# Potent Protective Effect Conferred by Four Bouts of Low-Intensity Eccentric Exercise

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## ABSTRACT

CHEN, T. C., H.-L. CHEN, M.-J. LIN, C.-J. WU, and K. NOSAKA. Potent Protective Effect Conferred by Four Bouts of Low-Intensity Eccentric Exercise. *Med. Sci. Sports Exerc.*, Vol. 42, No. 5, pp. 1004–1012, 2010. **Purpose**: It is known that submaximal eccentric exercise does not confer as strong a protective effect as maximal eccentric exercise. This study tested the hypothesis that four bouts of submaximal eccentric exercise would confer a similar protective effect to one bout maximal eccentric exercise. **Methods**: Thirty untrained men were placed into 4 × 40% (40%) or control (CON) groups ($n$ = 15 per group) by matching preexercise maximal voluntary isometric contraction strength (MVC). The 40% group performed 30 eccentric contractions with a load of 40% MVC (40% ECC) every 2 wk for four times followed 2 wk later by 30 maximal eccentric exercise (100% ECC) of the elbow flexors of the nondominant arm. The CON group performed two bouts of the 100% ECC separated by 2 wk. MVC at six angles, optimum angle (OA), concentric isokinetic strength ($30°\cdot s^{-1}$ and $300°\cdot s^{-1}$), range of motion, upper arm circumference, plasma creatine kinase activity and myoglobin concentration, muscle soreness, and echo intensity of B-mode ultrasound images were taken before to 5 d after each exercise. **Results**: No significant differences in the changes in any measures were evident between the 100% ECC of the 40% group and the second 100% ECC of the CON group. Changes in all measures except for OA and upper arm circumference after the second to the fourth 40% ECC bouts were significantly smaller than those after the first 40% ECC bout. The changes in the measures after any of the 40% ECC bouts were significantly ($P < 0.05$) smaller than those after the first 100% ECC bout of the CON group. **Conclusions**: These results suggest that repeating submaximal eccentric exercise confers the same magnitude of protective effect as one bout of maximal eccentric exercise against the subsequent maximal eccentric exercise. **Key Words:** MUSCLE DAMAGE, MUSCLE STRENGTH, OPTIMUM ANGLE, DELAYED ONSET MUSCLE SORENESS, REPEATED BOUT EFFECT, ELBOW FLEXORS

A bout of eccentric exercise confers a protective effect against muscle damage in the subsequent bout of the same or more demanding exercise, which is also known as the repeated bout effect (7,14,15,18). Typical signs of the protective effect include a faster recovery of muscle strength and range of motion (ROM), less swelling of the muscle, smaller increases in muscle proteins such as creatine kinase (CK) activity in the blood, less development of delayed onset muscle soreness (6,7,9,20), and less abnormality in echo intensity of B-mode ultrasound and/or magnetic resonance images (6,20,21).

It has been reported that the magnitude of the protective effect is dependent on the magnitude of muscle damage in the initial bout, and the greater the damage in the initial bout, the greater the attenuation of muscle damage in the second bout (5,19). Chen et al. (5) showed that a submaximal eccentric exercise bout consisting of 30 lengthening contractions with a load of 40% maximal isometric strength reduced the changes in indirect markers of muscle damage by 20%–60% after a subsequent bout of maximal eccentric exercise that was performed 2 wk later. However, the magnitude of the protective effect was significantly less than that conferred by the maximal eccentric exercise that attenuated the changes in the markers by 65%–100%.

In resistance training programs, maximal eccentric exercise is generally performed after submaximal-intensity eccentric exercise sessions (28). Thus, it seems likely that severe muscle damage can be avoided to some extent. However, it is not known to what extent submaximal-intensity eccentric exercise bouts can confer a protective effect against maximal eccentric exercise. It is possible that repeating low-intensity (e.g., 40%) eccentric exercise several times can confer more protective effect than a single bout of low-intensity eccentric exercise and provide the same magnitude of protective effect as one bout of maximal eccentric exercise.

Therefore, this study investigated the hypothesis that four bouts of submaximal (40%) eccentric exercise performed every 2 wk would confer a similar protective effect to one

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bout of maximal eccentric exercise against a subsequent bout of maximal eccentric exercise performed 2 wk later.

## METHODS

### Subjects and Study Design

Thirty men who had not performed regular resistance training in the past 1 yr provided informed consent to participate in this study that was approved by the institutional ethics committee. The study was conducted in conformity with the policy statement regarding the use of human subjects by *Medicine & Science in Sports & Exercise®*. The subjects were screened to confirm that they had no neuromuscular diseases and musculoskeletal problems for the nondominant upper extremity before their participation in this study. The subjects' mean ± SD age, height, and body weight were 22.2 ± 2.0 yr, 174.6 ± 5.6 cm, and 69.4 ± 9.0 kg, respectively. On the basis of the baseline maximal voluntary isometric contraction strength (MVC-ISO) of the elbow flexors at the elbow joint of 90° (1.57 rad), the subjects were placed into two groups ($n$ = 15 per group): 4 × 40% eccentric exercise (40%) and control (CON) groups. No significant differences in age, height, body mass, and MVC-ISO were evident between the groups. The subjects were asked to refrain from unaccustomed exercise or vigorous physical activity and not to take any antiinflammatory drugs or nutritional supplements during the experimental period.

The sample size was estimated using the data from a previous study in which a similar dumbbell eccentric exercise was performed by 41 men (4). On the basis of the effect size of 1, $\alpha$ level of 0.05, and a power ($1 - \beta$) of 0.80, with a potential difference of 10% for the MVC-ISO at 5 d after exercise after maximal eccentric exercise between groups, it was found that 12 subjects per group were necessary.

### Eccentric Exercise

The eccentric exercise protocol was adopted from a previous study (5). To determine a dumbbell weight for eccentric exercise, each subject was seated on a custom-made preacher curl bench with his shoulder joint angle at 45° (0.79 rad) flexion with 0° abduction, and a cuff connected to a load cell (Model DFG51; Omega Engineering, Stamford, CT) was attached to the wrist of the nondominant arm. The elbow joint angle was set at 90° (1.57 rad), and the subject was asked to flex the elbow joint maximally while keeping the forearm supinated. This measurement was taken immediately before the eccentric exercises (40% and 100% bouts), three times with a 45-s rest between trials for each occasion, and the average of the three measurements was used to determine a dumbbell weight for 40% and 100% bouts. The subjects in the CON group performed two bouts of exercise with the 100% load (100% ECC) separated by 2 wk, and the same weight was used for both bouts. In contrast, the subjects in the 40% group performed four bouts of exercise with the 40% load (40% ECC) once every 2 wk before performing a bout of 100% ECC 2 wk after the last 40% ECC bout.

The subjects were instructed to lower the dumbbell from an elbow flexed (50°, 0.87 rad) to an extended position (170°, 2.97 rad) in approximately 4–5 s. Subsequently, the investigator removed the dumbbell from the arm, and the subject returned the arm to the start position for the next eccentric contraction without the dumbbell. Subjects were verbally encouraged and guided to lower the dumbbell at a consistent velocity by following the count given by the investigator. The movement was repeated 30 times with a 45-s rest between contractions.

### Dependent Variables

The dependent variables consisted of MVC-ISO at six different elbow joint angles as described below, maximal isokinetic concentric contraction strength (MVC-CON) at $30° \cdot s^{-1}$ ($0.52\ rad \cdot s^{-1}$) and $300° \cdot s^{-1}$ ($5.22\ rad \cdot s^{-1}$), active ROM of the elbow joint, upper arm circumference, plasma CK activity and myoglobin (Mb) concentration, muscle soreness, and echo intensity of B-mode ultrasound images. All muscle strength measures, ROM, and upper arm circumference measures were taken before, immediately after, and at 1, 2, 3, 4, and 5 d after each exercise. The ultrasound images were taken before and at 2 and 5 d after each exercise, and blood samples for CK and Mb and muscle soreness measurements were taken before and at 1, 2, 3, 4, and 5 d after each exercise.

**Muscle strength.** Each subject was seated on the chair of the isokinetic dynamometer (Biodex System 3 Pro; Biodex Medical Systems, Shirley, NY), with their trunk stabilized by a pelvic strap and two shoulder straps to minimize the involvement of other body parts. The shoulder joint angle was set at 45° (0.79 rad) flexion with 0° abduction, and the forearm was kept supinated with the wrist placed on an attachment connected to the level arm of the isokinetic dynamometer. MVC-ISO was measured at 50° (0.87 rad), 70° (1.22 rad), 90° (1.57 rad), 110° (1.92 rad), 140° (2.44 rad), and 160° (2.97 rad) elbow joint angles (where the full elbow extension angle was considered as 180° = 3.14 rad) in a random order (6). The subjects were asked to generate maximal force for 3 s three times with a 45-s rest between attempts for each angle and a 2-min rest between different angles.

Using the same equipment and subject settings as those described for the MVC-ISO, MVC-CON was measured at an angular velocity of $30° \cdot s^{-1}$ and $300° \cdot s^{-1}$ in a random order for the ROM from 180° (3.14 rad) to 50° (0.87 rad). Two measurements were taken for each velocity with a 45-s rest between contractions and a 2-min rest between the velocities. In MVC-ISO and MVC-CON measures, strong verbal encouragement was provided during force generation. The peak torque of each contraction was identified using the software of the dynamometer (Systems 3 Application

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Software for Window XP; Biodex Medical Systems, Inc., Shirley, NY), and the highest value of the three MVC-ISO measurements for each angle and the higher value of the two MVC-CON measurements at each velocity were used for further analysis.

**Optimum angle.** The optimum angle (OA) of the elbow flexors was calculated from the MVC-ISO obtained from the six different angles using a fitted quadratic polynomial equation (5). Briefly, the OA was calculated by the fitted quadratic polynominal equation for the six MVC-ISO of each subject for each time point: force = $a + bA + cA^2$, and MVC-ISO at the OA was also obtained, where $A$ represents elbow joint angle and $a$, $b$, and $c$ are the fitted polynominal parameters.

**Elbow joint angles and ROM.** On the basis of a previous study (12), flexed elbow joint angle (FANG) was measured when the subject tried to touch his shoulder of the same side by flexing the elbow joint maximally while keeping the elbow joint at the side of the body. Extended elbow joint angle (EANG) was measured when the subject attempted to extend his elbow joint as much as possible with the elbow held by his side and the hand in mid pronation. The FANG and EANG were assessed with a plastic goniometer three times for each time point, and the average of the three measurements was calculated to obtain ROM, which was the difference between FANG and EANG.

**Upper arm circumference.** Upper arm circumference was assessed at 8 cm above the elbow joint with a Gulick tape measure while the subject was standing and allowed the arm to hang down by the side of the hips (5,18). The measurement point was marked on the subject's arm to ensure consistent placement of the tape measure. The average value of three measurements was used for further analysis.

**Muscle soreness.** The level of muscle soreness of the exercised arm was assessed using a visual analog scale consisting of a 100-mm line representing "no pain" at one end (0 mm) and "very, very painful" at the other (100 mm). The subjects were asked to indicate the level pain on the line when the investigator extended the elbow joint maximally. The same investigator assessed the muscle soreness over time for all subjects, and the procedure was standardized as described in previous study (5).

**Plasma CK activity and Mb concentration.** Approximately 10 mL of venous blood was withdrawn by a standard venipuncture technique from the cubital fossa region of the dominant arm and centrifuged for 10 min to extract plasma, which was stored at −80°C until analyses. Plasma CK activity was assayed spectrophotometrically by an automated clinical chemistry analyzer (Model 7080; Hitachi Co., Ltd., Tokyo, Japan) using a test kit (catalog no. 12132672; Roche Diagnostics, Indianapolis, IN). Plasma Mb concentration was measured by an automated clinical chemistry analyzer (Model Elecsys 2010; F. Hoffmann-La Roche, Ltd., Tokyo, Japan) using a test kit (catalog no. 12178214; Roche Diagnostics). Each sample was analyzed in duplicate, and the average value of two measures was used for subsequent statistical analysis (6). The reference ranges for plasma CK and Mb in men are 38–174 $\mathrm{IU{\cdot}L^{-1}}$ and <110 $\mu\mathrm{g{\cdot}L^{-1}}$, respectively, based on the manufacturer's information.

**Ultrasonography.** B-mode ultrasound images were obtained from the exercised upper arm by a Terason t3000 Ultrasound System (Terason Co., Burlington, MA) with a 7.5-MHz linear probe. The probe was placed on the upper arm between 4 and 8 cm from the elbow joint while each subject was sitting on a chair with the forearm on an armrest. The gains and contrast were kept constant during the experimental period, and all images were saved to a computer (HP Workstation xw4400; Singapore) and analyzed by a computer image analysis software (ULT File Reader for Windows; Broadsound Co, Taiwan). According to a previous study (6), the mean echo intensity of a histogram of gray scale (0 = black, 256 = white) for the region of interest (ROI: 2 × 2 = 4 $\mathrm{cm}^2$) located above the humerus was obtained, and the change in the echo intensity from the preexercise value was calculated for each subject. It was expected that the echo intensity would increase after eccentric exercise as shown in a previous study (6).

The coefficient of variation for MVC-ISO at six different angles, MVC-CON, ROM, upper arm circumference, muscle soreness, and plasma CK activity and Mb concentration was 8.4%–12.3%, 9.7%–12.2%, 5.0%, 4.3%, 0%, 15.8%, and 12.3%, respectively.

### Statistical Analyses

The preexercise values of each dependent variable were compared among all exercise bouts (first to fourth 40% ECC and the 100% ECC bouts of the 40% group, first and second 100% ECC bouts of the control group) by a one-way ANOVA with a Bonferroni *post hoc* test. Changes in the dependent variables after exercise were compared 1) among the four 40% ECC bouts (40% first to fourth), 2) between the first 40% ECC bout and the subsequent 100% ECC bout of the 40% group (40%–first, 40%–100%), 3) between the first 40% ECC bout of the 40% group and the first 100% ECC bout of the CON group (40%–first, 100%–first), and 4) among the 100% ECC bout of the 40% group and the first and second 100% ECC bouts of the CON group (40%–100%, 100%–first, 100%–second) by a two-way ANOVA. When the ANOVA indicated a significant effect (bout, time, or bout × time), a Bonferroni *post hoc* test was performed. Statistical significance was set at $P < 0.05$. Data are presented as mean ± SEM, unless otherwise stated.

## RESULTS

No significant differences in the preexercise values of any dependent variables were evident among bouts (40% first to fourth, 40%–100%, 100%–first, 100%–second).

This suggests that the subjects were fully recovered from the previous bout when performing the subsequent bout.

## Comparison among Four 40% ECC Bouts of the 40% Group and the First 100% ECC Bout of the Control Group

**OA, MVC, and ROM.** Figure 1A compares changes in OA among four 40% ECC bouts and the first 100% bout of the control group. OA changed significantly after each exercise bout with the greatest shift to a longer muscle length at 1–2 d after exercise, but the changes were not significantly different among the four 40% ECC bouts. The changes in OA after the first 100% bout of the control group were significantly greater than those after the 40% bouts. Figure 1B shows changes in MVC-ISO at the OA after each exercise bout. MVC-ISO decreased significantly immediately after each 40% ECC exercise bout (40%–first: 29% ± 4%, 40%–second: 25% ± 3%, 40%–third: 22% ± 3%, and 40%–fourth: 15% ± 2%), but the magnitude of the decrease was significantly smaller for the fourth bout compared with the first bout. The recovery of MVC-ISO after the second to fourth bouts was significantly faster than that after the first bout without significant difference across the second to fourth bouts. The magnitude of the decrease MVC-ISO after the first 100% bout of the control group was significantly greater than that of the 40% bouts. The time course of the changes in MVC-ISO was similar among the angles, and the changes in MVC-CON at two different angular velocities were similar to those in MVC-ISO. The preexercise MVC-CON was significantly greater for $30°\cdot s^{-1}$ compared with $300°\cdot s^{-1}$, but the normalized changes were not significantly different between the velocities.

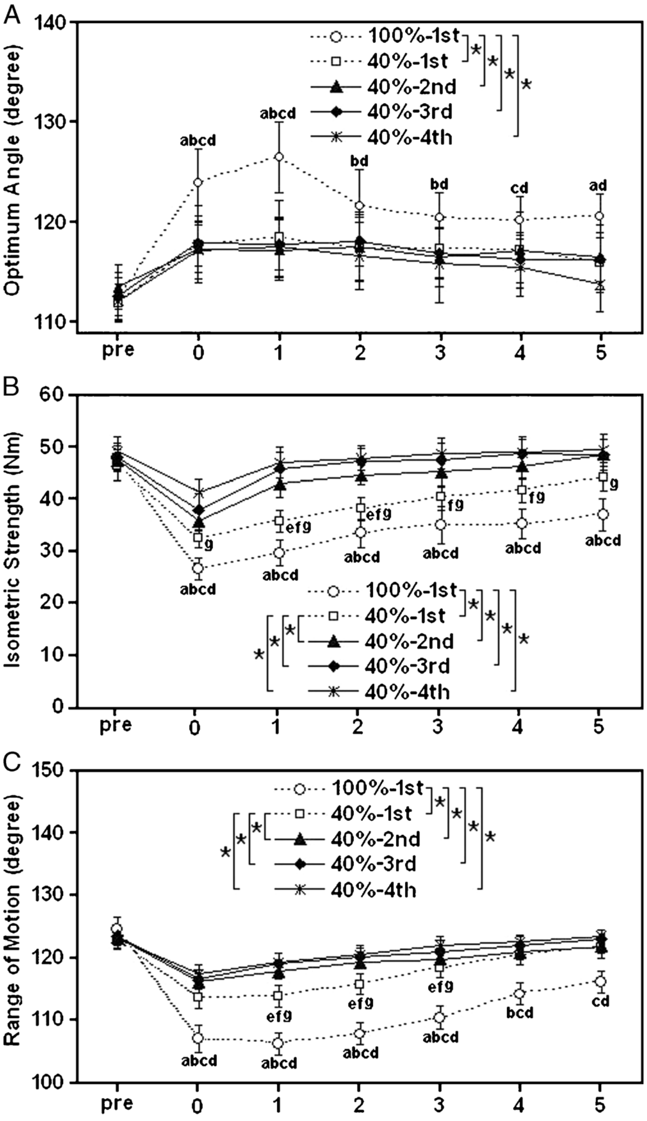

**FIGURE 1—Changes in OA (A), maximal voluntary isometric contraction strength at the OA (B), and ROM (C) before (pre), immediately after (0) and 1–5 d after first (40%–1st), second (40%–2nd), third (40%–3rd), and fourth 40% bouts (40%–4th) of the 40% group and the first 100% bout of the control group (100%–1st). *Significant difference ($P$ < 0.05) between bouts on the basis of the bout × time interaction effect shown by the ANOVA. On the basis of the *post hoc* tests, the time points showing a significant ($P$ < 0.05) difference between the 100%–1st and 40%–1st, 40%–2nd, 40%–3rd, or 40%–4th bout are shown in *a*, *b*, *c*, and *d*, respectively. Likewise, the time points showing a significant ($P$ < 0.05) difference between the 40%–1st and 40%–2nd, 40%–3rd, or 40%–4th bout are shown in *e*, *f*, and *g*, respectively.**

As shown in Figure 1C, ROM decreased significantly immediately after each 40% ECC exercise bout (40%–first: −9° ± 2°, 40%–second: −6° ± 1°, 40%–third: −6° ± 1°, and 40%–fourth: −5° ± 1°), but the decrease was significantly smaller for the fourth bout compared with the first bout. The recovery of ROM after the second to fourth bouts was significantly faster than that after the first bout. The decreases in ROM after the first 100% bout of the control group were significantly greater than those shown after the 40% bouts.

**Upper arm circumference, muscle soreness, and echo intensity.** As shown in Figure 2A, the first 40% ECC bout resulted in small (<10 mm) but significant increases in upper arm circumference without significant difference among the bouts. The increases in the circumference after the first 100% bout of the control group were significantly greater than those after the 40% bouts.

Muscle soreness developed after all bouts, but the extent of soreness was significantly smaller after the second to fourth 40% ECC bouts compared with the first bout (Fig. 2B). The magnitude of muscle soreness after the first 100% bout of the control group was significantly greater than that after the 40% bouts.

Echo intensity increased significantly after the first and second 40% ECC bouts, but the third and fourth bouts did not result in significant changes (Fig. 2C). No significant difference was evident between the first and second bouts and between the third and fourth bouts. The increases in the echo intensity were significantly greater after the first 100% bout of the control group compared with the 40% bouts.

**Plasma CK activity and Mb concentration.** As shown in Figure 3, significant increases in plasma CK activity and Mb concentration were evident after the first 40% ECC of the 40% group and the first 100% ECC of the control group, and no significant changes were evident after the second to fourth 40% ECC bouts. The increases in plasma CK activity and Mb concentration after the first 40% ECC bout (peak CK: 582 ± 92 $\text{IU}\cdot\text{L}^{-1}$; peak Mb: 178.8 ± 40.4 $\mu\text{g}\cdot\text{L}^{-1}$) were significantly smaller than those after the first 100% ECC bout of the control group (peak CK: 5958 ± 607 $\text{IU}\cdot\text{L}^{-1}$; peak Mb: 1271 ± 144 $\mu\text{g}\cdot\text{L}^{-1}$).

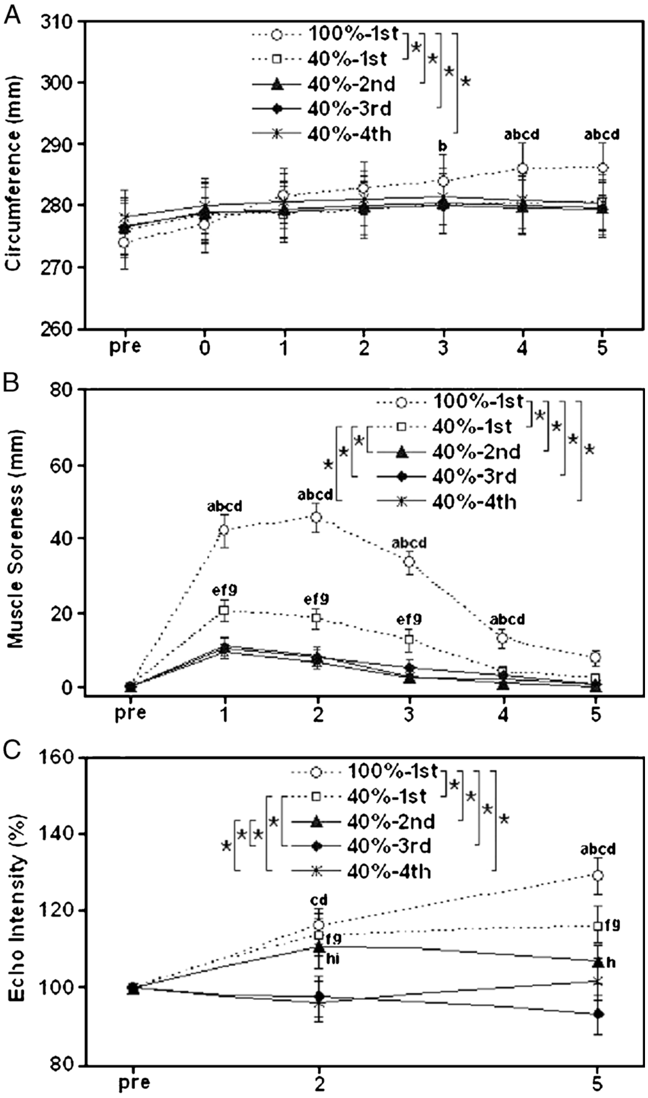

**FIGURE 2—Changes in upper arm circumference (A), muscle soreness (B), and echo intensity of B-mode ultrasound (C) before (pre), immediately after (0) and 1–5 d after first (40%–1st), second (40%–2nd), third (40%–3rd), and fourth 40% bouts (40%–4th) of the 40% group and the first 100% bout of the control (100%–1st) group. *Significant difference ($P$ < 0.05) between bouts on the basis of the bout × time interaction effect shown by the ANOVA. On the basis of the *post hoc* tests, the time points showing a significant ($P$ < 0.05) difference between the 100%–1st and 40%–1st, 40%–2nd, 40%–3rd, or 40%–4th bout are shown in *a*, *b*, *c*, and *d*, respectively. Likewise, the time points showing a significant ($P$ < 0.05) difference between the 40%–1st and 40%–2nd, 40%–3rd, or 40%–4th bout are shown in *e*, *f*, and *g*, respectively, and between 40%–2nd and 40%–3rd or 40%–4th bout are shown in *h* and *i*, respectively.**

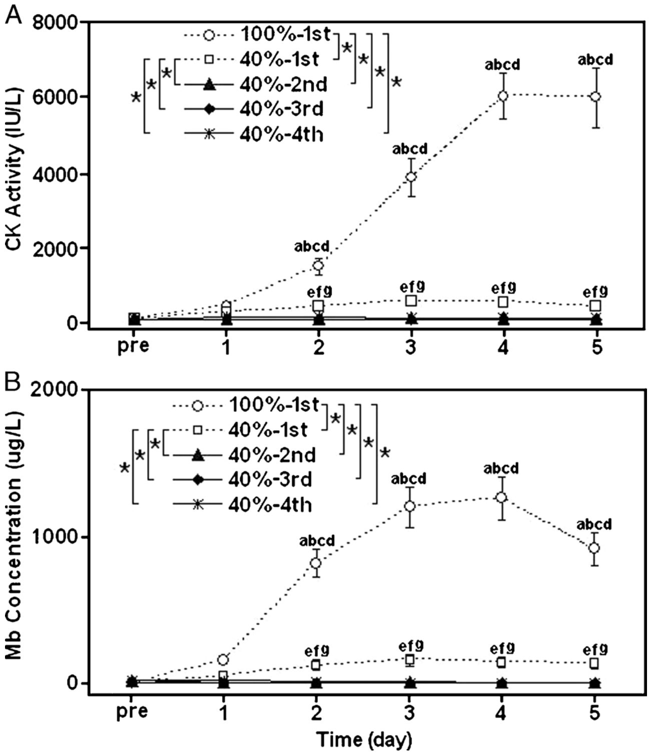

**FIGURE 3—Changes in plasma CK activity (A) and Mb concentration (B) before (pre) and 1–5 d after first (40%–1st), second (40%–2nd), third (40%–3rd), and fourth 40% bouts (40%–4th) of the 40% group and the first 100% bout of the control (100%–1st) group. *Significant difference ($P$ < 0.05) between bouts on the basis of the bout × time interaction effect shown by the ANOVA. On the basis of the *post hoc* tests, the time points showing a significant ($P$ < 0.05) difference between the 100%–1st and 40%–1st, 40%–2nd, 40%–3rd, or 40%–4th bout are shown in *a*, *b*, *c*, and *d*, respectively. Likewise, the time points showing a significant ($P$ < 0.05) difference between the 40%–1st and 40%–2nd, 40%–3rd, or 40%–4th bout are shown in *e*, *f*, and *g*, respectively.**

### Comparison among the First 40% ECC Bout and the 100% ECC Bout of the 40% Group, and the First and Second 100% ECC Bouts of the CON Group

When comparing between the first 40% ECC and 100% ECC bouts of the 40% group, changes in plasma CK activity and Mb concentration and echo intensity after the 100% ECC bout were significantly smaller than those after the first 40% ECC bout (Fig. 4). However, no significant differences were evident for the changes in other variables (i.e., OA, muscle strength, ROM, muscle soreness, upper arm circumference) between the bouts.

Figure 4 also depicts the comparisons among the 100% bout of the 40% group and the first and second 100% ECC bouts of the CON group. As shown in Figure 4A, OA changed significantly after exercise, but the changes were not significantly different among the bouts. Figure 4B shows changes in MVC-ISO at the OA after each maximal exercise bout. MVC-ISO decreased significantly immediately after 100% ECC bout (100%–first: 46% ± 5%, 100%–second: 35% ± 4%, 40%–100%: 32% ± 4%), but the

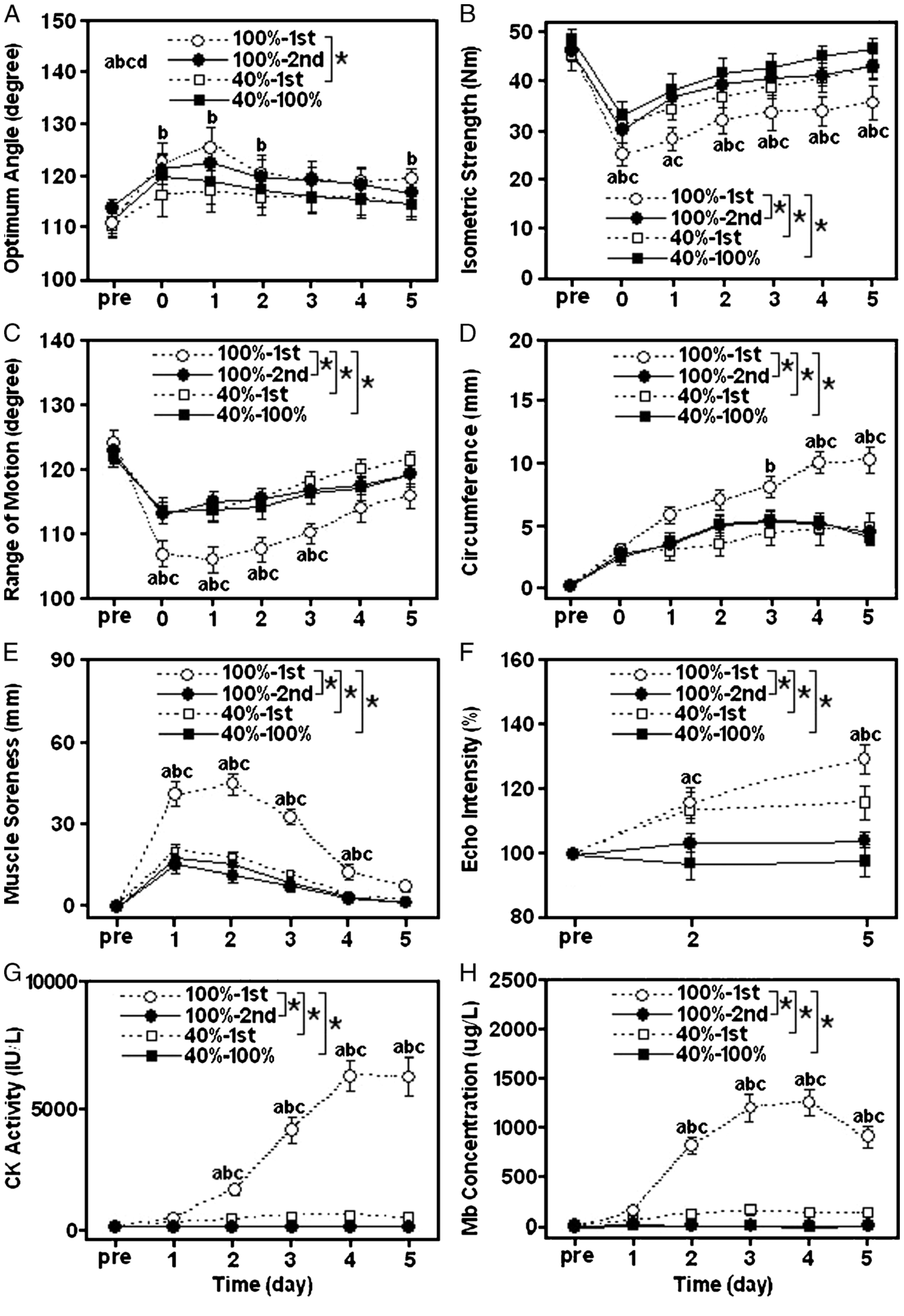

**FIGURE 4—Changes in OA (A), maximal voluntary isometric contraction strength at the OA (B), ROM (C), upper arm circumference (D), muscle soreness (E), echo intensity (F), plasma CK activity (G), and plasma Mb concentration (H) before (pre), immediately after (0), and 1–5 d after the first (100%–1st) and second maximal eccentric exercise (100%–2nd) bouts of the control group and the first 40% bout (40%–1st) and the 100% bout of the 40% group (40%–100%). *Significant difference ($P < 0.05$) between bouts on the basis of the bout × time interaction effect shown in the ANOVA. On the basis of the *post hoc* tests, the time points showing a significant ($P < 0.05$) difference between the 100%–1st and 100%–2nd, 40%–1st, or 40%–100% bout are shown in *a*, *b*, and *c*, respectively.**

magnitude of the decrease was significantly greater for the first 100% ECC bout of the CON group compared with others. The recovery of MVC-ISO after the second 100% ECC bout of the CON group and the 100% ECC bout of the 40% group was significantly faster than that of the first 100% ECC bout of the CON group. No significant difference was evident between the second 100% ECC bout of the CON group and the 100% ECC bout of the 40% group. This was also the case for MVC-CON.

ROM decreased significantly immediately after exercise (100%–first: −18° ± 2°, 100%–second: −10° ± 1°, 40%–100%: −8° ± 1°), but the decrease was significantly greater for the first 100% ECC bout of the CON group compared with others (Fig. 4C). The recovery of ROM after the second 100% ECC bout of the CON group and the 100% ECC bout of the 40% group was significantly faster than that after the first 100% ECC bout of the CON group. No significant difference between the second 100% ECC bout of the CON group and the 100% ECC bout of the 40% group was observed.

Significant increases in upper arm circumference were found after all bouts; however, the second 100% ECC bout of the CON group and the 100% ECC bout of the 40% group showed significantly smaller increases compared with the first 100% ECC bout of the CON group (Fig. 4D). No significant difference between the second 100% ECC bout of the CON group and the 100% ECC bout of the 40% group was evident.

Muscle soreness developed after all bouts, but the degree of soreness was significantly smaller after the second 100% ECC bout of the CON group and the 100% ECC bout of the 40% group compared with the first 100% ECC bout of the CON group, without significant difference between the two (Fig. 4E). As demonstrated in Figure 4F, significant increases in echo intensity were seen only after the first 100% ECC bout of the CON group.

Figures 4G and H show that increases in plasma CK activity and Mb concentration were significant only after the first 100% ECC bout of the CON group.

## DISCUSSION

This study examined whether four bouts of submaximal eccentric exercise (40% ECC) performed every 2 wk would confer a similar extent of protective effect to a bout of maximal eccentric exercise (100% ECC) against subsequent bout of maximal eccentric exercise. As shown in Figure 4, no significant differences in the changes in any measures were evident between the 100% ECC bout of the 40% group, which was performed after four 40% ECC bouts, and the second 100% ECC bout of the CON group. These results support the hypothesis and show that four bouts of the 40% ECC conferred a similar protective effect to one bout of 100% ECC. It is also important to note that the magnitude of muscle damage in the first 40% ECC bout was much less than that of the first 100% ECC bout of the CON group (Figs. 1–4).

Our previous study (5) showed that the magnitude of protective effect conferred by the first eccentric exercise bout was intensity-dependent such that the protective effect conferred by 40% ECC was significantly smaller than that by 100% ECC. However, the 40% ECC was still effective in providing approximately half of the protective effect of that induced by the 100% ECC (5). The changes in the dependent variables after the first 40% ECC bout (Figs. 1–3) were similar to those shown in our previous study (5) in which the same 40% eccentric exercise was performed. The present study showed that when the 40% ECC was repeated four times, the magnitude of the protective effect was similar to one bout of 100% ECC (Fig. 4). This suggests that the magnitude of the protective effect is not necessarily dependent on the magnitude of the initial muscle damage. It seems that the intensity-dependent aspect of the protective effect is not applicable to the situation when low-intensity eccentric exercise is repeated.

The question is why the additional protective effect was produced by the additional three 40% ECC bouts compared with the effect induced by a single 40% ECC bout shown in the previous study (5). As shown in Figures 1–3, changes in all measures except for OA and upper arm circumference were significantly smaller after the second to fourth 40% ECC bouts compared with the first 40% ECC bout. Similar results were found in our recent study (6) in which the responses to four maximal eccentric exercise bouts performed every 4 wk were compared. That study (6) showed that changes in the muscle damage markers were greatest after the first bout, and only minor differences existed among the second, third, and fourth bouts. It seems likely that the magnitude of muscle damage induced by the repeated 40% ECC bouts was less than that by the first 100% ECC of the CON group, even if the accumulated muscle damage in the four bouts were taken into account. Thus, it seems that the additional protective effect conferred by the second to fourth 40% ECC bouts was produced with little or no muscle damage.

Lavender and Nosaka (12) reported that a protective effect was conferred by eccentric exercise resulting in no significant changes in markers of muscle damage. In their study, one group of subjects performed eccentric exercise of the elbow flexors using a dumbbell set at 10% of MVC, followed 2 d later by an eccentric exercise with a dumbbell weighted at 40% MVC. No significant changes in muscle strength, ROM, upper arm circumference, muscle soreness, and plasma CK activity were found immediately and 1–2 d after the 10% exercise, but changes in muscle strength, ROM, and muscle soreness after the eccentric exercise with a heavier load (40% MVC) were significantly smaller compared with the group that performed the 40% exercise without the 10% eccentric exercise. It should be noted that some protective effect is induced without muscle damage. It is possible that repeating "nondamaging" eccentric exercise can provide an even greater protective effect than one bout of "nondamaging" eccentric exercise. It seems that the combination of the first 40% ECC bout that resulted in minor damage and the second to fourth 40% ECC bouts that resulted in little or no damage provided the same magnitude of protective effect as one bout of 100% ECC. It would be interesting to investigate further if two or three bouts of the 40% ECC can confer the same magnitude of protection to that shown by the four bouts.

McHugh (14) documented that the underlying mechanisms of the protective effect could be a combination of neural, mechanical, and cellular adaptations. The neural adaptations include more efficient recruitment of motor units, increased synchrony of motor unit firing, better distribution of the workload among muscle fibers, improved usage of synergist muscles, and increased slow-twitch fiber recruitment (3,15,27). Mechanical adaptations include increases in passive or dynamic muscle stiffness, remodeling of intermediate filament system, and increased intramuscular connective tissue (14). Because the present study did not include any measures to assess the neural and mechanical adaptations, it is unknown whether the four 40% ECC bouts induced any of these adaptations. Black and McCully (2) have recently shown that changes in T2 relaxation time in magnetic resonance image, which is indicative of inflammation, and muscle soreness after 80 eccentric contractions with electrical muscle stimulation were attenuated in the second bout that was performed 7 wk after the first bout. This may suggest the involvement of neural factors in the protective effect is minor. The possibility of mechanical adaptations should be investigated further.

Cellular adaptations refer to longitudinal addition of sarcomeres, adaptation in inflammatory response, adaptation to maintain excitation–contraction (E–C) coupling, strengthened plasma membrane, increased protein synthesis, increased stress proteins (e.g., heat shock proteins), and removal of stress-susceptible fibers (1,16,23,26). Proske and Morgan (23) stated that increases in sarcomere number in the series were related to the protective effect and that the increases in sarcomeres could be indirectly assessed by a shift of OA toward a longer muscle length. However, in the present study, the preexercise OA was not significantly different among the first and second 100% ECC bouts and the 100% ECC bout of the 40% group (Fig. 4A). Chen et al. (5) also showed that a shift of OA to a longer muscle length was not necessarily a prerequisite for the conferral of protective effect because the repeated bout effect was still evident without any shift of the OA. Thus, it seems unlikely that the longitudinal addition of sarcomeres was a main contributor for the protective effect. It has been speculated that cytoskeletal proteins such as titin, desmin, talin, and vinculin are remodeled after the initial eccentric exercise bout to protect future injury (2,13). Future studies are warranted to examine that these adaptations are induced by an eccentric exercise that does not result in severe muscle damage.

Previous studies (6,19) have documented that the magnitude of decrease in muscle strength immediately after eccentric exercise does not necessarily represent the extent of muscle damage. Ingalls et al. (10) showed that E–C coupling failure was the major contributor (57%–75%) to the force deficit in the first 5 d after lengthening contractions of mouse extensor digitorum longus muscles. If this is the case for the eccentric exercise of the elbow flexors in humans, it seems possible that E–C coupling failure is attenuated by repeating the 40% ECC bouts, although it is not understood how the adaptation is induced.

If the increases in CK and Mb indicate plasma membrane damage (7), no increases in these proteins after exercise (Figs. 4G and H) suggest that plasma membrane damage occurred only after the first 40% ECC bout. It may be that some adaptation to plasma membrane was induced by the first 40% ECC bout. Adaptations in inflammatory responses and maintenance of calcium ion homeostasis in muscle fibers, increased stress proteins, and removal of stress-susceptible fibers (11,16,22,26) may also be associated with no increases in plasma CK activity and Mb concentration. However, direct evidence to support these speculations is lacking and warrants further study.

Regardless of the underlying mechanisms, it is important that submaximal eccentric exercise can prevent severe muscle damage potentially induced by maximal eccentric exercise, and repeating the submaximal eccentric exercises provides an even more potent protective effect. In fact, most of the resistance training programs are made based on the principle of progressive overload (8). When applying this principle to resistance training consisting of lengthening contractions, it is obvious that maximal-intensity eccentric exercise should not be performed for the first time. Most of the muscle damage studies (24,25) have demonstrated that eccentric exercise results in severe muscle damage using "untrained subjects," which could take several weeks to recover, but it should be noted that this is an experimental setting and does not necessarily apply for real-world situations. In fact, Newton et al. (17) have recently reported that maximal eccentric exercise of the elbow flexors, which was performed by "trained" individuals who had no experience in performing maximal eccentric exercise, results in minor muscle damage, and the recovery was completed within a week. Thus, severe eccentric exercise-induced muscle damage can be avoided, if training with submaximal-intensity (load) lengthening contractions is performed before maximal-intensity eccentric exercise.

In conclusion, the present study showed that four bouts of submaximal (40%) eccentric exercise performed every 2 wk conferred a similar protective effect to one bout of maximal eccentric exercise against subsequent bout of maximal eccentric exercise performed 2 wk later. Further studies are warranted to understand the mechanisms underlying the same protective effect conferred by four bouts of submaximal eccentric exercise.

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