Effect of eccentric exercise-induced muscle damage on electromyography activity of quadriceps in untrained healthy females

Mandana Rezaei¹, Ismael Ebrahimi-Takamjani², Ali A. Jamshidi³
Behnoush Vassaghi-Gharamaleki⁴, Nosratollah Hedayatpour⁵, Naser Havaei⁶

Abstract
Background: The aim of this study was to investigate muscle damage indicators and electromyography activities of quadriceps muscles at 25° of hip flexion in untrained healthy females after an eccentric exercise induced muscle fiber damage.

Methods: A total of 14 healthy females participated in this pre-experimental study. The subjects performed maximal eccentric quadriceps contractions at 25° of hip flexion. Maximum voluntary extensor isometric and concentric moments, angle of maximum moment for concentric contractions, perceived pain intensity, and pain pressure threshold were examined before, immediately, 48 hours, 120 hours and 14 days after eccentric exercise. Additionally, electromyography of three parts of quadriceps muscle, knee flexion range of motion and thigh circumference were measured before and after eccentric exercise.

Results: Significant reductions in maximum isometric moment and maximum concentric moment were observed at angular velocity of 60° per sec immediately after eccentric exercise (p<0.05). Both maximum isometric moment and maximum concentric moment recovered to the baseline 48 hours after eccentric exercise. Increased pain intensity and decreased knee joint range of motion manifested 48 hours after eccentric exercise. Pain pressure threshold for the quadriceps was higher 14 days after exercise as compared to 48 and 120 hours (p<0.05). No significant changes observed in electromyography and thigh circumference (p>0.05).

Conclusion: Eccentric exercise performed at 25° of hip flexion resulted in muscle fiber injuries within the quadriceps muscle. However, electromyography of quadriceps muscle was not significantly different than the baseline. The result indicates that hip joint position may modify the effect of eccentric exercise on muscle activation.

Keywords: Muscles, electromyography, hip, muscle soreness, exercise.


Introduction
Extent of muscle damage after eccentric exercise can be dependent on muscle length (1-13). It has been shown that eccentric exercise performed in the long lengths of the elbow flexor and knee extensor muscles resulted in higher damage as compared to the short lengths (7, 9, 11, 12). For example, previous studies examined the effect of eccentric exercise on quadriceps muscle damage by manipulating the length of bi-articular rectus femoris (RF) muscle from the hip joint. These studies reported that the extent of muscle damage at short length...
was significantly greater than the long length (6).

Others studies have also reported that eccentric exercise performed at 90 ° hip flexion produced higher pain pressure thresholds and lower electromyographic activities (EMG) at the most distal portion of quadriceps muscle (e.g. vastus medialis oblique) (1, 2, 14, 15).

It has been reported that changes in hip and knee joint position has a predominant effect on the activation level of quadriceps muscle during maximal voluntary isometric (16-18) and concentric contractions and electrically evoked contractions (18). Moreover, the excitation level of the quadriceps muscle is dependent on hip joint angles (17). Some previous studies have also demonstrated a lower activation level (17, 18) and a lower moment output of quadriceps muscle (18, 20) in lying position of the hip joint. However, there are no studies available on quadriceps muscle activity following eccentric exercise performed in lying position of the hip joint. In this study, we hypothesized that change in muscle length may modify muscle activation following eccentric exercise induced muscle damage. This knowledge may be useful to design exercise training and or rehabilitation program.

Therefore, the purpose of this study was to investigate electromyographic activities of the quadriceps muscle in the untrained healthy females after an eccentric exercise performed at 25° of hip flexion.

**Methods**

**Participants**

Fourteen healthy females (age 23.93 ± 4.48 yr, body mass 55.89 ± 4.55 kg, and height 1.59 ± 4.58 m) randomly participated in this pre-experimental study. All subjects were right-leg dominant (defined as preferred kicking leg). Participants were not involved in regular exercise of their knee extensor muscles for at least 6 months before the experiment. They had no prior history of knee injuries. The study was approved by the research ethics committee of the Iran University of Medical Sciences (N 90/D/130D2800).

**General protocol**

The subjects performed eccentric exercise of knee extensors with the dominant leg on Biodex isokinetic dynamometer (Biodex Medical Systems 4, Shirley, NYTM). Participants were familiarized with moment measurement and eccentric exercise protocol 48 hours prior to the experiment. Muscle damage indicators including maximum isometric knee extension moment at 30°, 60°, 90°, and 120° of knee flexion, maximum concentric knee extension moments at angular velocities of 60 ° and 180 ° per sec, angle of maximum moment for concentric knee extension at each velocities were measured before, immediately after, 48 hours (h), 120 h, and 14 days after the eccentric exercise. Moreover, active and passive knee flexion range of motion, thigh circumference, perceived pain intensity, pain pressure thresholds (PPT) and associated EMG activities at the distal parts of quadriceps were measured at same day of testing sessions.

**Eccentric exercise protocol**

The participants sat comfortably on the adjustable chair of the Biodex isokinetic dynamometer (Biodex Medical Systems 4, Shirley, NYTM) with their hip in 25° flexion. The chair position was adjusted so that the axis of rotation of the knee (tibiofemoral joint) was aligned with the axis of rotation of the dynamometer’s attachment arm. The subjects were fixed with straps secured across the chest and hips. The dominant leg was secured into the attachment arm with a Velcro strap. The participants performed six bouts of 20 maximal voluntary eccentric contractions at a speed of 120 per sec between 10° to 90° of knee flexion with a three minute rest interval between each set. During the exercise, the subjects were provided with visual feedback of force and was encouraged to maintain maximal force.
Maximum voluntary moment

Maximal voluntary isometric contraction extensor moment (MVIM) and maximal voluntary concentric contraction extensor moment (MVCM) were measured using a Biodex Dynamometer at 5° reclined position (seated). The participants were fixed with straps secured across the chest and hips. The subjects were asked to perform three maximal isometric knee extensions (5 s in duration) at 30°, 60°, 90°, and 120° of knee flexion (full extension=0°). Verbal encouragement was used to exceed the previous force level. Visual feedback of force was provided on a screen positioned in front of the subject to monitor force level. The averaged moment of 3 measurements at each angle was computed and considered as MVIM for that angle. The rest period between MVIM trials was 30 seconds, and a three minutes recovery period was allowed between tests at different joint angles. MVCM was measured at 60 and 180° per sec between 10° to 90° of knee flexion. The angle of maximum moment (AOM) was measured using Biodex software. The mean value of MVIM, MVCM and AOM obtained from 3 and 5 trials were used as a representative value. Moment values at each condition were normalized to body mass and were expressed as a percentage change from pre-exercise value.

Pain assessment

A 100-mm visual analog scale, labeled with end points on the left (no pain) and right (worst pain imaginable), was used to assess the perceived pain intensity at immediately, 48 h, 120 h and 14 days after eccentric exercise. Lying supine, volunteers actively flexed and extended their knee. They then placed a mark on the scale representing the soreness experienced in the knee-extensor region.

Using a 20 mL syringe, PPT measured from the distal part of RF, VL, and VM muscles where the EMG signals were recorded. The 20 mL syringe contains a spring inside that was scaled from 0 to 10. The flattened circular end of the syringe with the diameter of 0.5 cm² was vertically placed over the distal part of RF, VL, and VM muscles, and the location was pressed down while the participant was in the long-sitting position with a relaxed quadriceps muscle. The participant was asked to announce any unpleasant sensation (i.e., pain), and then the number on the syringe was recorded as the PPT. Measurements of PPT were repeated three times for each location in random order and were averaged for data analysis. In addition, the percent difference in PPT for post exercise sessions with respect to baseline (pre-exercise value in day 1) was calculated, to compare changes across testing sessions.

Knee flexion range of motion

The active and passive knee flexion ranges of motion (AKROM and PKROM respectively) were examined while the participant lies in prone position on the table without any rotation or abduction, and adduction in lower extremity. A goniometer was used to measure ROM until pain or discomfort in quadriceps begins. Fulcrum of the goniometer was placed on the lateral epicondyle of femur and the stationary arm was adjusted to the greater trochanter. Measurements for ROM were repeated three times and were averaged for data analysis. The percent difference in ROM for post exercise sessions with respect to baseline (day 1) was calculated, to compare changes across testing sessions.

Thigh Circumference

Thigh circumference is one of the muscle damage indicators that are presenting the amount of swelling after unaccustomed eccentric exercise (6). Thigh circumference measured at 10% of the distance between the greater trochanter and lateral epicondyle of femur by an elastic tape measure while the participant was in the standing position. Three measurements were taken from each marked location and the mean value of the 3 measurements was used for statistical analysis. Thigh circumference was expressed as a percentage change from
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Electromyography
Surface EMG signals were recorded from three locations distributed over the quadriceps muscle by circular Ag–AgCl surface electrodes (Biometric LTD Data Log UK™). Surface EMG signals were band pass filtered at 20 to 450 Hz at a sampling rate of 1,000 Hz with a common-mode rejection ratio of 110 dB using the Biometric Data Log software. Surface electrodes were placed on quadriceps muscle at 10% of the distance between medial border (VM), superior border (RF) and lateral border (VL) of the patella and anterior superior iliac spine based on previous study (22). The electrodes were located in bipolar configuration (inter-electrode distance 20 mm) between the most distal innervation zone and the distal tendon region of the quadriceps muscle (22). Before electrode placement, the skin was shaved and lightly abraded at the selected locations. The positions of the electrodes were marked on the skin during the first session, enabling replication of electrode location 48 h, 120 h, and 14 days after eccentric exercise. Three-way ANOVA was also applied to assess change in EMG amplitude before and after eccentric exercise with muscle (RF, VL, and VM) and MVIM angle (30°, 60°, 90°, and 120°) as dependent factor. A four-ways ANOVA was applied to compute changes in PPT value and EMG amplitude across testing session with muscle and MVIM angle (30°, 60°, 90°, and 120°) as dependent factor. Pairwise comparisons were performed with the Bonferroni adjustment when ANOVA was significant. The significance level was set at P < 0.05 for all statistical procedures using SPSS version 18.

Statistical analysis
One-way repeated-measures ANOVA was applied to analyze MVCM, AOM, perceived pain intensity, AKROM, PKROM, and thigh circumference before (baseline), immediately after, 48 h, 120 h, and 14 days after eccentric exercise. Three-way ANOVA was also applied to assess change in EMG amplitude before and after eccentric exercise with muscle (RF, VL, and VM) and MVIM angle (30°, 60°, 90°, and 120°) as dependent factor. A four-way ANOVA was applied to compute changes in PPT value and EMG amplitude across testing session with muscle and MVIM angle (30°, 60°, 90°, and 120°) as dependent factor. Pairwise comparisons were performed with the Bonferroni adjustment when ANOVA was significant. The significance level was set at P < 0.05 for all statistical procedures using SPSS version 18.

Fig. 1. Changes in isometric peak moment (MVIM) at different angles (30°, 60°, 90°, and 120°) as % of pre-exercise following eccentric exercise. * denotes significantly different from pre-exercise (p< 0.05). £ denotes significantly different from 120 h (P < 0.05). # denotes significantly different from 14 days (p< 0.05).
Results
Isometric and Isokinetic extension moments (MVIM and MVCM)

MVIM was dependent on time and angle (F > 4.77, P < 0.05), with the lower values observed for all angles, immediately after eccentric exercise as compared with pre-exercise session (P<0.05) (Fig. 1). There was no significant difference in MVIM between 48 h, 120 h and 14 days post exercise (all p>0.05).

MVIM at 30° of knee flexion was significantly lower than the other three angles (F= 125.20, P < 0.0001), and at 60˚ was significantly higher than 120˚ (p<0.001) (Table 1).

MVCM at angular velocity of 60 ° per sec was dependent on time (F= 22.81, p<0.001), with lower values obtained immediately after eccentric exercise compared with pre-exercise value (p<0.05). There was no significant difference between 48 h, 120 h and 14 days post exercise (all p>0.05).

AOM at angular velocity of 180 ° per sec was significantly changed toward more flexed angles immediately compared to 14 days after performing eccentric exercise (F=4.18, p=0.030).

Pain assessment
Perceived pain intensity during flexion and extension of the knee joint was dependent on testing day. Perceived pain intensity obtained at 48 h after eccentric exercise was significantly higher than the pre-exercise (mean ± SD of flexion and extension are 17.5 ± 14.16 and 18.0 ± 18.68, respectively) (F > 4.30, P<0.05) (Fig. 2). Only PPT of RF was depended on day (F = 5.64, P=0.015). There was no interaction between muscle and time.

Knee flexion range of motion
AKROM and PKROM were dependent on testing day (F> 4.13, p<0.05) with a lower value observed at 48 h after eccentric exercise compared to pre-exercise value (p<0.05). No significant changes observed between immediately after, 120 h and 14 days post exercise (all p>0.05).

Thigh circumference
Thigh circumference was not altered after eccentric exercise (p>0.05).

Electromyography
EMG activity of the quadriceps muscle during MVIM was depended on knee angle (F = 62.64, p=0.000). EMG amplitudes ob-

<table>
<thead>
<tr>
<th>30° knee flexion</th>
<th>60° knee flexion</th>
<th>90° knee flexion</th>
<th>120° knee flexion</th>
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</thead>
<tbody>
<tr>
<td>Pre-exercise MVIM (N.m)</td>
<td>95.84±25.16</td>
<td>169.18±35.62</td>
<td>146.93±35.86</td>
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<tr>
<td>Immediately after MVIM (N.m)</td>
<td>73.19±29.54*</td>
<td>133.39±23.88*</td>
<td>129.02±21.05≠</td>
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<tr>
<td>48 h MVIM (N.m)</td>
<td>88.73±22.52</td>
<td>157.26±39.55</td>
<td>134.71±21.91</td>
</tr>
<tr>
<td>120 h MVIM (N.m)</td>
<td>82.94±27.07</td>
<td>162.92±37.77</td>
<td>144.77±17.76</td>
</tr>
<tr>
<td>14 days MVIM (N.m)</td>
<td>90.13±19.81</td>
<td>165.04±35.14</td>
<td>158.64±37.48</td>
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*Indicates significant difference to pre-exercise (P < 0.05). £Indicates significant difference to 120 h (P < 0.05). ≠ Indicates significant difference to 14 days (P < 0.05). MIVM, maximum voluntary isometric moment.

<table>
<thead>
<tr>
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<th>60° knee flexion</th>
<th>90° knee flexion</th>
<th>120° knee flexion</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre-exercise EMG(µv)</td>
<td>140.27±65.61</td>
<td>163.53±90.44</td>
<td>220.23±109.16</td>
</tr>
<tr>
<td>Immediately after EMG(µv)</td>
<td>151.13±72.94</td>
<td>183.30±104.72</td>
<td>247.72±136.74</td>
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<tr>
<td>48 h EMG(µv)</td>
<td>144.17±71.27</td>
<td>175.14±124.79</td>
<td>238.90±147.92</td>
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<tr>
<td>120 h EMG(µv)</td>
<td>141.12±72.84</td>
<td>172.73±128.99</td>
<td>250.76±176.74</td>
</tr>
<tr>
<td>14 days EMG(µv)</td>
<td>159.67±84.88</td>
<td>161.25±75.20</td>
<td>266.79±168.55</td>
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</tbody>
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EMG, electromyography as root mean square (RMS).
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Discussion

The result of this study demonstrated that delayed onset muscle soreness (DOMS) manifested 48 h after eccentric exercise and resulted in significant reduction in MVIM, MVCM and knee ROM, which is in agreement with previous studies (1, 2, 4, 7, 14, 15, 22). All these DOMS-related indicators (e.g., ROM, PPT) returned to the pre-exercise level 120 h after eccentric exercise. However, thigh circumference and EMG amplitude of quadriceps muscle at post-exercise session was not significantly different from the baseline. The lack of significant differences in muscle activation at different knee angles across testing session indicated that the eccentric exercise performed in lying position had no effect on quadriceps muscle activity.

Previous studies have also investigated the effect of eccentric exercise in the short vs. the long length of quadriceps muscle (7, 4). Child et al. reported that the altered quadriceps muscle length to a longer muscle length from the knee joint resulted in higher muscle damage after eccentric exercise (7). Paschalis et al. also reported that alteration in the length of quadriceps muscle from the hip joint can modify muscle damage after eccentric exercise (4). In agreement with these studies, eccentric exercise performed in 90° hip angle resulted in higher muscle damage as compared to 180° hip angle (4).

In the present study muscle damage indicators was not significantly changed after eccentric exercise performed at 25° of hip flexion. Similarly, other studies also reported no significant changes in muscle damage indicators in the seated and lying position with similar eccentric protocols (2, 22, 23, 4).

Moreover, MVIM reduced immediately after eccentric exercise and recovered to the baseline at 48 h post eccentric exercise. Subjects also reported uncomfortable muscle soreness at 48 hour post exercise but not at 120 hour and 14 days after eccentric exercise.

The results suggest that alteration in quadriceps length from the hip joint can modify quadriceps muscle responses to the maximal intensity eccentric exercise as compared to the seated position.
The lack of significant changes in EMG amplitude of the quadriceps muscle observed after eccentric exercise at 25° of hip flexion can also indicate that eccentric exercise performed at this knee angle has no significant effect on quadriceps activation. Quadriceps muscle reflected higher pain pressure threshold 14 days after eccentric exercise. To our knowledge, this the first study that has investigated interaction between quadriceps activation and muscle damage indicators at different knee angles after eccentric exercise performed at 25° of hip flexion.

Previous studies reported a higher PPTs and a lower EMG activity in the most distal parts of the quadriceps muscle and particularly at the medial aspect of it after eccentric exercise in the seated position (1, 2, 14, 15, 22).

Published literature also reported change in hip (18) and knee (16) joint position can effect on quadriceps muscle activity. For example a lower activation of RF muscle (18) and a lower quadriceps muscle moment (20) reported in lying position compared to the seated position. This may partly explain that why muscle damage indicators observed following our eccentric exercise protocol were different than those reported in the seated position (1, 2, 14, 15, 22).

Changes in hip and knee joint position can also have influence on the excitability of the quadriceps muscle as reported by previous studies (17, 24, 25). It has been shown that the excitability of quadriceps muscle at 112°, 135°, 157° of hip extension (180° being full extension), were lower than 90° and 180° (17), which may explain the lesser muscle damage observed following our eccentric exercise protocol. Other factors such as biomechanical changes influenced by joint position in bi- and mono-articular muscles may also change the magnitude of fiber damage within the quadriceps muscle (12) that was not assessed in this study. This study has not compared the effect of seated position versus lying position eccentric exercise and it is recommended to compare the effect of knee and hip joint position manipulation on damage response of quadriceps muscle and its excitability.

Conclusion
The result of this study shows that eccentric exercise performed in lying position produces lower muscle damage most probably due to a lower activation of quadriceps muscle and/or a lower moment produced by quadriceps muscle compared with the seated position. This knowledge may be useful to design exercise training and or rehabilitation programs.

Acknowledgements
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