NRC Research Press

ARTICLE

Low-intensity eccentric contractions of the knee extensors and flexors protect against muscle damage

Min-Ju Lin, Trevor Chung-Ching Chen, Hsin-Lian Chen, Bo-Han Wu, and Kazunori Nosaka

Abstract: This study investigated the magnitude and duration of the protective effect of low-intensity eccentric contractions (LowEC) against damage induced by maximal eccentric contractions (MaxEC) of the knee flexors (KF) and extensors (KE). Young men were assigned to 8 experimental groups and 2 control groups ($n = 13$/group); the experimental groups performed LowEC of KF or KE 2 days (2d), 1 week (1wk), 2 weeks (2wk), or 3 weeks (3wk) before MaxEC, while the control groups performed MaxEC of KF or KE without LowEC. The 2d, 1wk, 2wk, and 3wk groups performed 30 LowEC of KF or 60 LowEC of KE with a load of 10% of maximal voluntary isometric contraction strength on a resistance-training machine, and all groups performed 30 MaxEC of KF or 60 MaxEC of KE on an isokinetic dynamometer. Several muscle damage markers were measured from before to 2 days after exercise (LowEC) or from before to 5 days after exercise (MaxEC). No significant changes in any variables were evident after LowEC. The changes in all variables after MaxEC were smaller ($P < 0.05$) for the 2d and 1wk groups (e.g., peak creatine kinase activity: 1002 ± 501 IU/L; peak muscle soreness: 13 ± 5 mm) than for the control group (peak creatine kinase activity: 3005 ± 983 IU/L; peak muscle soreness 28 ± 6 mm) for both KE and KF. There were no significant differences between the 2d and 1wk groups or among the 2wk, 3wk, and control groups. These results show that LowEC provided 30%–66% protection against damage induced by MaxEC of KF and KE, and the protective effect lasted 1 week.

Key words: lengthening contractions, maximal voluntary concentric contraction strength, delayed onset muscle soreness, creatine kinase, myoglobin.

Résumé : Cette étude évalue la durée et l'intensité de l'effet protecteur procuré par des contractions pliométriques de faible intensité (« LowEC ») contre les lésions musculaires suscitées par des contractions pliométriques maximales (« MaxEC ») des fléchisseurs (« KF ») ou des extenseurs du genou (« KE »). On répartit des jeunes hommes dans 8 groupes expérimentaux (quatre KF et quatre KE) effectuant des LowEC 2 jours avant (« 2d »), 1 semaine avant (« 1wk »), 2 semaines avant (« 2wk ») ou 3 semaines avant (« 3wk ») MaxEc et dans deux groupes de contrôle (1 pour KF et 1 pour KE) effectuant des MaxEc sans LowEC ($n = 13$/groupe). Les groupes 2d, 1wk, 2wk et 3wk effectuent 30 KF ou 60 KE à une intensité sollicitant 10 % de la tension isométrique maximale volontaire sur un appareil d'entraînement contre résistance et tous les groupes effectuent 60 MaxEC (KE) ou 30 MaxEC (KF) sur un dynamomètre isokinétique. On note la présence de lésions musculaires au moyen de marqueurs, et ce, avant et jusqu'à 2 jours (LowEC) ou avant et jusqu'à 5 jours (MaxEC) après la séance d'exercice. On n'observe à la suite de LowEC aucune modification significative des variables quelles qu'elles soient. Les modifications parmi toutes les variables à la suite de MaxEC sont plus faibles ($P < 0,05$) dans les groupes 2d et 1wk (p. ex., activité de pointe de la créatine kinase : 1,002 ± 501 UI/L, douleur musculaire de pointe : 13 ± 5 mm) comparativement au groupe de contrôle (3005 ± 983 UI/L, 28 ± 6 mm), et ce, dans les deux conditions (KE et KF) sans aucune différence significative entre les groupes 2d et 1wk; aussi, on n'observe aucune différence significative entre les groupes 2wk, 3wk et des groupes de contrôle. D'après ces observations, LowEC procure 30–66 % d'effet de protection d'une durée d'une semaine à la suite de MaxEC dans les conditions KF et KE. [Traduit par la Rédaction]

Mots-clés : contractions avec étirement, force de contraction isométrique maximale volontaire, douleur musculaire d'apparition retardée, créatine kinase, myoglobine.

Introduction

Unaccustomed eccentric exercise can result in severe muscle damage, but the magnitude of the muscle damage is significantly attenuated when the unaccustomed eccentric exercise is preceded by non-damaging low-intensity eccentric contractions (Chen et al. 2012; Lavender and Nosaka 2008). For example, Chen et al. (2012) showed that eccentric contractions with a load of 10% of maximal voluntary isometric contraction strength reduced the magnitude of changes in muscle damage markers after maximal eccentric exercise of the elbow flexors by 57%–81%, and this protective effect was sustained for 7 days, decreased at 14 days, and disappeared at 21 days after the low-intensity eccentric contractions. However, it is not known whether this is also the case for other muscle groups such as knee extensors (KE) and flexors (KF). The findings of previous studies using elbow flexors as a model of eccentric exercise-induced muscle damage and adaptation may not be applicable to leg muscles.

Received 16 March 2015. Accepted 2 June 2015.

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Chen et al. (2011) reported that the magnitude of muscle damage was greater for elbow flexors and extensors when compared with KE and KF, and the magnitude of muscle damage was greater for KF than for KE. The lower degree of muscle damage in lower extremity muscles is likely due to the greater use of these muscles in daily activities involving submaximal eccentric contractions, such as walking and descending stairs (Chen et al. 2011; Jamurtas et al. 2005). Therefore, it may be that low-intensity eccentric contractions do not provide any protection against muscle damage induced by maximal eccentric contractions of KE and KF because these muscles may no longer respond to low-intensity eccentric contractions. Even if some protective effect is conferred by low-intensity eccentric contractions of these muscles, it is possible that the protective effect does not last as long as that observed for the elbow flexors (i.e., 2 weeks).

Hamstring muscle strains are the most frequent sporting injuries, particularly in sports that involve sprinting (Schmitt et al. 2012). In sprinting and kicking a ball, the knee is extended while the hip joint is flexed, which increases the risk of hamstring muscle tears (Kujala et al. 1997). Eccentric training of KF has been shown to be effective for preventing hamstring injuries (Arnason et al. 2008; Schmitt et al. 2012). However, there has been little research on eccentric exercise-induced muscle damage of KF.

Therefore, the purpose of the present study was to investigate the magnitude and time course of the protective effect of low-intensity eccentric contractions (LowEC) against damage induced by a subsequent bout of maximal eccentric contractions (MaxEC) of the KE and KF performed 2, 7, 14, or 21 days later. It was hypothesized that LowEC would attenuate changes in muscle damage markers after MaxEC of both KE and KF but that this effect would not last for 7 days.

Materials and methods

Subjects and study design

One hundred and thirty healthy, untrained young men (age: 21.4 ± 1.8 years; height: 172.0 ± 5.5 cm; body mass: 66.3 ± 6.8 kg) who had not regularly performed any resistance, aerobic, or flexibility training in the past year were recruited for this study. They had no previous muscle, joint, or bone injuries of the lower extremities, and they provided written informed consent to participate in the study. The study was approved by a local ethics committee and was conducted in conformity with the principles of the Declaration of Helsinki. All subjects were asked and reminded to refrain from unaccustomed exercises and/or vigorous physical activities, to maintain their normal dietary habits, and to not take any anti-inflammatory drugs or nutritional supplements during the experimental period, including 2 weeks prior to the first exercise bout and between the first and second exercise bouts. The subjects were instructed to drink enough water (more than 1 L/day) after exercise to reduce the risk of acute renal failure due to rhabdomyolysis and to not have any treatments (e.g., massage, stretching) of the exercised muscles during the study.

Based on the baseline maximal voluntary isometric contraction (MVIC) strength measured at 80° for KE or 30° for KF (0° = a full knee extension), the subjects were placed into 5 subgroups for the KE exercise and 5 subgroups for the KF exercise ($n = 13$ per group). The 5 subgroups included 1 control group and 4 experimental groups (2d, 1wk, 2wk, 3wk). The subjects in the 8 experimental groups (4 for KE and 4 for KF) performed 2 bouts of eccentric exercise of KE or KF with the nondominant leg 2 days (2d), 1 week (1wk), 2 weeks (2wk), or 3 weeks (3wk) apart. The first bout consisted of LowEC and the second bout consisted of MaxEC of either KE or KF. Subjects in the control groups performed only a single bout of MaxEC of KE or KF with their nondominant leg. There were no significant differences in age, height, body mass, or MVIC strength among the 5 groups for each exercise (i.e., KE or KF).

The experimental period included a familiarization session conducted 3 days prior to the first exercise bout, during which the subjects were familiarized with the measurements of the dependent variables described below. The measurements were taken 2 days before and immediately before the first eccentric exercise bout (LowEC for the experimental groups, MaxEC for the control groups) to establish the reliability of the measurements and again immediately after and 1–5 days after each exercise bout. Changes in the variables after MaxEC were compared among groups for the same exercise (KE or KF) and between KE and KF exercises for corresponding groups (e.g., 2d group for KE vs. 2d group for KF).

LowEC and MaxEC

There were 2 types of eccentric exercise (LowEC and MaxEC) for both KF and KE. For KF, subjects performed 30 LowEC and 30 MaxEC of the nondominant leg (the leg better for kicking was considered dominant) in a prone position. For KE, the number of contractions was 60 instead of 30 for both LowEC and MaxEC, and the exercise was performed in a seated position. The number of eccentric contractions was not large in the present study, especially for the KF exercise, because our previous study (Chen et al. 2011) showed that 30 MaxEC of KF induced large changes in markers of muscle damage. However, 30 MaxEC of KE resulted in only small changes in markers of muscle damage (Chen et al. 2011). Therefore, the present study used a larger number of eccentric contractions (60) for KE to induce greater changes in the markers (Chen et al. 2013).

LowEC of KF and KE were performed on a leg curl machine and a leg extension machine (Efit Sports Co. Ltd., Taiwan), respectively. To determine the weight to be loaded for each exercise, MVIC strength of the nondominant leg was measured at a knee joint angle of 30° for KF and 80° for KE on each machine using a load cell system (model SBLC, serial number 50J192, Acutek Precision Machinery Co. Ltd., Taiwan) immediately before the exercise (Chen et al. 2011, 2013). LowEC of KF and KE consisted of 3 sets of 10 and 6 sets of 10 eccentric contractions, respectively. For KF, the subjects were instructed to lower the weight from a flexed knee position (90°) to an extended position (0°) in 3 s as guided by the investigator, who counted "0, 1, 2, 3" for the movement. For KE, the subjects were instructed to lower the weight from an extended knee position (10°) to a flexed position (100°) in 3 s as guided by the investigator in the same way. After each contraction, the investigator returned the leg to the starting position while the subjects were instructed to relax their muscles. The interval between contractions was 10 s, and a 2-min rest between sets was provided.

MaxEC of KE and KF were performed on an isokinetic dynamometer (Biodex System 3 Pro, Biodex Medical Systems, Shirley, N.Y., USA), but the position setting was the same as that for LowEC. The preparation procedure for the dynamometer, including gravity correction, is described elsewhere (Chen et al. 2011, 2013). The knee joint of the exercised leg was aligned with the rotation axis of the dynamometer, and the ankle was strapped to the pad connected to the dynamometer lever arm. For KF, the subjects were instructed to contract the muscle maximally to resist the knee-extending action of the dynamometer, which moved the knee joint from a flexed position (90°) to an extended position (0°) at an angular velocity of 30°/s. In contrast, for KE, the knee joint was forcibly flexed from an extended position (10°) to a flexed position (100°) in 3 s at 30°/s. After each contraction, the lever arm passively returned the knee joint to the starting position at 9°/s, which allowed a 10-s rest between contractions. The rest between sets was 2 min. All subjects received strong verbal encouragement during contractions to generate maximal force. Torque and displacement signals of each contraction were saved on a computer (ASUSTeK Computer Inc., Taiwan) connected to the dynamometer, and peak torque and work of each contraction were calculated using Biodex Medical Systems software.

Dependent variables

The dependent variables included maximal voluntary isokinetic concentric contraction (MVC-CON) torque, knee joint angle at peak MVC-CON torque (peak torque angle), range of motion of the knee joint (ROM), plasma creatine kinase (CK) activity, myoglobin (Mb) concentration, muscle soreness, and echo intensity of B-mode ultrasound images for the exercised muscle. These variables were measured 2 days before, immediately before, and immediately after (except muscle soreness, CK, Mb, and echo intensity) exercise, 1–2 days after LowEC, and 1–5 days after MaxEC.

MVC-CON torque and peak torque angle

MVC-CON torque was measured 3 consecutive times at an angular velocity of 30°/s from 120° to 0° for KE and from 0° to 120° for KF on the same dynamometer with the same settings as those described for MaxEC of KE and KF. The investigator verbally encouraged the subjects to generate their maximal force for the whole range of motion. The torque, position (joint angle), and displacement signals of each contraction were saved on the computer connected to the dynamometer, and the highest torque of the 3 trials was used for further analyses (Chen et al. 2013). In previous studies (Brockett et al. 2001; Chen et al. 2011), the peak torque angle was calculated as the average of 3–6 measurements; therefore, in the present study, the peak torque angle was identified for each MVC-CON torque measurement, and the average of the 3 measurements was used instead of the peak torque angle of the highest MVC-CON torque.

ROM

Maximal voluntary flexed and extended knee joint angles were measured 3 times in standing position with a goniometer, and the ROM of the knee joint was obtained by subtracting the mean maximal voluntary flexed knee joint angle from the mean maximal voluntary extended knee joint angle (Chen et al. 2011; Tseng et al. 2012).

CK and Mb

Approximately 5 mL of venous blood was drawn using a standard venipuncture technique from the cubital fossa region of the arm and centrifuged for 10 min to extract plasma. Plasma samples were stored at −8 °C until analysis. Plasma CK activity and Mb concentration were assayed spectrophotometrically with an automated clinical chemistry analyzer (model 7080, Hitachi, Ltd., Tokyo, Japan) using commercial test kits (Roche Diagnostics, Indianapolis, Ind., USA). Each sample was analyzed in duplicate, and the average of the 2 measurements was used for subsequent analysis.

Muscle soreness

Muscle soreness was quantified using a visual analog scale (VAS) of a 100-mm continuous line with "not sore at all" on one side (0 mm) and "very, very sore" on the other side (100 mm). The investigator asked each subject to rate his perceived soreness on the VAS when the muscles were passively extended for KF and flexed for KE through the ROM used for the MVC-CON torque measurements (Chen et al. 2011, 2013).

Echo intensity

Echo intensity has been reported to increase after eccentric exercise and is considered to reflect muscle inflammatory response (Nosaka and Clarkson 1996). B-mode ultrasound images of the rectus femoris for KE or the long head of the biceps femoris for KF were taken at the midpoint between the greater trochanter and the epicondyle of the femur of the exercised leg using a Terason t3000 Ultrasound System with a 7.5-MHz linear probe (Terason Co., Burlington, Mass., USA) as described previously (Chen et al. 2011). Briefly, the probe was placed at the midportion of the long head of the biceps femoris for KF or the midportion of the rectus femoris for KE while the subject was lying prone or supine, respectively. Transverse images were obtained from the same sites over time, and all images were saved on a computer (HP xw4400 Workstation, Hewlett-Packard, Singapore). The saved images were analyzed using ULT File Reader for Windows (Broadsound Co., Taiwan), and the region of interest was set approximately 5 mm from the femur (Chen et al. 2011). The mean relative change in the echo intensity of a grayscale histogram (0: black; 256: white) was calculated for the region of interest (2 cm × 2 cm) from the pre-exercise value for each muscle.

Statistical analyses

Baseline measurements before LowEC and MaxEC were compared among the groups by a 1-way analysis of variance (ANOVA). Changes in the variables after LowEC were compared among the 4 experimental groups (i.e., 2d, 1wk, 2wk, and 3wk) for KE exercise and KF exercise, respectively, by a mixed-design 2-way ANOVA. Changes in all variables over time (before versus 1–5 days after MaxEC, excluding immediately postexercise because some variables were not measured immediately postexercise) were compared among groups by a multivariate ANOVA (MANOVA) with repeated measures. A mixed-design 2-way ANOVA with repeated measures was also performed to compare changes in each variable over time among the groups within KE or KF and between KE and KF for corresponding groups. When the ANOVA showed a significant interaction effect, Tukey's post hoc test was performed. The significance level was set at $P \leq 0.05$. The data are presented as the mean ± SD.

Results

Baseline values

The values of the dependent variables before LowEC or MaxEC were not significantly different among the groups (2d, 1wk, 2wk, 3wk, and control) for both KE and KF (Table 1). Significant differences between KE and KF (KF < KE) were found for MVC-CON torque, peak torque angle, and B-mode ultrasound echo intensity (Table 1).

Changes in variables after LowEC

No significant changes in any of the dependent variables were evident following LowEC of KE or KF (Table 1).

Peak torque and work during maximal eccentric exercise

Changes in peak torque and work over sets, the average peak torque, and the total work during 60 KE MaxEC or 30 KF MaxEC did not differ significantly among groups (2d, 1wk, 2wk, 3wk, and control) for both KE and KF. The average peak torque was significantly greater for KE (200 ± 20 N·m) than for KF (96 ± 9 N·m), and the total work was also significantly greater for KE (12 080 ± 1650 J) than for KF (2919 ± 324 J).

Changes in dependent variables after MaxEC

The repeated-measures MANOVA showed significant ($P < 0.05$) interaction effects for group × time, dependent variable × group, dependent variable × time, and group × dependent variable × time for KE and KF. These results show that when all dependent variables were combined, the responses of the groups (i.e., 2d, 1wk, 2wk, 3wk, and control) were different. To determine which groups differed, 2-way ANOVAs were run for each dependent variable.

MVC-CON torque

Significant changes in MVC-CON torque were found after MaxEC of both KE and KF for all groups (Fig. 1). For both KE and KF, the magnitude of the decrease in MVC-CON torque immediately after MaxEC was significantly smaller ($P < 0.05$) for the 2d and 1wk groups than for the other groups, and there was no significant difference between the 2d and 1wk groups. The recovery of MVC-CON torque to the baseline was faster ($P < 0.05$) for the 2d and 1wk groups when compared with the control, 2wk, and 3wk groups for both KE and KF, and the recovery to the baseline was significantly

Table 1. Maximal voluntary concentric contraction torque (MVC-CON), peak torque angle, range of motion (ROM), plasma creatine kinase activity (CK), myoglobin concentration (Mb), muscle soreness (SOR) with passive extension of knee flexors (KF) or passive flexion of knee extensors (KE), and B-mode ultrasound echo intensity (EI) before (Pre), immediately after (Post), and 1 and 2 days after low-intensity eccentric contractions (LowEC) of the KE (n = 52) and KF (n = 52).

Variable	Pre	Post	1 day	2 days
MVC-CON (N·m)				
KF	74.1±7.3 (72.1±7.2)	71.2±7.5	73.1±7.8	75.3±7.8
KE	169.2±9.3 (167.9±10.4)	165.9±9.5	169.3±9.2	168.6±9.6
Peak torque angle (°)				
KF	27.2±3.2 (26.9±3.4)	27.1±4.6	26.3±3.5	26.3±4.2
KE	75.7±3.9 (75.4±4.3)	74.3±4.2	75.2±4.2	75.0±4.2
ROM (°)				
KF	110.0±4.5 (110.7±4.5)	106.1±4.7	108.3±4.5	108.9±4.6
KE	110.0±4.6 (112.0±4.8)	108.5±5.1	111.3±4.6	111.1±4.7
CK (IU/L)				
KF	141.8±12.8 (138.1±11.0)	—	158.7±13.0	153.5±12.8
KE	135.8±9.4 (137.3±9.2)	—	165.4±11.0	169.8±13.0
Mb (μg/L)				
KF	29.3±3.3 (30.9±4.3)	—	33.3±4.8	31.4±3.7
KE	25.0±2.1 (25.6±3.1)	—	29.3±2.3	27.0±2.8
SOR (mm)				
KF	0.0±0.0 (0.0±0.0)	—	0.8±1.2	0.9±0.9
KE	0.0±0.0 (0.0±0.0)	—	0.5±0.2	0.0±0.0
EI (A.U.)				
KF	72.0±6.6 (74.4±7.1)	—	72.0±6.9	73.3±6.6
KE	88.4±6.0 (87.9±6.8)	—	88.7±7.5	88.3±6.0

Note: Values are means ± SD. The baseline values before maximum eccentric contractions (MaxEC) for all groups combined are shown in parentheses for KE (n = 52) and KF (n = 52). No significant changes (P > 0.05) in any of the dependent variables were seen after LowEC, and no significant differences in the baseline values were evident between LowEC and MaxEC.

faster for the 2d group than for the 1wk group. Relative changes in MVC-CON torque were similar between KE and KF for corresponding groups (e.g., 2d for KE vs. 2d for KF), including the control groups.

Peak torque angle

As shown in Figs. 2A–2B, all groups showed significant changes in peak torque angle after MaxEC for both KE and KF. The shift of the angle to a longer muscle length was smaller (P < 0.05) for the 2d and 1wk groups than for the control, 2wk, and 3wk groups for both KE and KF, and there was no significant difference between the 2d and 1wk groups.

ROM

Changes in ROM were significantly smaller for the 2d and 1wk groups when compared with the control, 2wk, and 3wk groups, and a significantly smaller change occurred for the 2d group than for the 1wk group for both KE and KF (Figs. 2C–2D). No significant differences in the changes were evident among the control, 2wk, and 3wk groups. The magnitude of changes in peak torque angle and ROM was similar between KF and KE for corresponding groups.

CK and Mb

Significant increases in plasma CK activity and Mb concentration after MaxEC were evident for all groups, but the increases were significantly smaller for the 2d and 1wk groups compared with the other groups and for the 2d group compared with the 1wk group for both KF and KE (Fig. 3). No significant differences were found among the control, 2wk, and 3wk groups. The changes in plasma CK activity and Mb concentration were not significantly different between KF and KE for corresponding groups.

Muscle soreness

Significant changes in the VAS representing muscle soreness were observed after MaxEC (Figs. 4A–4B). The extent of muscle soreness was significantly smaller for the 2d group compared with the control, 2wk, and 3wk groups and for the 1wk group compared with the control and 3wk groups, but no significant differences were evident between the 2d and 1wk groups, 1wk and 2wk groups, or 2wk and 3wk groups or among the 2wk, 3wk, and control groups for KF and KE. The increases in VAS were greater (P < 0.05) for KF than for KE for the 2wk, 3wk, and control groups.

Echo intensity

The increase in B-mode echo intensity was significantly smaller (P < 0.05) for the 2d and 1wk groups when compared with the 2wk, 3wk, and control groups for both KF and KE; significant differences (P < 0.05) were found between the 2d and 1wk groups for both KF and KE and between the 2wk and 3wk groups for KF, no significant differences were found between the 2wk and control groups, and 3wk and control groups for KF, and no significant differences were found among the 2wk, 3wk, and control groups for KE (Figs. 4C–4D). No significant difference was found between KF and KE for corresponding groups.

Discussion

The main findings of the present study were as follows: (*i*) although LowEC did not change any of the dependent variables, it significantly attenuated changes in all variables except KE muscle soreness after MaxEC of KE and KF performed 2 or 7 days later, and (*ii*) this protective effect decreased between 2 and 7 days and was abolished at 2 weeks for both KF and KE (Figs. 1–4). Thus, the hypothesis that LowEC can attenuate changes in muscle damage markers after MaxEC of KE and KF was supported, but the effect lasted longer (7 days) than expected (less than 7 days).

The changes in the variables after MaxEC of KE and KF in the control groups (Figs. 1–4) were similar to those reported in previous studies in which a similar MaxEC of KE (Brown et al. 1997; Chen et al. 2013) or KF (Chen et al. 2011; Paschalis et al. 2008) was performed. As expected, the extent of MaxEC-induced KE muscle damage in the present study was greater than that in our previous study (Chen et al. 2011) in which 30 MaxEC were performed, suggesting that the increase in the number of eccentric contractions from 30 to 60 resulted in greater muscle damage. However, the magnitude of the decrease in muscle strength after KE eccentric exercise in the present study was smaller than that in previous studies (Crameri et al. 2007; Paulsen et al. 2010; Xin et al. 2014) in which a larger number of eccentric contractions (e.g., 100–300) were performed.

LowEC of KE and KF did not change any of the dependent variables from the baseline values (Table 1), suggesting that LowEC did not induce muscle damage. Previous studies showed that a bout of low-intensity (10% of MVIC) eccentric contractions of elbow flexors (Chen et al. 2012; Lavender and Nosaka 2008) or knee extensors (Chen et al. 2013) did not change any muscle damage markers. Because the total work and peak torque produced during MaxEC were similar among all groups (i.e., 2d, 1wk, 2wk, 3wk, and control) for KE and KF, it

Fig. 1. Normalized changes (mean ± SD) in maximal voluntary concentric contraction torque of the knee flexors (KF: A) and extensors (KE: B) from the baseline (pre), immediately after (post), and 1, 2, 3, 4, and 5 days after maximal eccentric exercise (MaxEC) for the 2-day (2d), 1-week (1wk), 2-week (2wk), 3-week (3wk), and control (CON) groups. *, Significant ($P < 0.05$) difference between groups for the interaction effect shown by a 2-way ANOVA.

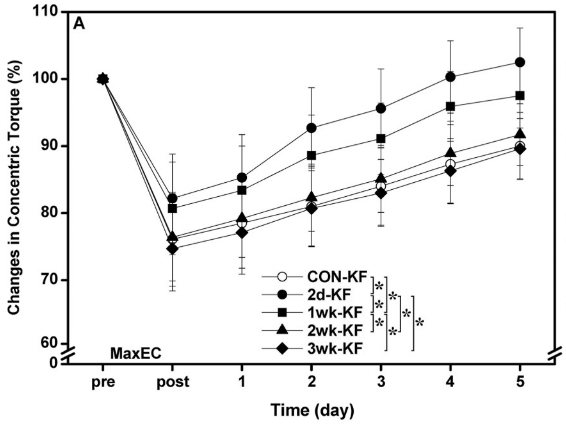
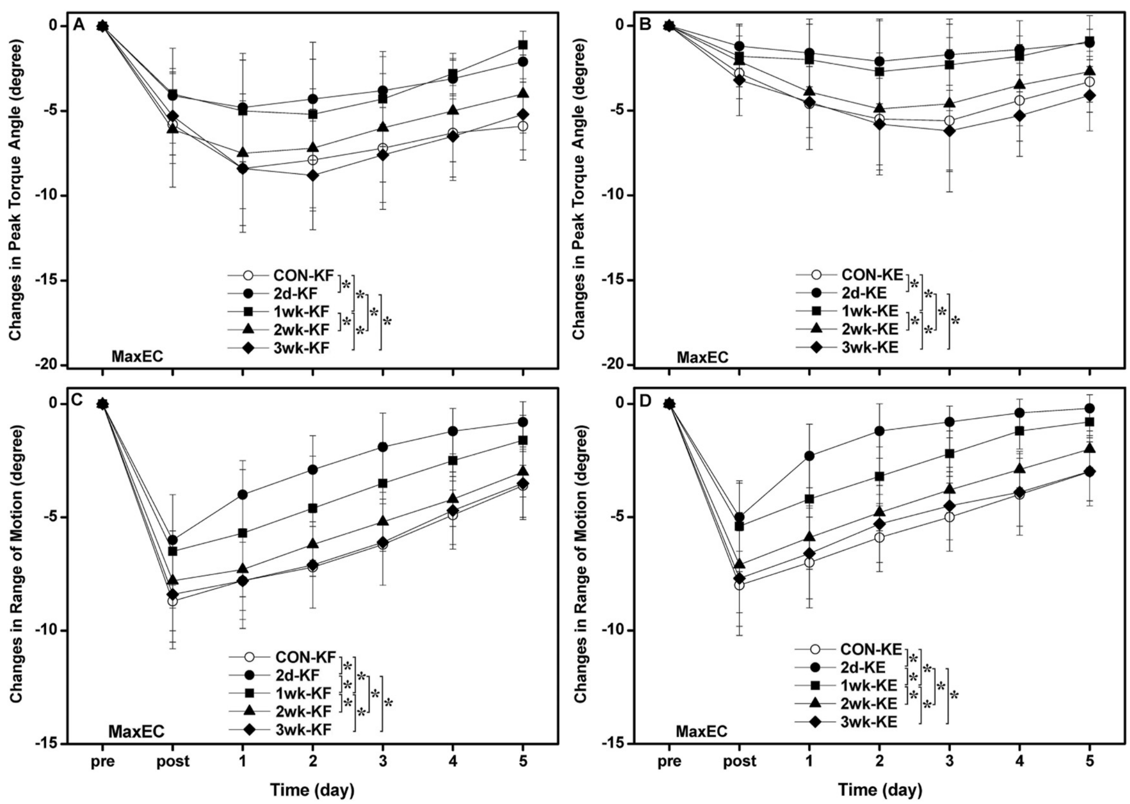


Fig. 2. Normalized changes (mean ± SD) in peak torque angle for the knee flexors (KF: A) and extensors (KE: B) and range of motion of KF (C) and KE (D) from the baseline (pre), immediately after (post), and 1, 2, 3, 4, and 5 days after maximal eccentric exercise (MaxEC) for the 2-day (2d), 1-week (1wk), 2-week (2wk), 3-week (3wk), and control (CON) groups. *, Significant ($P < 0.05$) difference between groups for the interaction effect shown by a 2-way ANOVA.



seems reasonable to assume that the experimental groups (i.e., 2d, 1wk, 2wk, 3wk) would have shown responses similar to those demonstrated by the control group had there been no effect of LowEC. Therefore, the differences in the changes in variables seen in some of the experimental groups (i.e., 2d, 1wk) in comparison with the control group were most likely due to the protective effect conferred by LowEC.

The time course of changes in the protective effect conferred by LowEC was similar between KE and KF, such that the protective effect against damage induced by MaxEC was evident for 7 days but decreased from 2 to 7 days and was abolished by 14 days after LowEC (Figs. 1–4). The protective effect conferred by LowEC was typically presented by the 2d group, which showed more than 50% faster recovery of MVC-CON torque and ROM, more than 70% lower peak CK activity and Mb concentration, more than 60% lower peak muscle soreness, and more than 60% smaller increases in echo intensity when compared with the control group for both KE and KF. These effects were similar to those reported for elbow flexors in a previous study (Chen et al. 2012) in which the magnitude of changes in the dependent variables was attenuated by 57%–81% or

Fig. 3. Changes (mean ± SD) in plasma creatine kinase (CK) activity (A, B) and myoglobin (Mb) concentration (C, D) before (pre) and 1, 2, 3, 4, and 5 days after maximal eccentric exercise (MaxEC) of the knee flexors (KF: A, C) and extensors (KE: B, D) for the 2-day (2d), 1-week (1wk), 2-week (2wk), 3-week (3wk), and control (CON) groups. *, Significant ($P < 0.05$) difference between groups for the interaction effect shown by a 2-way ANOVA.

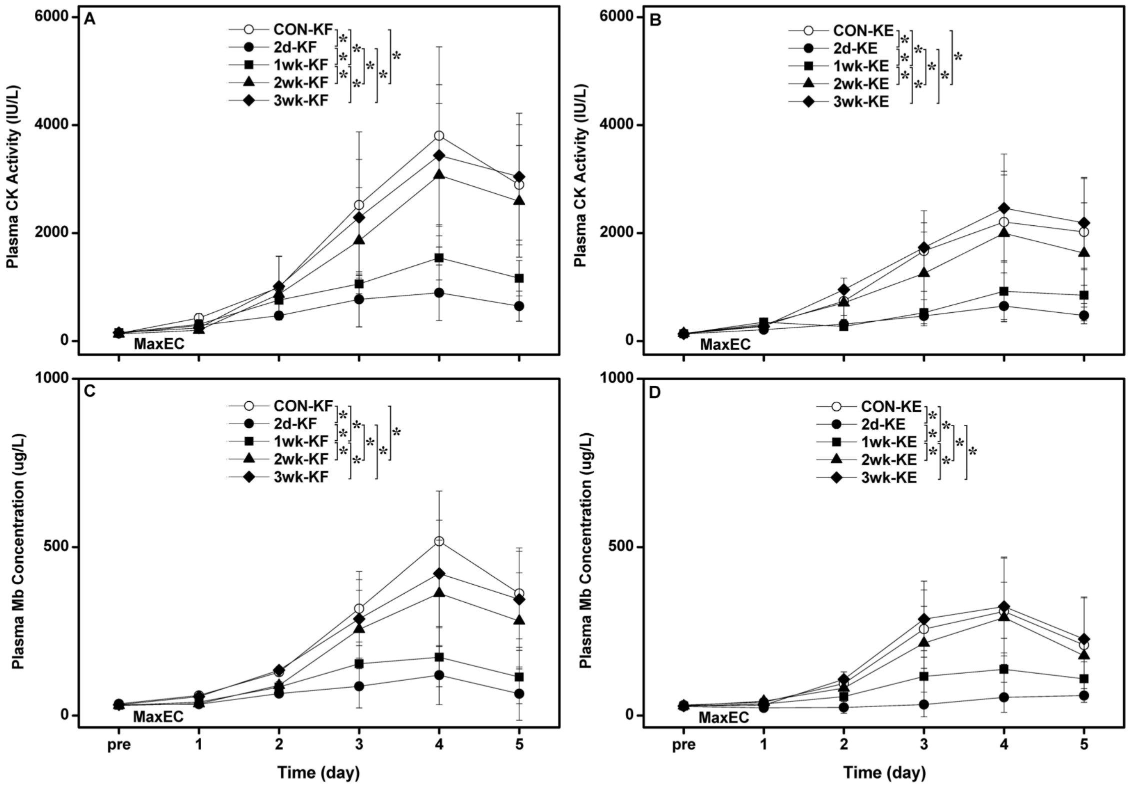
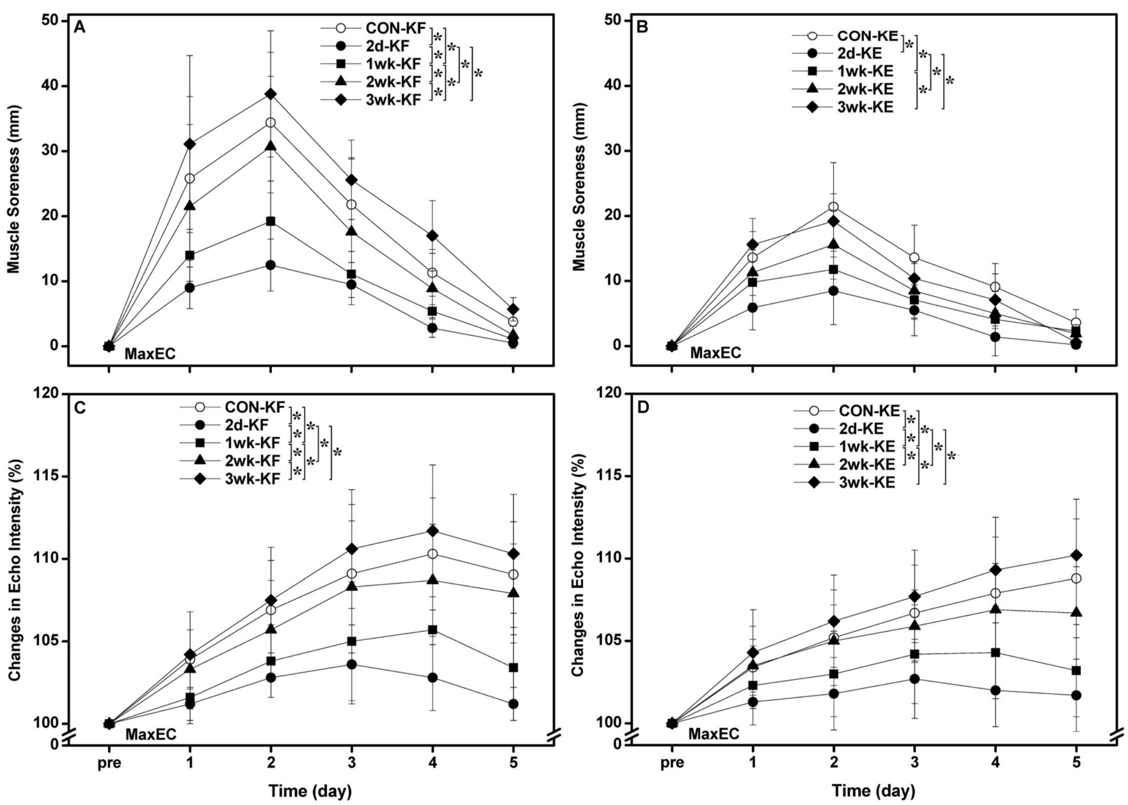


Fig. 4. Changes (mean ± SD) in muscle soreness (A, B) and normalized changes in echo intensity (C, D) before (pre) and 1, 2, 3, 4, and 5 days after maximal eccentric exercise (MaxEC) of the knee flexors (KF: A, C) and extensors (KE: B, D) for the 2-day (2d), 1-week (1wk), 2-week (2wk), 3-week (3wk), and control (CON) groups. *, Significant ($P < 0.05$) difference between groups for the interaction effect shown by a 2-way ANOVA.



45%–68% for the group that performed LowEC 2 or 7 days before MaxEC. Because lower extremity muscles are more exposed to submaximal eccentric contractions than upper extremity muscles in daily activities (Chen et al. 2011; Jamurtas et al. 2005), it was thought that the protective effect conferred by LowEC would be minimal; however, the present study showed that leg (KE and KF) and arm (elbow flexors) muscles respond similarly to LowEC. Moreover, LowEC provided a protective effect even though the intensity of eccentric contractions was lower than that during walking, since the eccentric loading during normal walking was reported to be about 25% of maximal voluntary contraction for KE (Dutton 2008). It should be noted that the maximal flexion of the knee joint is less than 20° during walking (Dutton 2008), but the knee joint was flexed to 100° during LowEC of KE. It may be that the large change in muscle length during LowEC contributed to the protective effect. It will be interesting to investigate whether LowEC with a smaller range produces a similar protective effect and whether downhill walking or stair descending exercise provides any protection against muscle damage induced by MaxEC.

While the protective effect of LowEC disappeared within 2 weeks for KE and KF, the effect reported for elbow flexors lasted for 2 weeks (Chen et al. 2012). It is not known why the duration of the protective effect is shorter for leg muscles than for elbow flexors. It has been reported that the "repeated bout effect" is long-lasting (e.g., several months) for elbow flexors (Nosaka et al. 2001), but it does not appear that this is the case for leg muscles (Black and McCully 2008). Considering that greater muscle atrophy with ageing is observed for leg muscles than for arm muscles (Frontera et al. 1991), it might be that protein turnover is more rapid, and thus the protective effect is shorter, for leg muscles than for arm muscles. This possibility should be investigated in future studies.

The mechanisms underpinning the protective effect conferred by LowEC were not investigated in the current study, but the potential mechanisms of the repeated bout effect may also underlie the protective effect. McHugh (2003) stated that a combination of neural, mechanical, and cellular adaptations are likely to be associated with the repeated bout effect. It is possible that some neural adaptations, such as changes in motor unit recruitment patterns and improved use of synergist muscles, were produced after performance of LowEC. In relation to mechanical adaptation, if ROM indicates changes in muscle stiffness (Clarkson et al. 1992), it does not appear that LowEC changed muscle stiffness (Table 1). It has been postulated that a longitudinal addition of sarcomeres contributes to the repeated bout effect, and this hypothesis was indirectly supported by a shift in peak torque angle to a longer muscle length (Brockett et al. 2001; Proske and Morgan 2001). However, no shift of the angle was evident after LowEC (Table 1); therefore, this mechanism does not appear to explain the protective effect conferred by LowEC. It is possible that LowEC induced cellular adaptations to protect muscle fibers and/or the extracellular matrix (ECM) from degeneration. Mackey et al. (2011) reported increased expression of the ECM de-adhesive protein tenascin C, collagens (I, II, IV, XII), laminins (b1, b2, a5), and matrix growth factors such as transforming growth factor β1 and insulin-like growth factor 1 after an initial bout of isometric contraction-induced muscle damage of the gastrocnemius medialis muscle. It should be investigated whether any molecular changes in muscle fibers and ECM are induced after LowEC.

In practice, an exercise consisting of LowEC can be used as a "preconditioning" exercise to prevent severe muscle damage. In the present study, MaxEC was performed after LowEC, and thus there was a large change in intensity from the first "preconditioning exercise" to the subsequent exercise. However, if the principle of progression is applied, MaxEC may not be performed only after a bout of LowEC. If the intensity of eccentric contractions is gradually increased from low to high in the process of training, it seems likely that muscle damage will be minimal after each eccentric exercise session, including MaxEC. Considering that the protective effect of LowEC against damage induced by MaxEC lasted only 1 week for KF and KE, it may be better to perform the subsequent submaximal eccentric contractions within a week. In the present study, the same number of eccentric contractions was performed for LowEC and MaxEC, but a smaller number of LowEC may provide a protective effect similar to that found in the present study. It would be interesting to determine how many submaximal eccentric contractions are necessary to produce the protective effect, whether the magnitude of the protective effect is dependent on the intensity of the contractions, and whether the duration of the protective effect is affected by the number and intensity of eccentric contractions.

In conclusion, the current study showed that LowEC of KF and KE was effective in attenuating the magnitude of muscle damage induced by MaxEC performed 2 or 7 days later but not 14 or 21 days later. The magnitude of the protective effect was similar between KE and KF, but the effect lasted only a week. Further studies are necessary to investigate the mechanisms underpinning the protective effect conferred by LowEC against eccentric contraction-induced muscle damage.

Conflict of interest statement

The authors declare that we have no any potential conflict of interest.

Acknowledgements

We thank Miss Jui-Hsin Lin, Mr. Kuan-Chieh Chen, and Mr. Shueh-Chien Ko for their assistance with data collection. This research was supported by the National Science Council (NSC100-2628-H-415-001-MY2), the "Aim for the Top University Plan" of National Taiwan Normal University, and the Ministry of Education, Taiwan.

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