Effect of single bout versus repeated bouts of stretching on muscle recovery following eccentric exercise

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Abstract

Objectives: To analyze the effects of a single bout and repeated bouts of stretching on indirect markers of exercise-induced muscle damage.

Design: A randomized controlled clinical trial at a university human research laboratory was conducted.

Methods: Fifty-six untrained males were randomly divided into four groups. (I) a single stretching group underwent a single bout of stretching on the quadriceps muscle; (II) an eccentric exercised group underwent eccentric quadriceps muscle contractions until exhaustion; (III) an eccentric exercise group followed by a single bout of stretching; (IV) an eccentric exercised group submitted to repeated bouts of stretching performed immediately and 24, 48, and 72 h post-exercise. Muscle stiffness, muscle soreness, maximal concentric peak torque, and plasma creatine kinase activity were assessed before exercise and 1, 24, 48, 72, and 96 h post-exercise.

Results: All exercised groups showed significant reduction in maximal concentric peak torque and significant increases in muscle soreness, muscle stiffness, and plasma creatine kinase. There were no differences between these groups in all assessed variables, with the exception of markers of muscle stiffness, which were significantly lower in the eccentric exercise group followed by single or repeated bouts. The single stretching group showed no change in any assessed variables during the measurement period.

Conclusions: Muscle stretching performed after exercise, either as single bout or as repeated bouts, does not influence the levels of the main markers of exercise-induced muscle damage; however, repeated bouts of stretching performed during the days following exercise may have favorable effects on muscle stiffness.

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1. Introduction

Exercise-induced muscle damage (EIMD) frequently occurs after exhaustive and/or unaccustomed exercise, particularly if the exercise involves eccentric muscle contractions. Moreover, it is clinically expressed by a relevant set of indirect markers of muscle damage such as muscle soreness, decreased maximal voluntary muscle contractions and increased muscle stiffness with reduced range of motion.\textsuperscript{1,2} It has been reported that passive muscle stiffness increases immediately after EIMD and remains elevated for the next 4–5 days.\textsuperscript{3}

Eccentric exercise can damage muscle cells in terms of disrupted structures and cytoskeletal components, loss of desmin, and permeabilization of the muscle cell plasma membrane. The structural damage to the sarcolemma or to the membranes of the sarcoplasmic reticulum, due to high mechanical tension, favors the loss of Ca\textsuperscript{2+} ion homeostasis interfering with length-tension relation in the relaxed muscle.\textsuperscript{1,5–7}

However, alterations in the electromyography activity found after EIMD \textsuperscript{8,9} suggest that the increase in muscle stiffness could also be related to alterations in the muscle reflex sensitivity. Alterations in the sensitivity of the muscle spindles of the damaged muscle could lead to an interference in sensory inputs (Ia and II afferent fibers), increasing the number of motor-units recruited at rest, and resulting in increased muscle stiffness.

Aiming to recover the exercised muscles, passive muscle stretching is commonly recommended.\textsuperscript{10} It is suggested that stretching might induce alterations in both mechanical and neural properties, leading to faster recovery of damaged muscles. However, the influence of stretching on muscle stiffness after EIMD has been insufficiently studied. DeVries,\textsuperscript{9} in 1966, showed that muscle stretches reduced muscle spasms after intense exercise. Subsequently, McGlynn et al.,\textsuperscript{9} in 1978, found a reduction of muscle spasm induced by stretching, based on a decrease in...
electromyographic activity (EMG) in stretched muscles following EIMD. These results are in accordance with those obtained by Torres et al.\textsuperscript{2} who found a reduction in muscle stiffness after performing a single bout of muscle stretching immediately post-exercise.

Taking into account that EIMD develops its clinical manifestation beyond 48 h post-exercise, it seems reasonable that a single stretching intervention performed immediately after exercise should be insufficient to have beneficial effects on the development of EIMD and it would be more logical to assume that repeated stretching, performed daily during the course of EIMD, might have accumulative beneficial effects on EIMD markers.

Thus, the purpose of this study was to compare the effect of passive muscle stretching, either as a single bout or as repeated bouts applied daily after eccentric exercise, on indirect markers of EIMD: muscle soreness; maximal muscle strength; muscle stiffness; and plasma creatine kinase activity.

2. Methods

This study was performed in accordance with the ethical standards.\textsuperscript{11} Moreover, the local Ethics Committee, in accordance with the Helsinki Declaration, approved all procedures prior to the start of this investigation. After completing a medical screening questionnaire and providing written informed consent prior to participation, a total of 56 young, healthy, and untrained men were included for this study [age = 21.4(1.9) years old; weight = 74.1(7.2) Kg; height = 179.8 (7.0) cm and body mass index 22.3 (1.4) Kg/m\textsuperscript{2}]. Exclusion criteria included a history of muscle tear, neurological disease involving the lower limbs, and current lower-limb musculoskeletal injury.\textsuperscript{12,13}

Subsequently, the sample was randomly divided into four groups: (I) a single stretching group (SSG, n = 14) that performed a single bout (SS) of stretching; (II) an eccentric exercised group (ECCG, n = 14) that performed only an eccentric exercise; (III) an eccentric exercise group followed by SS (ECC + SSG, n = 14) that was submitted to an identical eccentric exercise program as the ECCG, followed by an SS; and (IV) an eccentric exercised group submitted to repeated bouts (RS) of stretching (ECC + RSG, n = 14) that was submitted to a similar protocol as ECC + SSG; however, the RS was performed immediately after exercise and at 24, 48, and 72 h post-exercise.

The stretching, which was performed on the dominant quadriceps muscle, included ten passive stretches lasting 30 s each with a 10-s rest between stretches. All passive stretching was overseen by the same examiner, who continued the stretch until he felt reasonable resistance or the participant reported discomfort.\textsuperscript{10,14} The participant was in a standing position with one knee resting on a chair. The dominant leg was kept relaxed, with the hip in hyperextension; the examiner passively stretched the quadriceps, flexing the participant’s knee and extending the hip to a neutral position. If maximal knee flexion did not produce a sensation of stretch or resistance against the movement, hip extension would be added in order to increase the stretch.\textsuperscript{15}

To perform the eccentric muscle contractions an isokinetic dynamometer (Biodex System 3 Pro\textsuperscript{TM}, Biodex Medical Systems, Inc., Shirley, NY, USA) was used. All participants (except the SSG), after familiarization with the dynamometer, performed sets of thirty eccentric contractions with a target of 60% of the maximal concentric peak torque (MCPT).\textsuperscript{16} The exercise was interrupted whenever the participant could not complete two sets (which was defined as a criterion for incapacity to maintain the contractions at the given intensity and frequency). The participants were instructed to perform an eccentric quadriceps contraction against the moving lever of the system at 60 degrees per second, using visual feedback from the dynamometer software as a means to maintain strength intensity. The range of motion was set between 20 and 100 °C of knee flexion; resting time between contractions and sets was set at 1 and 30 s, respectively.

Before the procedure previously described, each participant had to warm up on a cycle ergometer (Monark E-824, Monark Exercise Ab, Varber, Sweden) at a resistance of 2% of body weight for 5 min. Then the participants were seated with 100° hip flexion on the dynamometer chair. The standard Biodex knee unit attachment was used to restrain the chest, pelvis, thigh, and ankle in accordance with the manufacturer’s instructions (Biodex Pro Manual, Applications/Operations, Biodex Medical Systems Inc, Shirley, NY, USA). The resistance pad was placed as distally as possible on the tibia while still allowing full dorsiflexion of the ankle.

The dependent variables, which were assessed before exercise and 1, 24, 48, 72, and 96 h after EIMD, included: muscle soreness; the first angle of knee flexion and the final resting angle in the pendulum test\textsuperscript{17}; plasma CK activity; EMG collected during the pendulum test; and the MCPT, collected after assessing all other variables.

Muscle soreness was measured with the Visual Analog Scale that is commonly used in delayed onset muscle soreness research.\textsuperscript{12,16,18} The patient was instructed to perform a squat (active movement) and rate his perception of soreness marking the line at a distance corresponding to the intensity of present pain.

Following the methodology previously used,\textsuperscript{13} the dynamometer Biodex was used to evaluate the knee extensor muscle peak torque. The range of motion of the knee joint was set at 0–100 °C. Three maximal concentric isokinetic knee extensions at an angular velocity of 60°/s were made to assess muscular strength. Three submaximal warm-up trials preceded the maximal muscle actions.

Alterations in muscle stiffness were assessed using the Wartenberg Pendulum Test\textsuperscript{17} with the aid of an electronic goniometer and SG150 sensor (Biometrics Ltd, Gwent, UK) following the guidelines proposed by Badj and Vodovnik.\textsuperscript{20,21} This test has been applied with success in the assessment of the functional properties of muscle under conditions of altered tonus in spastic or dystonic muscles or after muscle contractures\textsuperscript{22} by evaluating the longitudinal muscle stiffness during a natural passive stretching.

The sensor was placed on the lateral side of the leg in line with the greater trochanter and the lateral malleolus. Analog signals were digitalized using an MP 150 converter (Biopac Systems Inc., Santa Barbara, CA, USA) and analyzed with the accompanying Acknowledge software version 3.9.0.

The Wartenberg Pendulum Test was conducted with the participants in a prone position on a treatment table and the knee joint tested at about 8 cm away from the edge of the table. The other leg was flexed with the heel supported on the table to stabilize pelvis rotation. The examiner lifted the calf to full knee extension with the hip point remaining in place and let it fall down and oscillate in decreasing flexion/extension movements until it came to rest. The test was repeated three times with 30 s intervals, and the mean value of the three tests was used for analysis.\textsuperscript{20} All participants became sufficiently accustomed to the described procedure before testing that they were able to completely relax their thighs and refrain from any voluntary oscillation movements. The first angle of knee flexion and the final resting knee angle were the parameters used to characterize muscle stiffness. Before this assessment, a pilot study was conducted with 15 participants in order to determine the intraclass correlation coefficient (ICC), and an excellent intraobserver reliability for the first angle of knee flexion and the final resting angle (ICC2, 3 = 0.986 and 0.998, respectively) was found.

Electromyography data was collected\textsuperscript{23} at the same time as the pendulum test was conducted. EMG signals from each participant’s dominant quadriceps muscle, in particular from vastus medialis and rectus femoris were recorded using surface EMG MP150. The
Table 1

Intragroup alterations over time in absolute values for muscle soreness, maximal concentric peak torque, angle of first knee flexion, final resting knee angle (expressed as means and standard deviations) and plasma creatine kinase activity (expressed as median and interquartile range); (SSG = Single stretching group; ECCG = Eccentric exercised group; ECC + SSG = Eccentric exercise group followed by Single Stretching; ECC + RSG = Eccentric exercised group submitted to Repeated Stretching); (p < 0.05).

<table>
<thead>
<tr>
<th>Variables</th>
<th>Groups</th>
<th>Before</th>
<th>1 h</th>
<th>24 h</th>
<th>48 h</th>
<th>72 h</th>
<th>96 h</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Muscle soreness</td>
<td>SSG</td>
<td>0.0(0.0)</td>
<td>0.0(0.0)</td>
<td>0.0(0.0)</td>
<td>0.0(0.0)</td>
<td>0.0(0.0)</td>
<td>0.0(0.0)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>ECCG</td>
<td>0.0(0.0)</td>
<td>0.1(0.4)a</td>
<td>2.3(0.8)a</td>
<td>3.8(1.8)b,c</td>
<td>1.8(1.2)d</td>
<td>0.7(0.5)c,d</td>
<td>p &lt; .001</td>
</tr>
<tr>
<td></td>
<td>ECC + SSG</td>
<td>0.0(0.0)</td>
<td>1.2(0.7)a</td>
<td>2.3(1.1)a</td>
<td>3.5(1.4)b,a</td>
<td>1.8(1.3)a</td>
<td>0.9(1.0)c,d</td>
<td>p &lt; .001</td>
</tr>
<tr>
<td></td>
<td>ECC + RSG</td>
<td>0.0(0.0)</td>
<td>1.3(0.6)a</td>
<td>2.4(0.8)a</td>
<td>3.3(0.8)b,a</td>
<td>1.4(0.7)d,c</td>
<td>0.4(0.2)b,c,d</td>
<td>p &lt; .001</td>
</tr>
<tr>
<td>Maximal concentric peak torque (60°/s)</td>
<td>SSG</td>
<td>219.0(24.6)</td>
<td>215.5(27.1)</td>
<td>223.3(38.8)</td>
<td>220.7(23.5)</td>
<td>221.8(32.6)</td>
<td>222.0(24.7)</td>
<td>p = .986</td>
</tr>
<tr>
<td>Angle of first knee flexion (degrees)</td>
<td>ECCG</td>
<td>221.3(16.7)</td>
<td>181.7(30.7)a</td>
<td>186.8(27.0)</td>
<td>188.5(40.3)</td>
<td>203.8(29.3)</td>
<td>219.8(24.9)b,c</td>
<td>p &lt; .001</td>
</tr>
<tr>
<td></td>
<td>ECC + SSG</td>
<td>216.9(33.5)</td>
<td>173.4(33.7)a</td>
<td>183.2(42.5)</td>
<td>189.7(44.5)</td>
<td>204.7(26.2)</td>
<td>217.3(35.1)b</td>
<td>p = .013</td>
</tr>
<tr>
<td></td>
<td>ECC + RSG</td>
<td>238.1(40.8)</td>
<td>200.2(31.9)a</td>
<td>204.4(35.1)</td>
<td>214.1(47.4)</td>
<td>227.5(44.8)</td>
<td>241.3(42.4)</td>
<td>p = .03</td>
</tr>
<tr>
<td>Final resting knee angle (degrees)</td>
<td>SSG</td>
<td>75.7(7.5)</td>
<td>19.7(12.0)</td>
<td>56.5(6.4)a</td>
<td>65.6(6.6)a</td>
<td>73.4(7.0)b,c</td>
<td>73.8(7.8)b,c</td>
<td>p &lt; .001</td>
</tr>
<tr>
<td></td>
<td>ECCG</td>
<td>76.7(6.5)</td>
<td>63.2(6.7)a</td>
<td>69.2(6.3)a</td>
<td>69.9(7.8)</td>
<td>73.5(8.1)b</td>
<td>76.3(5.6)b</td>
<td>p &lt; .001</td>
</tr>
<tr>
<td></td>
<td>ECC + SSG</td>
<td>76.7(5.0)</td>
<td>63.4(4.0)a</td>
<td>68.8(7.4)a</td>
<td>73.0(6.6)b</td>
<td>75.6(7.2)b,c</td>
<td>77.0(5.6)b</td>
<td>p &lt; .001</td>
</tr>
<tr>
<td>Plasma creatine kinase activity</td>
<td>SSG</td>
<td>55.2(4.2)</td>
<td>54.2(4.2)</td>
<td>54.7(5.4)</td>
<td>55.1(4.5)</td>
<td>56.1(3.8)</td>
<td>55.3(5.2)</td>
<td>p = .906</td>
</tr>
<tr>
<td></td>
<td>ECCG</td>
<td>56.7(2.6)</td>
<td>46.3(4.4)a</td>
<td>48.8(4.9)a</td>
<td>50.3(4.8)a</td>
<td>52.6(3.7)b</td>
<td>55.7(2.7)b,c,d</td>
<td>p &lt; .001</td>
</tr>
<tr>
<td></td>
<td>ECC + SSG</td>
<td>57.5(5.2)</td>
<td>49.8(5.3)a</td>
<td>51.9(5.2)</td>
<td>51.1(5.5)</td>
<td>54.3(6.3)</td>
<td>56.2(5.1)b</td>
<td>p = .001</td>
</tr>
<tr>
<td></td>
<td>ECC + RSG</td>
<td>55.5(5.2)</td>
<td>48.2(4.7)a</td>
<td>51.5(5.6)</td>
<td>51.6(5.2)</td>
<td>53.7(4.4)</td>
<td>53.3(5.0)b</td>
<td>p = .001</td>
</tr>
</tbody>
</table>

Note: p < 0.05, a vs. before; b vs. 1 h; c vs. 24 h; d vs. 48 h.

The analyzed parameters resulting from the pendulum test as well as muscle soreness, MCPT and plasma CK activity did not change over time in SSG (Table 1). After performing eccentric exercise, all groups showed a significant increase in muscle soreness (p < 0.05) up to 72 h post-exercise. The assessment of the MCPT revealed a significant decrease 1 h post-exercise, while plasma CK activity increased in the following three days.

It can be seen that stretching applied immediately post-exercise (ECC + SSG) or repeated at 24 h (ECC + RSG) contributed to recovery from muscle stiffness. Indeed, at 48 h only the ECCG demonstrated significant differences in “first knee flexion” relative to baseline values.

The knee angle at which the calf finally came to rest was also considerably reduced in ECCG, ECC + SSG and ECC + RSG at 1 h post-exercise. The assessment at 24 h showed significant recovery in the ECC + SSG and ECC + RSG. However, in the following assessment (at 48 h) the ECC + RSG did not differ significantly from the ECCG.

For electromyographic activity, apart from one negligible exception, no changes were found over time in all groups in the standard assessed parameters.

No significant differences were found between groups in any of the studied variables evaluated at baseline (Fig. 1). The intergroup analysis of “first knee flexion” in the assessment made at 1 h post-exercise showed that all groups which performed eccentric exercise had a significant (p < 0.05) and similar reduction in this parameter (~20%), compared to SSG. However, at 24 h, both...
Fig. 1. A–E – intergroup comparisons for each measurement time for muscle soreness, maximal concentric peak torque, first angle of knee flexion and resting angle of the pendulum test (expressed as means and standard deviations), and plasma creatine kinase activity (expressed as median and interquartile range); (SSG = single stretching group; ECCG = eccentric exercised group; ECCC + SSG = eccentric exercise group followed by single stretching; ECC + RSG = eccentric exercised group submitted to repeated stretching).
groups that had carried out stretching (ECC + SSG and ECC + RSG) immediately post-exercise showed a significant recovery in the first knee flexion angle and in the resting knee angle (≈10%), without significant differences compared to SSG (p > 0.05). At 48 h post-exercise, significant differences were only observed between SSG and ECC + SSG, suggesting that the favorable effect of a single stretching episode on muscle stiffness, observed at 24 h, had been lost.

Although some differences were found between exercised groups (ECCG, ECC + SSG, and ECC + SRG) and the SSG, no significant differences were found among these three groups in all assessed variables, not even in EMG (p > 0.05).

4. Discussion

Our findings demonstrated no significant differences in muscle soreness, MCPT or plasma CK activity after performing one or multiple bouts of stretching. However, both interventions contributed to recovery from muscle stiffness, suggesting that stretching applied repetitively during this myogenic condition produced a cumulative effect on this indirect marker of muscle damage.

There is increasing evidence that both extrafusal and intrafusal fibers are affected by eccentric contractions. Consequently, injury to intrafusal fibers would lead to muscle spindle dysfunction with consequent increase in muscle stiffness. These alterations in the sensitivity of the muscle spindles, leading to an increase in sensorial inputs (Ia and II afferent fibers), might lead to an increase in motor units recruited at rest, which could explain the increase in muscle stiffness.

It has been argued that repeated stretching diminishes the sensitivity of the muscle spindles, leading to a reduction of muscle reflex sensitivity. Evidence of this effect was obtained when Avela et al. detected a clear reduction (≈85%) in the stretch-reflex peak-to-peak amplitude after 1 h of muscle stretching, suggesting that increased compliance of the muscle-tendon unit after stretching might influence neural activation patterns. Moreover, these authors suggested that the origin of the reduced reflex sensitivity could be a reduction in the activity of the large-diameter afferent fibers, resulting from reduced mechanical sensitivity of muscle spindles to repeated stretch. In this sense, the use of muscle stretching has been claimed to contribute to the muscle recovery, by reducing muscle stiffness. The beneficial effects on muscle stiffness found in our results corroborate this suggestion.

However, the EMG activity data collected during the “first knee flexion”, and particularly during the “final resting angle” tests, revealed no increase in EMG activity at rest after EIMD, contrary to the findings of DeVries and McGlynn. In this sense, we are of the opinion that EMG probably does not have sufficient sensitivity to detect alterations in the muscle reflex activities. Therefore, the EMG which was done simultaneously with the pendulum test allowed us only to verify an eventual muscle activation, minimizing the disturbance created by active movement.

More recently, we carried out a study using a pendulum test, and found a reduction in muscle stiffness after applying SS immediately after exercise, which suggests acute effects of stretching on reflex activity, probably related to alterations in muscle spindle function. However, this previous study suggested that SS is not sufficient to induce long-term effects, raising the hypothesis that repeated stretching applied daily during EIMD might be effective on the maintenance of low levels of muscle stiffness. In the present study, we found similar acute positive effects and found that repeated muscle stretching accelerates recovery from muscle stiffness, confirming the findings of our previous study. Our results showed that both parameters of the pendulum test improved after muscle stretching: the ECC + RSG demonstrated a greater recovery compared to the ECC + SSG.

Nevertheless, the increase in the muscle stiffness after EIMD is not only due to alterations in reflex activity but it is also evidenced by structural alterations in the skeletal muscle, which are mainly expressed as irregularities in cross-striated patterns. Passive muscle stiffness rises as a consequence of the damage to the sarcolemma or to the membranes of the sarcoplasmatic reticulum, leading to the loss of Ca ion homeostasis, and developing an injury contracture. Theoretically, due to the fact that stretching has a similar mechanical tension as eccentric exercise in the elastic components of the muscle and in the intermediate filament that compose the cytoskeleton, it seems to be irrelevant to stretch the muscle in order to avoid the signs and symptoms of EIMD. Furthermore, Lund et al. reported that stretching post-exercise does not prevent secondary pathological alterations, suggesting even that it could aggravate some markers of exercise induced muscle damage.

The increase of muscle stretching after EIMD, reducing range of motion in the involved joints, could interfere with the better posture of the body segments. Although there is absence of stretching effects on MCPT, muscle soreness, and CK activity, stretching seems to be a favorable intervention post-exercise as it normalizes muscle tonus and contributes to improve posture. Nevertheless, more studies are needed in order to analyze the effect of EIMD on body segments and posture and the true effect of stretching after EIMD.

Yet this does not allow us to state that stretching after EIMD is an effective intervention. Indeed, no significant differences in muscle soreness among the ECCG, ECC + SSG or ECC + RSG were found: results showed similar behavior over time in all groups, corroborating other studies in which no difference in this variable was found after stretching intervention. Therefore, our results do not support the hypothesis proposed by Armstrong, that stretching enhances the threshold excitability of type III and IV free nerve endings with consequent decrease in pain sensation.

Similar results were found for muscle strength and plasma CK activity, confirming the ineffectiveness of stretching in improving muscle damage recovery. Knowing that prolonged strength loss after eccentric exercise is considered to be one of the most valid and reliable indirect markers of EIMD in humans, the absence of stretching effects on this marker allowed us to conclude that stretching the damaged muscle seems to be irrelevant to improvement of function.

The discomfort caused by EIMD leads the authors involved in this type of studies to include a reduced number of participants, increasing the risk of a type II error. Therefore, the analysis of these results should have these facts in consideration due to the fact that they could be regarded as limitations of the present study.

5. Conclusions

Although stretching applied repeatedly after exercise has a positive effect on the relief of muscle stiffness, the absence of positive effects on muscle soreness, MCPT, and plasma CK activity suggests that stretching intervention is ineffective in relieving the harmful effects that can result from eccentric exercise.

6. Practical implications

- Wartenberg test is a valid tool that can be used to identify alterations in the quadriceps muscle stiffness after EIMD.
- Static muscle stretching performed after EIMD contributes to reduce muscle stiffness.
- Although stretching applied repeatedly after exercise has a positive effect on the relief of muscle stiffness, its application as a
single bout or as repeated bouts, does not influence other indirect markers of EIMD.

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References