2008

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ORIGINAL PAPER

A light load eccentric exercise confers protection against a subsequent bout of more demanding eccentric exercise

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Summary   This study investigated the hypothesis that a light eccentric exercise (ECC) that does not induce a loss of muscle function and delayed onset muscle soreness would confer a protective effect against a more strenuous ECC. Eighteen young men were randomly placed into two groups: 10—40% ($n = 9$) and 40% ($n = 9$). Subjects in the 10—40% group performed ECC of the elbow flexors (six sets of five reps) using a dumbbell set at 10% of maximal isometric strength (MVC) at an elbow joint angle of 90°, followed 2 days later by ECC using a dumbbell weight of 40% MVC. Subjects in the 40% group performed the 40% ECC only. Changes in MVC, range of motion (ROM), upper arm circumference (CIR), plasma creatine kinase (CK) activity and muscle soreness before, immediately after, 1—5 and 7 days following the 40% ECC were compared between groups by a two-way repeated measures ANOVA. No significant changes in any of the criterion measures were found immediately and 1—2 days after the 10% ECC. Following the 40% ECC, the 10—40% group showed significantly ($P < 0.05$) smaller decreases in MVC and ROM, and smaller increases in muscle soreness compared with the 40% group, but no significant differences between groups were evident for CIR and plasma CK activity. These results suggest that the 10% ECC induced some protection against a subsequent bout of 40% ECC performed 2 days later. It appears that the light eccentric exercise preconditioned the muscles for exposure to the subsequent damaging eccentric exercise bout.
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Introduction

Performing an unaccustomed exercise or overdoing an exercise causes symptoms characterised by muscle weakness, stiffness, soreness and swelling.[1]

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It is known that eccentric exercise produces these symptoms greater than concentric exercise,[2] and the symptoms are often used as markers of muscle damage.[1,3] When eccentric exercise is repeated, the magnitude of changes in the markers is smaller and recovery of muscle function is faster compared to the initial bout.[1,4] This protective effect is often referred to as the repeated bout effect,[1,4] and is known to last for several months for the case of maximal eccentric exercise of the elbow flexors.[5]

It has been reported that a reduced number of maximal eccentric contractions can provide a protective effect against a larger number of maximal contractions.[6,7] For example, Nosaka et al.[7] showed that two maximal eccentric contractions of the elbow flexors, which induced small but significant changes in the markers of muscle damage, conferred protection against muscle damage induced by 24 maximal eccentric contractions of the same muscle performed 2 weeks later. It has been documented that some degree of muscle damage is required to initiate the protective effect;[4,7] however, it is not known how much muscle damage is necessary for producing such an effect. It seems possible that muscle damage is not a prerequisite for the protective effect to be conferred, as some animal studies[8,9] have shown that protective effect is conferred by non-damaging exercise, such as isometric contractions or passive stretches.

Another characteristic of the repeated bout effect is that a full recovery of muscle function is not required for the protective effect to be conferred.[10] It is known that a second bout of eccentric exercise performed in an early recovery stage (1—5 days) after the first exercise bout does not exacerbate muscle damage nor retard recovery from the initial exercise.[10–12] It has been also reported that the repeated bout effect is conferred as early as 2 days after the initial bout.[11,12] However, no previous study investigated whether or not protective effect is conferred by performing exercise that does not result in loss of muscle function and muscle soreness in this time frame.

This study tested the hypothesis that a light eccentric exercise with a dumbbell set at 10% of maximal isometric strength, which would result in no or little effects on muscle function and muscle soreness,[13] could still confer protection against a subsequent bout of more strenuous eccentric exercise with a dumbbell set at 40% of maximal isometric strength carried out 2 days later. In the present study, the submaximal eccentric exercise (40%) was used as a damaging exercise model, which has been shown to result in significant changes in all markers of muscle damage in a previous study,[14] because it was assumed that protective effects, if any, could be better demonstrated by this model.

Methods

Subjects

Eighteen men volunteered for this study. The Ethics Committee of the local institute approved the study, and all subjects gave informed consent in accordance with the Declaration of Helsinki. Their mean ($\pm$S.D.) age, height, body mass and percentage of body fat were 21.4 ± 2.6 y, 171.2 ± 5.4 cm, 63.7 ± 8.8 kg and $15.8 \pm 4.7\%$, respectively. None of the subjects had been involved in any resistance training for at least 12 months prior to this investigation, and all of them were free from any musculoskeletal disorders of the upper extremities. They were asked to refrain from taking medications and dietary supplements for at least 7 days prior to, and 7 days subsequent to a bout of eccentric exercise of the elbow flexors.

Subjects were randomly placed into one of the two groups: the 40% group ($n = 9$) or the 10—40% group ($n = 9$) as described below. No statistically significant ($P = 0.29$—0.87) differences between the groups were evident for the age, height, body mass and percentage of body fat. The number of subjects was determined using the data of changes in maximal isometric strength from our previous study[14] in which a similar eccentric exercise was performed and subjects from the same population were used, and nine subjects per group were shown to be necessary based on the effect size of 1, alpha level of 0.05 and a power (1-β) of 0.80.

Exercise

Maximal voluntary isometric contraction (MVC) was measured at 90° (1.57 rad) elbow flexion using a transducer (model 100, Takei Scientific Instruments Co. Ltd., Niigata, Japan) connected to an Apple computer (Macintosh Performer Mac G4, Apple Computer Inc., Cupertino, USA) via a Power Lab system with a provided software programme (PowerLab/8SP, ADInstruments, Castle Hill, Australia). Subjects were seated on a bench with the shoulder and elbow of the exercise arm flexed at 90° (1.57 rad). A wristband worn by the subject was attached to the transducer via a steel cable. After two practice attempts to produce submaximal force, in which subjects were instructed to generate approximately 60 and 80% of maximal force by

their own perception, they performed two maximal isometric contractions for 3 s with 30 s rest between trials. The average of the two MVC values was used for the determination of the exercise load.

All subjects performed six sets of five eccentric contractions of the elbow flexors with non-dominant arm using a dumbbell set at 40% of MVC. The dumbbell weight used for the 40% load eccentric exercise (40% ECC) was between 8 and 13 kg (average 9.7 kg) for both groups, without a significant difference ($P = 0.40$) between groups. The dumbbell weight was adjusted by sticking small lead bars (100 g each) to a dumbbell (8–13 kg) with tape. The 10–40% group performed a bout of light eccentric exercise (10% ECC) 2 days prior to the 40% ECC in which the subjects completed six sets of five eccentric contractions with a dumbbell weighing 10% of MVC (2.0–3.2 kg, average 2.4 kg). The dumbbell weight adjustment was made in a similar way to that of the 40% ECC with a lighter dumbbell (2, 3 kg). The first MVC measurement was performed within 30 min before the 10% ECC bout (10–40% group) or the 40% ECC bout (40% group) and the weight for the 40% ECC bout of the 10–40% group was determined by the MVC measured at approximately 30 min before the 40% ECC. For the exercise of the elbow flexors, subjects were seated on a standard arm curl bench with the shoulder flexed at 45° (0.79 rad) and the forearm in a supinated position. The forearm was moved from an elbow joint angle of approximately 90° (1.57 rad) to a fully extended position (180°, 3.14 rad) in 3–4 s, thus the angular velocity was roughly 20–30° s^{-1} (90°/3–4 s), but the velocity was not necessarily consistent throughout the movement. The dumbbell was taken from the subject's hand at the end of each eccentric action and returned to the starting position by an investigator so that subjects did not perform concentric actions with weight. The time between each eccentric action was approximately 3 s, and subjects rested for 2 min between sets. The investigator spotted subjects if necessary, and assisted them in keeping the velocity of the eccentric muscle actions as constant as possible.

Criterion measures

All criterion measures described below have been used previously as indirect markers of muscle damage for eccentric exercise of the elbow flexors.[1,5–7,12] The reliability of the measures had been analysed prior to this study by an Intraclass correlation (ICC) by taking measurements of the criterion measures for 2 consecutive days from 10 men who were from the same subject population as those of the present study. The R-values of ICC for maximal voluntary isometric contraction, range of motion, upper arm circumference, plasma CK activity and muscle soreness were 0.87, 0.91, 0.89, 0.96 and 0.99, respectively.

MVC

As explained previously in the section of exercise, MVC was measured at 90° (1.57 rad) elbow flexion using the force transducer system. Two measurements for maximal effort were taken after submaximal attempts (approximately 60 and 80% of subject's perception), and the average of the two values was used for further analysis.

Range of motion (ROM)

A plastic goniometer was used to measure elbow joint angles when subjects actively extended (extended angle) and flexed (flexed angle) the elbow joint maximally. Subjects stood in a relaxed position with their arm held at their side with the upper arm remaining parallel to the trunk while the goniometer was applied for both extended and flexed measures. A semi-permanent ink pen was used to mark a point over the proximal apex of the deltoid, the axis of rotation of the elbow, the styloid process and dorsal tubercle of the radius. Two measurements were taken for both angles and averaged, and the difference between the extended and flexed angles was considered as ROM.

Upper arm circumference

Upper arm circumference was measured using a Gulik constant tension tape measure at 3, 5, 7, 9 and 11 cm proximal from the elbow crease of the cubital fossa. During this measurement, subjects stood with their arm hanging in a relaxed position by their side while the investigator applied the tape. Two measurements were taken from each site, and the mean value of the five sites was used for further analysis.

Plasma creatine kinase (CK) activity

Approximately 5 ml of blood was drawn from an antecubital vein of the dominant arm (non-exercised arm) by a standard venipuncture technique using a disposable needle and vacutainer containing lithium heparin. The blood was centrifuged for 10 min to obtain plasma, and the plasma samples were stored at −40 °C until being analysed for CK activity using an automatic blood analyser (Model 7170; Hitachi, Tokyo, Japan) with a test kit (Shikarikid CK; Kanto Chemical Co. Ltd., Tokyo, Japan). The normal reference range for male adults with this method is 57–197 IU L^{-1}, according to the information provided in the test kit.

Muscle soreness

Muscle soreness was assessed by palpating the biceps brachii, while the arm lay relaxed on a table. The palpation was applied by the investigator to the upper arm by placing the tips of four fingers to the proximal, middle and distal part of the biceps brachii, and the same investigator assessed the muscle soreness for all time points for all subjects. In this way, the pressure applied to the marked sites on the elbow flexors was kept as consistent as possible. Muscle soreness during passive extension of the elbow joint was also measured while subjects were in the same seated position, and the elbow joint being passively extended by the investigator. Subjects were asked to indicate their pain level on a visual analogue scale with a 50-mm line on which the left-hand end indicated no pain and the right-hand end indicated the worst pain experienced.

MVC, ROM and upper arm circumference were measured prior to each exercise bout and then immediately and 1 and 2 days after 10% ECC. MVC, ROM and upper arm circumference were measured immediately before and after, and 1—5 days and 7 days following the 40% ECC bout. Plasma CK activity and muscle soreness were assessed before and 1 and 2 days after 10% ECC, and before and 1—5 and 7 days after 40% ECC. The order of the measurements was standardised, starting with muscle soreness (excluding immediately after), followed by ROM, upper arm circumference and MVC. A blood sample was taken prior to the muscle soreness assessment.

Statistical analyses

The pre-exercise values for all criterion measures were compared between groups by a Student's t-test. Changes in the criterion measures following 40% ECC were compared between groups by a two-way analysis of variance (ANOVA) with repeated measures. The ANOVA was performed for both raw and normalised data of MVC, ROM and upper arm circumference, but only for raw data of CK and muscle soreness. For the ANOVA using the normalised data, the pre-exercise values (100 for MVC, 0 for

ROM and upper arm circumference) were excluded; thus 2 (group) $\times$ 7 (time; post, 1, 2, 3, 4, 5 and 7) design was used. When the ANOVA showed a significant interaction (group $\times$ time) effect, a Tukey's HSD test was employed as post hoc analysis to locate the time points of significant differences between groups. A one-way ANOVA was employed to assess changes in MVC, ROM and upper arm circumference within group from the baseline using the raw data. Changes in all criterion measures following 10% ECC were also analysed by a one-way ANOVA. Significance was set at $P < 0.05$.

For some of the selected values of the criterion measures, effect size (ES) was calculated by the ratio of the mean difference between 10—40 and 40% groups over the standard deviation of the 10—40% group. Data are presented as mean $\pm$ standard error of the mean (S.E.M.).

Results

Effect of 10% ECC on criterion measures

Table 1 shows the effect of the 10% ECC on criterion measures at immediately, and 1 and 2 days post-exercise in comparison with the pre-exercise values. None of the measures changed significantly ($P = 0.56$—0.97) after exercise.

Comparison between groups for changes in criterion measures following 40% ECC

No significant difference ($P = 0.22$) in MVC between groups existed prior to the 40% ECC (10—40% = 244.0 ± 11.8 N; 40% = 229.3 ± 11.8 N). The reduction in MVC from pre- to immediately post-exercise was not significantly different ($P = 0.59$) between groups. Fig. 1 shows normalised changes in MVC from baseline. A significant ($P < 0.001$) interaction effect was evident for changes in MVC from immediately to 7 days post-exercise. MVC was significantly ($P = 0.002$—0.009) lower for the 40% group compared with the 10—40%

Table 1 Changes (mean ± S.E.M.) in MVC, ROM, upper arm circumference (CIR), plasma CK activity and muscle soreness with passive extention (SOR) before (Pre), immediately after (Post) and 1 (d1) and 2 days (d2) after the 10% MVC eccentric exercise

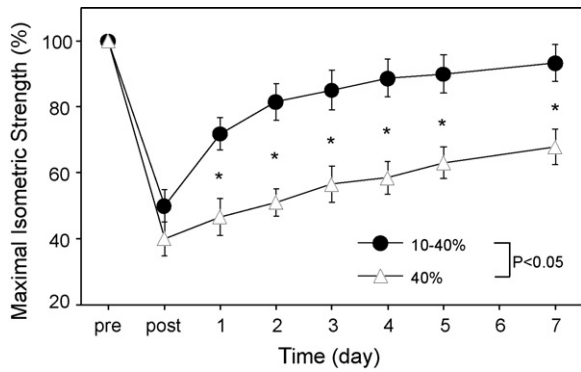|	Pre	Post	d1	d2
MVC (N)	243.0 ± 9.8	240.1 ± 11.8	242.1 ± 15.7	244.0 ± 11.8
ROM (°)	142.9 ± 2.8	140.4 ± 3.1	141.0 ± 2.7	140.0 ± 2.8
CIR (mm)	249.7 ± 5.7	253.0 ± 5.3	252.7 ± 5.3	251.4 ± 5.3
CK (IU L^{-1})	131.6 ± 18.4	—	136.8 ± 16.9	141.4 ± 16.7
SOR (mm)	0	—	2.3 ± 1.3	1.1 ± 1.1

Figure 1 Normalized changes (mean ± S.E.M.) in maximal isometric strength from baseline (pre: 100%) immediately (post) and 1—7 days following the 40% eccentric exercise bout for the 10—40 and 40% groups. A significant interaction effect ($P < 0.05$) was evident for the changes from immediately to 7 days post-exercise. *$P < 0.05$ based on post hoc test.

group between 1 (ES = 2.07) and 7 days (ES = 1.68) after exercise. When comparing with the pre-exercise value, MVC recovered to the pre-exercise level for the 10—40% group at 7 days post-exercise, but it was still significantly ($P = 0.004$) lower than baseline (68% of pre-exercise) for the 40% group.

ROM was not significantly different ($P = 0.77$) between groups prior to the 40% ECC (10—40% = 140.3 ± 2.8°; 40% = 141.7 ± 1.4°). No significant ($P = 0.64$) difference between the 10—40 and 40% groups was evident for the magnitude of decrease in ROM immediately after exercise. A significant interaction effect ($P < 0.001$) was found for changes in ROM from immediately to 7 days post-exercise (Fig. 2), and the 10—40% group had a significantly ($P = 0.03—0.05$) smaller decrease between 2 (ES = 1.08) and 7 days (ES = 0.65) follow-
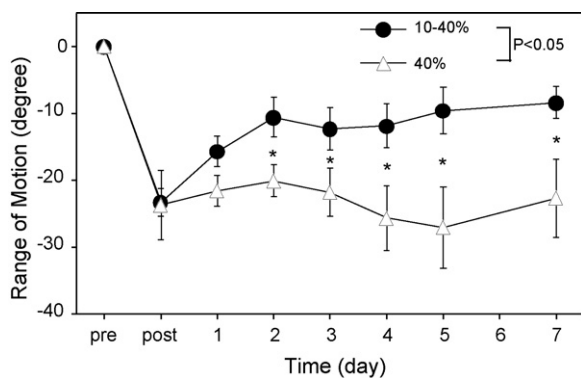

Figure 2 Changes (mean ± S.E.M.) in range of motion from baseline (pre: 0) immediately (post) and 1—7 days following the 40% eccentric exercise bout for the 10—40 and 40%. A significant interaction effect ($P < 0.05$) was evident for the changes from immediately to 7 days post-exercise. *$P < 0.05$ based on post hoc test.
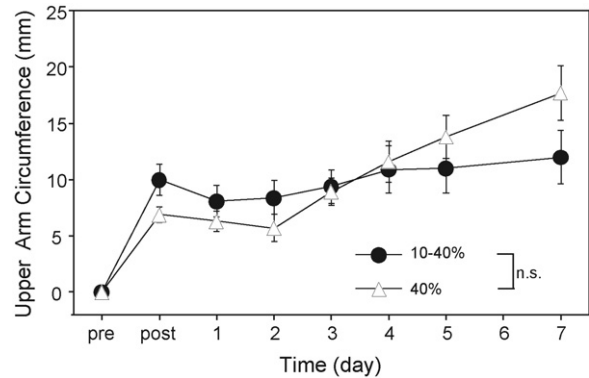

Figure 3 Changes (mean ± S.E.M.) in upper arm circumference from baseline (pre: 100%) immediately (post) and 1—7 days following the 40% eccentric exercise bout for the 10—40 and 40%. No significant interaction effect (n.s.) was evident for the changes from immediately to 7 days post-exercise.

ing exercise compared with the 40% group, which showed no recovery.

The baseline upper arm circumference was not significantly different ($P = 0.31$) between the 10—40% (239.3 ± 8.2 mm) and 40% (249.7 ± 5.7 mm) groups. Both groups showed significant ($P < 0.001$) increases in upper arm circumference following exercise. Fig. 3 shows changes in upper arm circumference from the pre-exercise value. The changes in the circumference after exercise were not significantly different ($P = 0.50$) between groups, and the amount of increase in the circumference at 7 days post-exercise from the baseline for the 10—40 and 40% group was 12.0 ± 3.7 and 15.8 ± 2.6 mm, respectively (ES = 0.24).

Plasma CK activity prior to exercise was within the normal range for both groups without significant difference ($P = 0.53$) between groups. Although the magnitude of increase in plasma CK activity after exercise was smaller for the 10—40% group than for the 40% group, the difference did not reach a statistical significance ($P = 0.08$) (Fig. 4). The peak CK activity after exercise for the 10—40 and 40% group was 2898 ± 1344 and 5501 ± 1758 IU L^{-1}, respectively (ES = 0.49).

Changes in muscle soreness upon passive extension are shown in Fig. 5. The peak soreness occurred 1—3 days after exercise for both groups, but the value was significantly ($P = 0.02$) smaller for the 10—40% group (25.1 ± 4.4 mm) compared with the 40% group (39.0 ± 3.0 mm) (ES = 1.52). Muscle soreness upon palpation of the biceps brachii was also significantly ($P = 0.002$) less for the 10—40% group compared with the 40% group. The peak palpation soreness for the 10—40% group (28.0 ± 4.2 mm) was significantly ($P = 0.002$) smaller than that of the 40% group (41.7 ± 3.0 mm) (ES = 1.20).
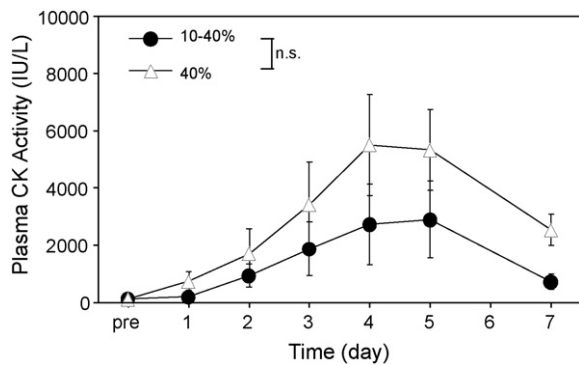
Figure 4 Changes (mean ± S.E.M.) in plasma creatine kinase activity before (pre) and 1—7 days following the 40% eccentric exercise bout for the 10—40 and 40%. No significant interaction effect (n.s.) was evident for the changes.
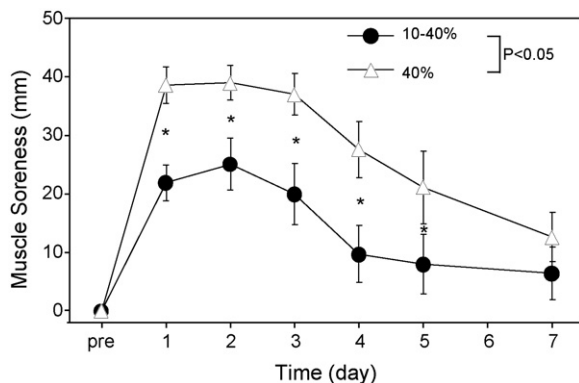

Figure 5 Changes (mean ± S.E.M.) in muscle soreness upon passive extension of the elbow flexors before (pre) and 1—7 days following the 40% eccentric exercise bout for the 10—40 and 40%. A significant interaction effect ($P < 0.05$) was evident for the changes. *$P < 0.05$ based on post hoc test.

Discussion

All parameters changed significantly from the baseline after the 40% ECC for both groups; however, muscle soreness was significantly less (Fig. 5), and the decreases in MVC (Fig. 1) and ROM (Fig. 2) from the baseline were significantly smaller for the 10—40% group compared with the 40% group, and the differences in these measures between groups were also shown by large ES values. Although no significant differences between groups were evident for changes in upper arm circumference (Fig. 3) and plasma CK activity (Fig. 4), the ES values showed a small difference between groups for the increase in circumference from pre- to 7 days post-exercise and peak plasma CK activity. These results suggest that the 10% ECC conferred some protective effect against the 40% ECC.

It is important to note that the 10% ECC did not induce significant changes in any of the criterion measures after exercise (Table 1). This is different from previous human studies reporting the repeated bout effect, since all of the studies showed that the initial eccentric exercise bout resulted in significant changes in markers of muscle damage. For example, Nosaka et al.[7] showed that two maximal eccentric actions of the elbow flexors conferred protective effect against 24 maximal eccentric actions of the same muscle performed 2 weeks later, but the initial exercise bout resulted in 20% decrease in MVC immediately post-exercise, maximum of 5° decrease in ROM and 3.5 mm increase in upper arm circumference, peak plasma activity of approximately 500 IU L^{-1} and peak muscle soreness of 10 mm. The time interval between the 10 and 40% bouts (2 days) was the same as that of previous studies;[11,12] however, the first exercise bout of these studies resulted in significant changes in the markers of muscle damage, and the second bout was performed when all of the markers were still changing. Thus, the protective effect found in the present study seems to be different from the repeated bout effect documented in previous studies, all of which used loads greater than 10% MVC.

This is the first study to show that a protective effect against muscle damage is conferred by a light eccentric exercise that does not significantly change markers of muscle damage. It should be noted that the markers of muscle damage used in the present study were indirect, and might not be sensitive enough to detect any cellular events after the 10% ECC. Therefore, the possibility that the 10% ECC induced muscle damage cannot be ruled out, but it is important that the 10% ECC did not cause any discomfort to the subjects. It should be also noted that the interval between the 10 and 40% bouts was 2 days. It has been shown that changes in markers of muscle damage are significantly attenuated after the second bout compared with the initial bout, when the two bouts of maximal eccentric exercise of the elbow flexors are separated by more than 2 weeks.[1,5—7] It remains uncertain how long the protective effect conferred by the 10% bout lasts, and further study is necessary to examine whether or not the protective effect conferred by the 10% bout lasts more than 2 weeks.

McHugh[4] categorised the potential mechanisms underlying the repeated bout effect into neural, mechanical, cellular and others. It seems that several mechanisms are involved in the process of producing the repeated bout effect,[4] but it is questionable whether or not the results of the present

study can be explained by the existing mechanism theories. Armstrong et al.[15] proposed that a pool of weak muscle fibres were damaged by the initial eccentric exercise bout and replaced by stronger fibres, inducing the repeated bout effect. Since no changes in MVC and plasma CK activity were evident following 10% ECC (Table 1), it is unlikely that weak fibres, if any, were injured by the 10% ECC. It has been also speculated that strengthening of connective tissue made muscle fibres more resistant to subsequent injury.[16] However, it does not seem that the 10% ECC bout was strenuous enough to trigger a remodelling of connective tissue or cytoskeletal proteins. It was reported that a rightward shift in optimum angle to generate maximal isometric force was evident after eccentric exercise.[17,18] Morgan and Proske[18] hypothesised that the repeated bout effect would involve addition of sarcomeres in series. In the present study, maximal isometric strength was measured only at 90° and no data are available to show the changes in optimal angle after the 10% ECC exercise. It would be interesting to investigate whether or not a shift in optimum angle occurs after low-load eccentric exercise.

Koh and Brooks[8] reported that maximal isometric contractions or passive stretches of the extensor digitorum longus (EDL) muscles in mice did not cause degeneration of muscle fibres but induced protection against muscle damage by maximal eccentric actions performed 3 days later. They speculated that upregulation of cytoskeletal proteins and/or upregulation of free radical scavenging pathways might be associated with the protective effect conferred by isometric contractions or passive stretches.[8] It is interesting that the decreases in muscle strength and ROM immediately after exercise were not protected by the light eccentric exercise, but the decreases were significantly attenuated in the recovery days (Figs. 1 and 2). This may suggest that the light eccentric exercise contributed to attenuation of the secondary damage, which is likely to be related to the inflammatory response and free radical damage. McArdle et al.[9] reported that non-damaging isometric contractions of soleus and extensor digitorum longus muscles by electrical stimulation conducted 4 or 12 h prior to damaging protocol reduced CK release from the muscles of mice. They proposed that activation of the haemoxygenase-1 (HO-1) gene resulting from increased reactive oxygen and nitrogen species (ROS) production was associated with the protective effect.[9] The results of the present study appear to be in line with the findings by McArdle et al.,[9] since a tendency ($P = 0.08$) of attenuated CK responses for the 10—40% group

was detected by ANOVA, and the ES value (0.49) showed a small difference between groups for peak plasma CK activity. It is possible to assume that the 10% ECC exercise increased ROS, producing a similar protective effect through increased expression of HO-1. Mikkelsen et al.[19] have recently shown that stimulation of the Na^+-K^+ pump with β_2-adrenoceptor agonists improved force recovery of rat EDL muscles by 40—90% following a 30-min electrical stimulation protocol. It may be that the 10% ECC resulted in upregulation of molecules including HO-1 and/or β_2-adrenoceptor agonists. More research is required to understand the mechanisms underlying the protective effect conferred by the light eccentric exercise.

It is important to note that the 10—40% group performed greater number of maximal and submaximal isometric contractions (8 maximal and 16 submaximal contractions) before the 40% ECC compared with the 40% group (2 maximal and 4 submaximal contractions). Since the animal studies[8,9] reported that isometric contractions induced protection against muscle damage as described previously, it is possible that the MVC measurements produced the protective effect, and the greater number of isometric contractions performed before the 40% ECC in the 10—40% group contributed to the protective effect. It is interesting to investigate if maximal or submaximal isometric contractions, instead of the light eccentric exercise, could induce the protective effect.

In summary, the light eccentric exercise was effective for attenuating muscle damage against a subsequent bout of more intense eccentric exercise performed 2 days later. It appears that the light eccentric exercise preconditioned the muscle for damaging eccentric exercise bout, producing a protective effect.

Practical implications

- Eccentric exercise with a light weight reduces muscle soreness and attenuates decreases in muscle strength and range of motion after strenuous eccentric exercise performed 2 days later.
- It seems that muscles are preconditioned by the light eccentric exercise to be protected against muscle damage.
- To implement resistance training with eccentric loading, especially for beginners, it is better to start with a light weight that does not result in muscle soreness or decrease in muscle function.

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