findings of those two studies (Foley et al., 1999; Nosaka et al., 1991) reporting that a second eccentric exercise bout performed more than 4 weeks after the first bout resulted in a faster recovery of strength and range of motion, reduced swelling, less development of muscle soreness, and smaller increases in muscle proteins in the blood compared with the

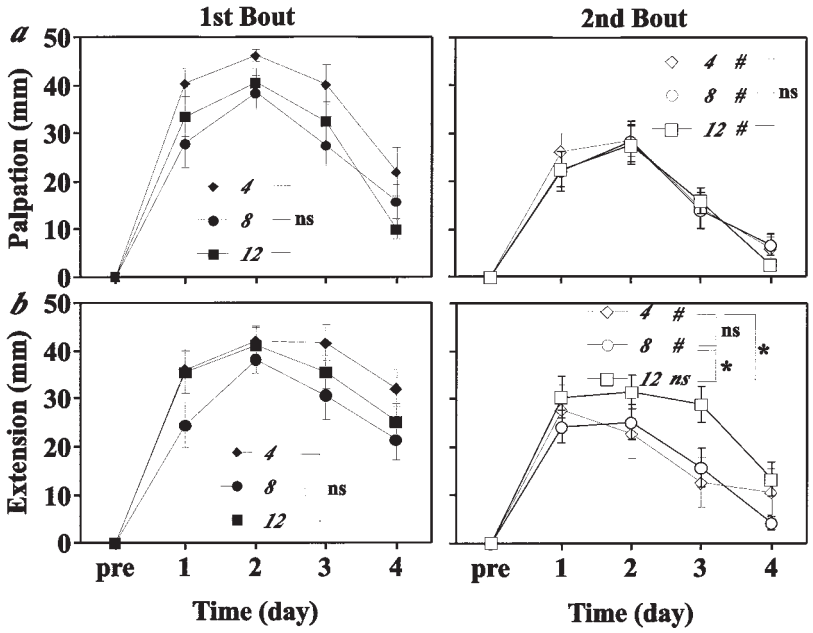

Figure 4. Changes in muscle soreness upon palpation (*a*) and extension (*b*) before (pre), and for 4 days (1–4) after the first and second exercise bouts for the 4-wk (4), 8-wk (8), and 12-wk (12) groups. Values are mean ± *SEM*. The 2nd bout graph (right) shows comparisons between bouts for each group: # = significant difference ($p < 0.05$) from the first bout; ns = no significant difference between bouts. The results of comparisons among groups for first (left) and second bout (right) are also shown. *$p < 0.05$.

initial bout. The new findings of the present study are as follows: (a) no significant differences in the protective effect were evident between 4 and 8 weeks; (b) the magnitude of the protective effect is attenuated after 8 weeks; and (c) the time course of attenuation of the protective effect varies among the criterion measures.

It is important to note that changes in the peak force and work during eccentric exercise were similar between the first and second bouts for all groups, and maximal isometric strength was similarly decreased immediately after both exercise bouts (Figure 1a). This contrasts with previous studies (Golden and Dudley, 1992; Hortobágyi et al., 1998; Warren et al., 2000) which reported smaller decreases in muscle strength immediately after the second bout compared to the first bout. These studies suggested that the protective effect stemmed from the exercise itself, and neural adaptations such as an increased activation of slow motor units were hypothesised. Alternation in recruitment in the second bout might have occurred, but the same magnitude of decrease in muscle strength in the present study (Figure 1a) does not appear to support the neural adaptation hypothesis.

Most of the previous studies (Clarkson and Tremblay, 1988; Newham et al., 1987; Nosaka et al., 1991) which used the elbow flexors to investigate the protec-

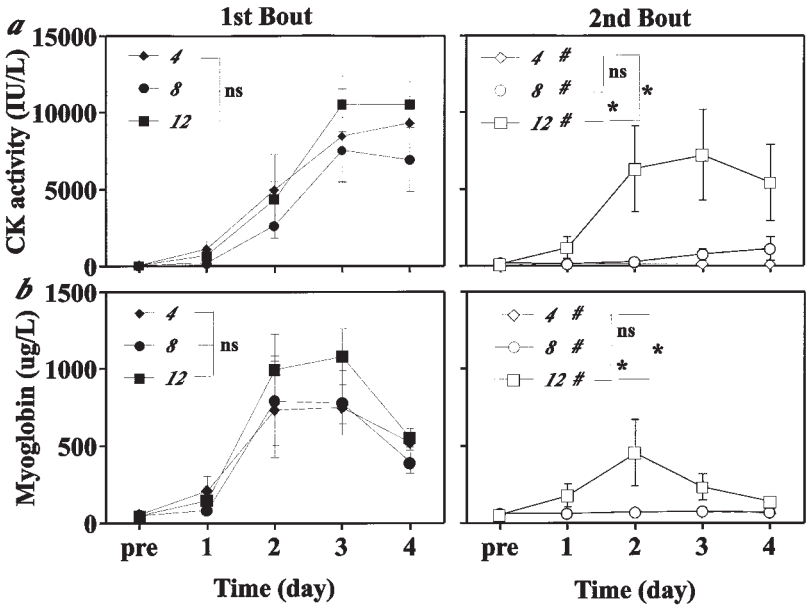

Figure 5. Changes in plasma CK activity (*a*) and myoglobin concentration (*b*) from preexercise value (pre: 0), and for 4 days (1–4) after the first and second exercise bouts for the 4-wk (4), 8-wk (8), and 12-wk (12) groups. Values are mean ± *SEM*. The 2nd bout graph (right) shows comparisons between bouts for each group: # = significant difference ($p < 0.05$) from the first bout; ns = no significant difference between bouts. The results of comparisons among groups for first (left) and second bout (right) are also shown. *$p < 0.05$.

tive effect also support findings of the present study. It is interesting that the studies reporting significantly smaller strength loss immediately after the second bout used leg muscles such as knee extensors (Golden and Dudley, 1992; Hortobágyi et al., 1998) or ankle dorsiflexors (Warren et al., 2000). The magnitude of strength loss after eccentric exercise of these leg muscles does not appear to be as large as that of the elbow flexors. It may be that leg muscles respond differently to eccentric exercise from the elbow flexors or other arm muscles, thus we should be cautious when talking about the protective effect with muscles. It is unlikely that the information provided by the elbow flexors concerning the protective effect can apply directly to other muscles.

Together with the similar strength loss between bouts, the similar changes in flexed elbow joint angle between bouts support the notion that the exercise in the present study was performed similarly between bouts (Figure 1). It is difficult to determine how much the changes in the markers immediately after exercise reflects muscle damage, but it is true that the changes were caused by exercise. Therefore the same magnitude of change in some markers immediately after exercise between bouts indicates that the initial impact of eccentric exercise on the elbow flexors was not different between bouts. It should be noted that smaller

changes in the relaxed elbow joint angle, ROM (Figure 2), and upper arm circumference (Figure 3) were already seen immediately after the second bout compared to the first bout. This would suggest that the protective effect is already functioning during exercise to reduce the changes in these measures. It seems unlikely that factors responsible for the protective effect in strength/flexed elbow joint angle, relaxed elbow joint angle, and upper arm circumference are the same.

Although the loss of strength was similar between bouts, the recovery of strength from immediately after to 1 day or 4 days after exercise was significantly larger for the second bout than the first bout (Figure 1a). It is generally accepted that increases in CK or myoglobin in the blood reflect some form of membrane damage (Newham et al., 1987). It appears that there was little membrane damage after the second exercise bout, since increases in plasma CK activity and myoglobin concentration were hardly observed after exercise which was performed 4–8 weeks after the initial bout (Figure 5). The large recovery of strength in the first 24 hrs after the second exercise bout, together with the faster recovery rate, may suggest that the affected muscle fibres were restored more rapidly to block the series of events leading to the loss of contractile proteins.

In the recovery period 1 to 4 days following the second exercise bout, all criterion measures were attenuated for the 4-w and 8-w groups, and no significant differences were found between those groups for all criterion measures (Figures 1–5). It appears there was no deterioration of the protective effect between 4 and 8 weeks. In contrast, the 12-w group did not show significant protective effects on flexed (Figure 1b) and relaxed elbow joint angles (Figure 2a), ROM (Figure 2b), circumference (Figure 3), and muscle soreness upon extension (Figure 4b).

Changes in these measures following the second bout differed significantly between the 12-w and other two groups. Although the protective effect still remained, the magnitude of this effect for the 12-w group did not appear to be strong as that demonstrated by the 4-w and 8-w groups for maximal isometric strength (Figure 1a) and CK and Mb (Figure 5). The measure that did not show any difference between the 12-w and other two groups for the second bout was only palpation soreness (Figure 4a). These results suggest that some aspects of the protective effect diminish between 8 and 12 weeks. It seems that adaptations associated with the protective effect obtained after the first bout begin to be impaired after 8 weeks, but we cannot speculate as to what factors are responsible for the adaptation.

The magnitude of protective effect on most measures except muscle soreness appears to be similar to that shown in the 4-w group when the second bout was performed at 7 days (Chen, 2003) or 14 days (Clarkson and Tremblay, 1988; Ebbeling and Clarkson, 1990; Nosaka et al., 2001a). It should be noted that eccentric exercise-induced muscle damage cannot be completely avoided even when the interval between bouts is less than 4 weeks. Some degree of muscle damage is induced after performing strenuous eccentric exercise, but it is interesting that little if any muscle damage occurs when the second bout is performed during an early recovery phase such as 5 days after the initial bout (Ebbeling and Clarkson, 1990). The protective effect observed after complete recovery of muscle function seems to differ from that conferred in the process of the recovery.

All criterion measures returned to the baseline in 4 weeks, and no differences in preexercise values for the measures were evident in the present study. The

recovery process is contingent upon the magnitude of muscle damage. When the second exercise bout is undertaken within 2 weeks, no muscle soreness occurs after exercise (Nosaka et al., 2001b). This suggests that the protective effect on muscle soreness diminishes more rapidly than other parameters of muscle damage. If one wishes to avoid delayed muscle soreness completely, he or she should perform the second exercise bout within 2 weeks after the first bout.

The present study found that muscle soreness was reduced for 8 weeks for both palpation and extension soreness, and for 12 weeks for palpation soreness only (Figure 4). Once experiencing long-lasting decreases in muscle function, the second bout does not induce as much muscle damage as the first bout, even if the same exercise is not performed for 12 weeks (Figures 1 and 2). This is encouraging for people who are afraid of suffering from severe muscle damage again. It has been shown that some of the protective effects last more than 6 months (Nosaka et al., 2001a). This long-lasting protective effect should be considered for individuals who are involved in novel or unaccustomed physical activity (Cleary et al., 2002).

Neural, mechanical, and cellular adaptations have been proposed for the underlying mechanism of the protective effect (McHugh, 2003; McHugh et al., 1999). Newham et al. (1987) have postulated that muscle fibres become more resilient and can withstand the same eccentric exercise after stress-susceptible fibres are removed and replaced by regenerated fibres. This theory seems to explain the repeated-bout effect very well, if the newly regenerated fibres become susceptible again to eccentric exercise in 8 to 12 weeks. Another theory has been suggested by Proske and Morgan (2001), that increases in sarcomere number in series are associated with the protective effect. This theory is indirectly supported by a shift of optimum angle toward a longer muscle length probably caused by increases in sarcomere number in series (Proske and Morgan, 2001); however, the duration of this adaptation has yet to be determined. It is interesting to examine whether the shift of optimum angle is maintained for 8 weeks. It seems unlikely that the protective effect can be explained by increases in sarcomere number in series alone. The different time course of the attenuation of the repeated bout effect among criterion measures described in the present study support the statement by McHugh et al. (1999) that several mechanisms are involved in the protective bout effect.

In summary, the present study showed that responses of all measures were significantly smaller than the initial eccentric exercise of the elbow flexors when the second bout was performed 4 or 8 weeks after the first bout, but only for strength, soreness upon palpation, CK, and Mb when the interbout interval was 12 weeks. This indicates that some aspects of the protective effect were attenuated after 8 weeks, but the time course of attenuation of the protective effect varies among the measures. It seems there are multiple factors involved in the protective effect, and some of the factors are associated with remodeling of muscle fibres and/or connective tissue that may occur through the process of degeneration/regeneration. As a practical application of this study, the fact that the prophylactic benefits of the protective effect are retained for 2 months without any attenuation after performing an unaccustomed exercise can be considered when designing a training program.

References

Chen, T.C. (2003). Effects of a second bout of maximal eccentric exercise on muscle damage and electromyographic activity. **Eur. J. Appl. Physiol.** 89: 115-121.

Clarkson, P.M., Nosaka, K., and Braun, B. (1992). Muscle function after exercise-induced muscle damage and rapid adaptation. **Med. Sci. Sports. Exerc.** 24: 512-520.

Clarkson, P.M., and Tremblay, I. (1988). Exercise-induced muscle damage, repair, and adaptation in humans. **J. Appl. Physiol.** 65: 1-6.

Cleary, M.A., Kimura, I.F., Sitler, M.R., and Kendrick, Z.V. (2002). Temporal pattern of the repeated bout effect on eccentric exercise on delayed-onset muscle soreness. **J. Athl. Train.** 37: 32-35.

Ebbeling, C.B., and Clarkson, P.M. (1990). Muscle adaptation prior to recovery following eccentric exercise. **Eur. J. Appl. Physiol.** 60: 26-31.

Foley, J.M., Jayaraman, R.C., Prior, B.M., Pivarnik, J.M., and Meyer, R.A. (1999). MR measurements of muscle damage and adaptation after eccentric exercise. **J. Appl. Physiol.** 87: 2311-2318.

Golden, C.L., and Dudley, G.A. (1992). Strength after bouts of eccentric or concentric actions. **Med. Sci. Sports Exerc.** 4: 926-933.

Hortobágyi, T., Houmard, J., Fraser, D., Dudek, R., Lambert, N., and Tracey, J. (1998). Normal forces and myofibrillar disruption after repeated eccentric exercise. **J. Appl. Physiol.** 84: 492-498.

McHugh, M.P. (2003). Recent advances in the understanding of the repeated bout effect: The protective effect against muscle damage from a single bout of eccentric exercise. **Scand. J. Med. Sci. Sports** 13: 88-97.

McHugh, M.P., Connolly, D.A.J., Eston, R.G., and Gleim, G.W. (1999). Exercise-induced muscle damage and potential mechanisms for the repeated bout effect. **Sports Med.** 27: 157-170.

Newham, D.J., Jones, D.A., and Clarkson, P.M. (1987). Repeated high-force eccentric exercise: Effects on muscle pain and damage. **J. Appl. Physiol.** 63: 1381-1386.

Nosaka, K., and Clarkson, P.M. (1996). Changes in indicators of inflammation after eccentric exercise of the elbow flexors. **Med. Sci. Sports Exerc.** 28: 953-961.

Nosaka, K., Clarkson, P.M., McGuiggin, M.E., and Byrne, J.M. (1991). Time course of muscle adaptation after high force eccentric exercise. **Eur. J. Appl. Physiol.** 63: 70-76.

Nosaka, K., Newton, M., and Sacco, P. (2001b). How long does the protective effect on eccentric exercise-induced muscle damage last? **Med. Sci. Sports Exerc.** 33: 1490-1495.

Nosaka, K., Sakamoto, K., Newton, M., and Sacco, P. (2001a**)**. The repeated bout effect of reduced-load eccentric exercise on muscle damage of the elbow flexors. **Eur. J. Appl. Physiol.** 85: 34-40.

Pizza, F.X., Davis, B.H., Henrickson, S.D., Mitchell, J.B., Page, J.F., Bigelow, N., DiLauro, P., and Naglieri, T. (1996). Adaptation to eccentric exercise: Effect on CD64 and CD11b/CD18 expression. **J. Appl. Physiol.** 80: 47-55.

Proske, U., and Morgan, D.L. (2001). Muscle damage from eccentric exercise: Mechanism, mechanical signs, adaptation and clinical applications. **J. Physiol.** 537: 333-345.

Warren, G.L., Hermann, K.M., Ingalls, C.P., Masselli, M.R., and Armstrong, R.B. (2000). Decreased EMG median frequency during a second bout of eccentric contractions. **Med. Sci. Sports Exerc.** 32: 820-829.

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