Comparison of Muscle Damage Response among Different Submaximal Repeated Eccentric Exercise Bouts: A Dose Response Study

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Abstract

**Purpose:** To compare the effect of different frequencies of initial eccentric exercise (40% MVIC) on protection from muscle damage by subsequent maximal eccentric exercise.

**Methods:** Thirty two healthy collegiate males were recruited and randomly assigned into one of the four groups: control, 1 bout, 2 bouts and 3 bouts. The control group received no preconditioning exercise, prior to a maximal bout of eccentric elbow flexor contraction at 100% MVIC that induced muscle damage. The 1 bout group performed a bout of submaximal exercise at 40% MVIC preceding the maximal bout of eccentric elbow flexor contraction at 100% MVIC. Similarly, the 2 and 3 bouts groups performed 2 and 3 bouts, respectively, of submaximal exercise, prior to a muscle damaging maximal bout. Each exercise bout consisted of six sets of five eccentric contractions of the elbow flexors with non-dominant arm using a dumbbell set. MVIC, ROM of elbow, Upper Arm Circumference, Biceps Muscle Soreness, Serum Creatine Kinase and LDH activity were measured immediately before, and at 24, 48 and 72 hours following the eccentric exercise of the elbow flexors.

**Results:** The overall findings of the study suggest that the group which received 3 bouts of submaximal eccentric exercise showed the most effective protection against subsequent muscle damage, when compared to those that received no or only one bout of preconditioning exercise.

**Conclusion:** Increasing the frequency of initial submaximal preconditioning eccentric exercise provides greater protection against a subsequent bout of maximal eccentric exercise.

ABBREVIATIONS

MVIC: Maximum Voluntary Contraction; ROM: Range of Motion; CK: Creatine Kinase; LDH: Lactate Dehydrogenase; UAC: Upper Arm Circumference; EIDM: Exercise Induced Muscle Damage; RBE: Repeated Bout Effect; SOR: Soreness

INTRODUCTION

Eccentric muscle contractions are performed regularly in everyday lives, and are important considerations for training and rehabilitation because of their potential to produce large forces with a low metabolic cost [1]. It has been well documented that unaccustomed eccentric exercise leads to damage in the exercised muscle fibers [2-5]. Exercise Induced Muscle Damage (EIMD) is followed by sensations of stiffness and soreness in the exercised muscles the next day [6].

One of the most recognized and studied adaptations to muscle damage is the “repeated bout effect” (RBE). The repeated bout effect refers to the protection or attenuation in muscle damage markers, observed following a second bout of exercise. It is well known that a damaging bout of exercise through eccentric
actions will result in a protective effect in subsequent repeated bouts; however, such adaptation is induced even when the initial exercise bout is different from the subsequent exercise bouts [7]. Although the RBE mechanisms remain to be fully elucidated, it is thought that mechanical [8], neural [9] and cellular [10] responses (or combination of these), are responsible [5,11,12]. RBE is characterized by faster recovery of strength, smaller restriction in range of motion about a joint, reduced swelling and muscle soreness, smaller increases in muscle proteins in the blood, fewer abnormalities on magnetic resonance or ultrasound images, and blunted immune responses after repeated exercise bouts.

Previous studies have compared the different intensities [13], set repetitions [14], velocity of eccentric exercise [15], and type of exercise (eccentric vs. different angle isometric) [16], used to produce RBE. However, as per our knowledge, there is no study that establishes an optimal volume of submaximal eccentric exercise required to confer this protective effect. Therefore, the purpose of this study was to compare the effect of different frequencies of initial eccentric exercise (40% MVIC) on protection from muscle damage by subsequent maximal eccentric exercise. We hypothesized that changes in markers of muscle damage would be smaller with greater volume of preconditioning.

MATERIAL AND METHODS

**Subjects:** Thirty two healthy collegiate males who had not participated in any resistance training for the last 12 months were recruited for the study (age 21.59±2.36 years, height 169.05±6.5 cms, weight 61.3±14.0 kgs and BMI 22.1±3.4 kg/m²). For the duration of the study, participants were asked to abstain from resistance exercise, use of heat or cold treatment, and from resistance exercise prior to a muscle damaging maximal bout. There was an interval of 2 weeks between each bout. Each exercise bout consisted of six sets of five eccentric contractions of the elbow flexors, using a dumbbell set, with non-dominant arm to minimize any functional disability. For all the groups, the criterion measurements were assessed by the same examiner, prior to, and at 24, 48 and 72 hours following the eccentric exercise bout.

**CRITERION MEASURES**

Maximum Voluntary Isometric Contraction (MVIC): MVIC of the elbow flexors was measured using Lafayette MMT system (Model no. 01165; accuracy ±1% over full scale or ±0.2% lbs). The dynamometer test consisted of isometric "make contractions", in which the patient uses each tested muscle group to push maximally against the plate and the piston of the hand-held dynamometer for 4-5 seconds [17]. To test for the elbow flexor (biceps brachii), the subject was positioned supine with the elbow flexed to 90 degrees, and the forearm supinated. The shoulder of the subject was manually stabilized, and the dynamometer was placed just proximal to wrist on the volar surface of forearm [18].

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. Two measurements were taken for both the angles and averaged, and the difference between the extended and flexed angles was considered as ROM [7].

**Upper Arm Circumference (UAC):** UAC was measured using a Gulik constant tension tape. Measurement was taken at 8cm proximal from elbow crease of cubital fossa [7]. Subjects were standing with their arm hanging in a relaxed position by their side while the tape was applied to get the measurement of circumference.

Serum Creatine Kinase (CK) and Lactate Dehydrogenase (LDH) Activity: Approximately 1-2ml of blood was drawn from antecubital vein of the arm by venipuncture technique. The blood was centrifuged for 10 minutes to obtain plasma and the plasma samples were stored at -40 degrees. The serum CK and LDH activity was assessed by a spectrophotometer at 340nm set wavelength using a CK kit (NAC act, Crest Biosystems, CORAL) and LDH kit (LDH, P-L, Crest biosystems CORAL), respectively. For both CK and LDH activity, 1ml of their respective working reagent was incubated at 37 degrees C for 1 minute, followed by addition of 0.02ml of serum for analysis. Changes in absorbance per minute during 4 minutes were measured, following which the summation of changes in absorbance was multiplied by the given factor. (Normal reference values: CK= 38-174 U/L, LDH= 230-460 U/L).

Muscle Soreness Measurement: 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 [7]. The subjects were asked to indicate the level of pain using a 10cm visual analog scale, where ‘0’ represents no pain and ‘10’ represents intense pain [19]. The same investigator assessed the muscle soreness for all time points for all subjects.
Statistical Analyses: In the present study, data was assessed by a Shapiro- Wilk test for the normality of the distribution scores. UAC and CK scores that demonstrated non-normal distribution were log-transformed for further analysis. SPSS for Windows (Version 21; SPSS Inc., Chicago, IL) was used for statistical analysis. Data was analyzed using a 4x4 (group x time) multivariate analysis of variance (MANOVA; α ≤ 0.05). Dependent variables included- maximum voluntary isometric contraction, range of motion of elbow joint, upper arm circumference, soreness of biceps muscle, serum creatine kinase and lactate dehydrogenase activity. Independent variables were groups with varying volume of initial preconditioning exercise (control, 1 bout, 2 bouts and 3 bouts) and time (pre-exercise, 24hr, 48hr, and 72hr). If MANOVA was found significant, a Turkey’s HSD post-hoc analysis was done as a follow-up to locate significant differences between the bouts as well as between time points (pre-exercise, 24hr, 48hr and 72hr post exercise). Significance level was set at \( p < 0.05 \), and the results reported as mean ± standard error of measurement (SEM) unless otherwise stated.

RESULTS AND DISCUSSION

Pre-exercise criterion measures

No significant differences among the groups were evident for any of the pre-exercise criterion measures except for LDH (Table 1).

Maximum Voluntary Isometric Contraction (MVIC)

Repeated measure MANOVA revealed a statistically significant difference in MVIC for different bouts of exercise with \( F(12, 66.435) = 2.66; \ p = 0.006 \). Post hoc analyses showed that the mean scores for MVIC were significantly different between the control group and 3 bouts group at 24 hour, with \( p=0.02 \) (Table 2 &3).

Range of Motion (ROM) and Upper Arm Circumference (UAC)

ROM and UAC showed a significant difference following different bouts of exercise, with \( F(12, 66.435) = 2.49; \ p = 0.009 \), and \( F(12, 66.435) = 2.95; \ p = 0.002 \), respectively. At 24 hour, ROM showed greatest difference between the control and 3 bouts groups (\( p=0.004 \)), and the difference between the 1 bout group and 3 bouts group was also significant (\( p = 0.04 \)) (Table 2&3).

Soreness (SOR)

Soreness for different volumes of exercise was significantly different between groups, with \( F(12, 66.435) = 3.11; \ p = 0.004 \). The post-hoc analyses showed that at 24 hours, the mean scores for soreness were different between the control group and 3 bouts group (\( p=0.02 \), and also between 1 bout group and 3 bouts group (\( p=0.01 \)). The 1 bout group and 3 bouts group remained significantly different at 48 and 72 hours as well, with \( p=0.02 \) and \( p=0.01 \), respectively.

Creatine Kinase (CK) and Lactate Dehydrogenase (LDH)

There was a statistically significant difference in plasma CK and LDH activity for different bouts of exercise, with \( F(12, 66.435) = 2.46; \ p =0.01 \), and \( F(12, 66.435) = 3.86; \ p =0.001 \), respectively. The post-hoc analyses showed that mean scores for CK were significantly different between the control group and 3 bouts group at all time points. At 24 hours, a difference between 1 bout group and 3 bouts group was also obtained (\( p=0.01 \)). The post-hoc analyses for LDH revealed differences between control group and 3 bouts group at 48 and 72 hours, with \( p=0.03 \) and \( p=0.04 \), respectively (Table 2 &3).

Discussion: This is a preliminary study comparing the effects of different volumes (1, 2 and 3 bouts) of initial submaximal eccentric exercise (40% MVIC) performed after every 2 weeks, on markers of muscle damage. The overall findings of the study suggest that the group which received 3 bouts of submaximal eccentric exercise showed the most effective protection against subsequent muscle damage, when compared to those that received no or only one bout of preconditioning exercise.

In the present study, it was observed that changes in criterion measures following maximal eccentric contraction were significant for all 4 groups. MVIC and ROM decreased, while upper arm circumference, soreness and plasma CK and LDH activity increased from the baseline, after performing eccentric exercise. However, the between group analyses revealed that the magnitude of the repeated bout effect increased in line with the

Table 1: Baseline Comparison of Groups Using One Way ANOVA.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Control</th>
<th>1 Bout</th>
<th>2 Bouts</th>
<th>3 Bouts</th>
<th>F value</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>MVIC (N)</td>
<td>196.01</td>
<td>209.6</td>
<td>202.86</td>
<td>203.02</td>
<td>0.197</td>
<td>0.898</td>
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<td></td>
<td>(17.35)</td>
<td>(48.2)</td>
<td>(37.32)</td>
<td>(32.21)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ROM (°)</td>
<td>141.38</td>
<td>137.38</td>
<td>142.00</td>
<td>143.88</td>
<td>2.09</td>
<td>0.123</td>
</tr>
<tr>
<td></td>
<td>(3.20)</td>
<td>(6.61)</td>
<td>(6.50)</td>
<td>(4.22)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>UAC (cm)</td>
<td>3.29</td>
<td>3.25</td>
<td>3.25</td>
<td>3.22</td>
<td>0.741</td>
<td>0.537</td>
</tr>
<tr>
<td></td>
<td>(0.04)</td>
<td>(0.08)</td>
<td>(0.07)</td>
<td>(1.48)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SOR (cm)</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>CK (U/L)</td>
<td>5.06</td>
<td>5.10</td>
<td>4.99</td>
<td>4.99</td>
<td>0.192</td>
<td>0.901</td>
</tr>
<tr>
<td></td>
<td>(0.39)</td>
<td>(0.36)</td>
<td>(0.31)</td>
<td>(0.21)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>LDH (U/L)</td>
<td>363.51</td>
<td>378.09</td>
<td>392.29</td>
<td>240.92</td>
<td>5.017</td>
<td>0.007</td>
</tr>
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<td></td>
<td>(100.26)</td>
<td>(79.40)</td>
<td>(96.83)</td>
<td>(68.45)</td>
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</tr>
</tbody>
</table>

Abbreviations: MVIC: Maximal Voluntary Isometric Contraction of Biceps; ROM: Range of Motion of Elbow; UAC: Upper Arm Circumference; SOR: Soreness of Biceps; CK: Creatine Kinase; LDH: Lactate Dehydrogenase; F: Variance of the group means / mean of the within group variances; p>0.05 is non-significant.
increase in the number of initial submaximal eccentric exercise, such that, with an increase in frequency there was a greater level of protection conferred. This suggests that even less-damaging initial submaximal exercise (40% MVIC) can provide a better protective effect to the exercising muscle, when repeated, before initiation of lengthening contraction with maximal weight.

Maximum Voluntary Isometric Contraction

In the present study, there was a significant difference among all the four groups for the decrease in the maximal voluntary isometric contraction. The group and time interaction was found only between the 1 bout and 3 bouts groups at 24 hours (Figure 1a), unlike a similar study by Chen et al. [20] that reported maximum difference after the first bout itself. The percentage decrease in muscle strength was less in groups that received preconditioning (3 bouts- 22%, 2 bouts- 33%, 1 bout- 38%), as compared to the control group that showed a decrement of 45% in MVIC. The exact mechanism of this loss of force is not well understood, but it is thought to be due to mechanical reflex inhibition of the muscle experiencing pain [21]. It has been speculated that strength decrement following exercise induced muscle damage were due to soreness which prevents subjects from fully activating their muscles [22]. But many researchers have found poor correlation of strength and muscle soreness [23,24]. Muscle soreness does not develop immediately after exercise whereas muscle strength shows the largest decrements in MVIC torque immediately following the exercise activity with a gradual recovery of force generating ability over subsequent days or weeks [25].

Previous studies [20,26] 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. [27] 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.

Range of motion

The results of the present study suggest significant Group × Time interaction, $p = 0.04$, indicating difference between the control and 3 bouts group at 24 hours, following which the ROM tends to recover after 48-72 hours. (Figure 1b) The percentage decrease in ROM was found to be 6.17% in those who received three bouts of initial submaximal exercise as compared to...
7.46%, 8.62% and 11.84% in 2 bouts, 1 bout and control groups respectively. This much lesser decrease in ROM can be attributed to the extent of pain and muscle stiffness experienced by subjects. The aetiology of the decreased ROM following eccentric exercise remains to be fully elucidated, however, previous research suggests that shortened non-contractile components, change in calcium homeostasis due to muscle damage, decreased strength, and/or swelling may be implicated [28, 29].

**Upper Arm Circumference**

Changes in upper arm circumference (Figure 1c) showed a 4.2% increase in the control group as compared to 1.3% in the 3 bouts group. The Group × Time interaction was non-significant suggesting that no further adaptation was produced by the additional eccentric exercise bouts for UAC. It may be that the development of delayed onset muscle soreness and swelling do not necessarily relate to skeletal muscle fibre injury, but connective tissue injury and inflammation. Crameri et al. [30] documented that tears within the extracellular matrix and subsequent inflammatory responses induced by lengthening contractions were associated with delayed onset muscle soreness. It has been recently shown that enlargement of interstitial space between muscle fibers (i.e. oedema) is associated with an increase in ultrasound echo intensity [31]. Thus, it is possible to assume that the increases in upper arm circumference indicate connective tissue damage and inflammation resulting in edema. It may be that muscle soreness and swelling are indicative of connective damage and inflammation, and if so, the different adaptive responses between muscle function variables (strength and ROM) and others (upper arm circumference, muscle soreness)
suggest that adaptation process is different between the skeletal muscle fibres and the extracellular matrix.

**Soreness**

As shown in Figure 1d, muscle soreness developed after each exercise and peaked 1–2 days post-exercise. A significant difference was located between control and 3 bouts groups at 24 hours. Additionally, there was also a significant difference between 1 bout and 3 bouts groups at 24 and 48 hours, suggesting that 3 bouts group showed a relatively less increase in pain post exercise even compared to 1 bout group which already had some preconditioning effect.

Muscle soreness can be attributed to pain experienced by the subjects. As demonstrated by previous studies that after eccentric exercise, resting muscle activation increases [32,33], indicating a tonic localized spasm of motor units. Muscle spasm causes muscle pain directly by stimulating mechanosensitive pain receptors or indirectly by compressing the blood vessels resulting in ischaemia [34]. A vicious cycle is created which probably prolongs ischemia. Structural disruption leads to the normal inflammatory response-an increase in chemical mediators such as histamine, bradykinin, prostaglandin and serotonin [35] causing pain and swelling. The products of the inflammatory response sensitize the free nerve endings in muscle, thus increasing soreness. Strauber et al. [36] concluded that the DOMS after repeated muscle action is not because of actual myofiber damage but more likely results from inflammation. The increased intramuscular edema, which activates the mechanoreceptor might be causing pain.

**Plasma CK and LDH activities**

In all the groups, there was an increase in activity of CK and LDH activity at 24 hours, peaking within 24 hours and then gradually decreasing from 48 to 72 hours. A significant difference in both CK and LDH, was evident in between the control and 3 bouts group, emphasizing the protective effect of three preconditioning bouts. The increase in plasma CK level was approx. 4 times lesser in the 3 bouts group than that found in the control group (Figure 1e & 1f).

Commonly accepted mechanisms of CK release are damage to muscle tissue and changes in myocyte membrane permeability. With regard to membrane permeability there are various theories of ion-distribution change, enzyme deficiency and ATP depletion [37]. When the exercise intensity is within the normal range of metabolism, the muscle tissue is exercised without marked changes in membrane permeability. However, when the exercise intensity exceeds this permissible range, the membrane permeability temporarily changes resulting in CK release from the active muscle.

If the increases in CK and LDH indicate plasma membrane damage, no difference between the control and 1 bout group and lesser increases after performing 3 bouts of submaximal eccentric exercise suggest that some adaptation to plasma membrane was induced by the first-third 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 [38-41] may also be associated with no increases in plasma CK activity and LDH concentration.

The study variables showed greatest difference between the control and 3 bouts groups but no difference were found in control vs. 1 bout or 2 bouts groups. This implies the absence of RBE in the 1 and 2 bouts groups. These results were in contrast to the findings of Chen et al. [20] who also examined the muscle damage responses to 4 maximal eccentric exercise bouts performed every 4 weeks and reported greatest adaptation after the first bout. This difference could be attributed to the fact that they used maximal eccentric exercise at each of the exposures and this high magnitude of muscle damage induced RBE in the first bout itself. Moreover, some variables in the present study showed differences between 1 and 3 bouts groups reinforcing the effect of dosimetry. Although all preconditioning groups showed improvements in the markers of muscle damage in line with the volume of exercise, the results were statistically significant only for the 3 bouts group. Therefore, we propose that submaximal exercise when increased to a certain threshold volume demonstrated RBE.

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 [42]. When applying this principle to resistance training consisting of eccentric contractions, it is obvious that maximal-intensity eccentric exercise should not be performed for the first time. A preceding load of lighter intensity provides preconditioning without causing muscle damage and multiple bouts produce superior results. These findings have direct implications for coaches, athletes and sports scientists where for instance, performing 3 submaximal eccentric bouts before competitive events will help to alleviate muscle damage encountered during competition phase. The present dose response study provides scope for modulating repeated bout effect by altering the volume (no. of exposure) at submaximal exercise intensity. Thus, severe eccentric exercise-induced muscle damage can be avoided, if training with submaximal intensity (load) eccentric contractions is performed before maximal-intensity eccentric exercise. Moreover, increasing the number of bouts or exposures of a submaximal preconditioning exercise results in greater benefits.

**CONCLUSION**

In conclusion, the present study showed that three bouts of submaximal (40%) eccentric exercise performed every 2 week conferred a greater protective effect against those who did not receive any preconditioning exercise prior to maximal eccentric exercise. Increasing the frequency of initial submaximal preconditioning exercise provides greater protection against a subsequent bout of maximal eccentric exercise on the markers of muscle damage. Further studies are warranted to understand the mechanisms underlying the same protective effect conferred by these bouts of submaximal eccentric exercise.

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REFERENCES


