A Gender Comparison of Central and Peripheral Neuromuscular Function After Exercise

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Context: Central and peripheral muscle fatigue during exercise may exacerbate neuromuscular factors that increase risk for noncontact anterior cruciate ligament injury. Objective: To compare lower extremity motor-evoked potentials (MEPs), muscle strength, and electromyography (EMG) activation after an exercise protocol. Design: Pretest, posttest group comparison. Setting: University laboratory. Participants: 34 healthy volunteers (17 female, age = 21.9 ± 2.3 years, weight = 77.8 ± 3.0 kg, height = 171.1 ± 6.6 cm, and 17 male, age = 23.4 ± 6.5 years, weight = 81.6 ± 3.3 kg, height = 179.6 ± 7.3 cm). Intervention: A standardized 30-min exercise protocol that involved 5 repeated cycles of uphill walking, body-weight squatting, and step-ups. Main Outcome Measures: Quadriceps and hamstring MEP amplitude (mV) and transmission velocity normalized to subject height (m/s) were elicited via transcranial magnetic stimulation and measured via surface EMG. Quadriceps peak EMG activation (% MVIC) and peak torque (Nm/kg) were measured during MVICs. Separate ANCOVAs were used to compare groups after exercise while controlling for baseline measure. Results: At baseline, males exhibited significantly greater knee-extension torques (males = 2.47 ± 0.68 Nm/kg, females = 1.95 ± 0.53 Nm/kg; P = .036) and significantly higher hamstring MEP amplitudes (males = 223.5 ± 134.0 mV, females = 89.3 ± 77.6 mV; P = .007). Males exhibited greater quadriceps MEP amplitude after exercise than females (males = 127.2 ± 112.7 mV, females = 32.3 ± 34.9 mV; P = .016). Conclusions: Males experienced greater peripheral neuromuscular changes manifested as more pronounced reductions in quadriceps torque after exercise. Females experienced greater central neuromuscular changes manifested as more pronounced reduction in quadriceps MEP amplitude. Reduced central neural drive of the quadriceps coupled with knee-extension torque preservation after exercise may increase risk of knee injury in females.

Keywords: transcranial magnetic stimulation, knee joint, fatigue

Noncontact anterior cruciate ligament (ACL) injuries are common in athletes participating in sports that involve rapid changes of directions, sudden deceleration, pivoting, and jump landings.1–3 Investigators have consistently documented gender differences in rates of noncontact injury and risk factors for ACL injury. Specifically, female athletes are 2 to 8 times more likely to sustain this type of injury than their male counterparts.4–8 This gender disparity is considered multifactorial and may be due to anatomic, hormonal, and neuromuscular differences between males and females.1,3,9–13

Females have been shown to have weaker quadriceps and hamstring muscles, higher quadriceps-to-hamstrings strength ratios, and decreased hamstrings activation.13–16 These differences have been reported among subjects who are in a nonfatigued state; however, injuries frequently occur late in athletic competition, suggesting an influence of exercise-related fatigue on injury risk.17–19 Therefore, the effect of exercise on physical characteristics that may lead to injury risk over the course of exercise may help explain the knee-injury gender bias.

Fatigue has been broadly defined as a decreased ability of muscles to produce force. This definition clearly describes the outcome of fatigue but is not specific regarding the activity responsible for inducing the fatigue or the site along the neuromuscular pathway responsible for the reduction in force. Fatigue may occur either peripherally or centrally. Previous research has focused primarily on peripheral fatigue due to ease of measurement and reproducibility.20,21 Changes in lower extremity neuromuscular function such as increased tibial anterior translation,23 increased knee-valgus moment,24 and increased internal tibial rotation,25 have been reported.26,27 While these changes help clarify the potential effects of fatiguing exercise, the lack of a central component may limit the generalizability of the findings to alterations that may occur during a more functional activity.

Research that is more recent has attempted to investigate both the peripheral and central components of fatigue to construct a better model of the neuromuscular pathway responsible for the reduction in force. Therefore, the effect of exercise on physical characteristics that may lead to injury risk over the course of exercise may help explain the knee-injury gender bias.
effects resulting from generalized fatigue. Quantification of central fatigue can be more difficult than for the peripheral component; however, recent investigations have attempted to overcome this limitation by studying motor-evoked potentials (MEPs) using transcranial magnetic stimulation. MEPs describe the nervous system’s response to a direct stimulus of the motor cortex. As the signal descends from the motor cortex along the pathways in the central nervous system to the muscle, its transmission characteristics are used to assess central fatigue.\(^{28-31}\) It has been suggested that MEPs may aid in measuring a component of injury risk that is related to a subconscious deficit in motor control. These deficits are thought to be derived from changes in motor-cortex excitability, which may be a driving factor in the central component of fatigue.\(^{28,31-33}\)

The purpose of this study was to compare lower extremity muscle strength, EMG activation, and MEP transmission characteristics after a submaximal protocol including aerobic and anaerobic exercise in males and females. We hypothesized that females would exhibit less quadriceps and hamstrings strength and activation than males after exercise.

### Methods

#### Design

This was a descriptive laboratory study with a pretest, posttest, repeated-measures design. The independent variables were gender (male, female) and time (before functional exercise, after functional exercise). The main outcome variables included isometric torque of the knee extensors and flexors, EMG muscle activation of the knee extensors and flexors, MEP amplitude, and MEP latency. This study was approved by the University of Virginia institutional review board, and all subjects gave their written, informed consent before participation.

#### Participants

Thirty-four healthy, recreationally active (exercised 3 times/wk at least 30 min/session) volunteers (17 females, 17 males) participated in this study (Table 1). Potential subjects were assessed for eligibility and completed a general health-history questionnaire after enrollment. Participants were excluded if they had a history of a concussion within the past 2 years, lower extremity joint surgery within the past 6 months, lower extremity joint sprain or muscle strain within the past 6 weeks, cardiopulmonary disorders, pregnancy, or the perceived inability to complete the prolonged aerobic exercise.

#### Instrumentation

A MagStim Novametrix 200 transcranial magnetic stimulator (The MagStim Co, Ltd, Wales, UK) with a flat 70-mm figure-of-8 (double) magnetic coil that provided a maximum magnetic field strength of 2.2 T (teslas) was used to elicit MEPs during the preexercise and postexercise testing. A monophasic pulsed transcranial magnetic stimulation was used to evoke a motor potential. A Biodex multijoint System II isokinetic dynamometer (Biodex Medical Systems Inc, Shirley, NY) was used to provide resistance during the maximum voluntary isometric contractions (MVICS) for knee flexion and knee extension. Electromyography (EMG) signals were amplified (1000 gain) and digitized using a 16-bit data-acquisition system (MP150, Biopac Systems Inc, Santa Barbara, CA). EMG signals were sampled at 2000 Hz with an input impedance of 1000 MΩ. Data acquisition and analysis were completed using AcqKnowledge software (Version 3.9.2-150M, Biopac).

#### Procedures

**EMG Preparation.** The skin over the anterolateral aspect of the right thigh was gently shaved, abraded, and cleaned with isopropyl alcohol to minimize electrode–skin impedance before the application of the surface EMG electrodes. Ag/AgCl disposable self-adhesive disc-surface EMG electrodes for the vastus lateralis were placed over the muscle belly at a vertical angle of 15°, approximately 10 cm proximal and 3 cm lateral to the superior aspect of the patella, with an interelectrode distance of 2 cm. Electrodes for the biceps femoris were placed over the muscle belly at a vertical angle of 20°, approximately 10 cm proximal and 2 cm lateral to the popliteal fossa. The electrodes were left in place throughout the testing session and were traced in permanent marker on the skin to ensure accurate replacement if they fell off during exercise.

**Transcranial Magnetic Stimulation Preparation.** Each subject was fitted with a nonlatex swim cap on which 2 reference lines were marked to aid in locating the stimulation site over the primary motor cortex. One line traveled in the sagittal plane from the nose to the external occipital protuberance, and the other traveled in the frontal plane connecting the tragi of the external ears (Figure 1).

#### Baseline Testing

Before baseline measurements subjects performed a brief warm-up including 5 minutes of bike riding at a self-selected pace. Baseline measurements included EMG muscle activation during MVIC of the knee extensors and knee flexors, torque during MVIC of the knee extensors and knee flexors, and MEPs evoked before exercise.

### Table 1 Demographic Information

<table>
<thead>
<tr>
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<th>Females</th>
<th>Males</th>
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<tbody>
<tr>
<td>Age (y)</td>
<td>21.9 ± 2.3</td>
<td>23.4 ± 6.5</td>
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<tr>
<td>Mass (kg)</td>
<td>77.8 ± 3.0</td>
<td>81.6 ± 3.3</td>
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<tr>
<td>Height (m)</td>
<td>1.7 ± 0.1</td>
<td>1.8 ± 0.1</td>
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<tr>
<td>Body-mass index</td>
<td>21.5 ± 2.5</td>
<td>24.8 ± 2.5</td>
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and knee flexors, MEP transmission latency, and MEP amplitude. Subjects then completed a standardized functional-exercise protocol intended to induce generalized lower extremity fatigue. Baseline measures were repeated immediately after exercise in the order of MEP transmission latency and MEP amplitude, EMG muscle activation during an MVIC of the knee extensors, and EMG muscle activation during an MVIC of the knee flexors.

**Muscle Activation and MVIC Torque.** To assess muscle activation and torque during MVIC, each subject was seated in the Biodex chair, with his or her trunk perpendicular to the floor, the hip flexed to roughly 85°, and the knee flexed to 60°. The distal cuff was fastened approximately 2 cm proximal to the malleoli, and a seatbelt was fastened around the subject’s waist to limit accessory movement. The subject completed 3 consecutive MVICs for the knee extensors with roughly 15 seconds of rest between them. These methods were then repeated for the knee flexors. Verbal encouragement (“Push harder” and “Keep going”) was provided by the same researcher during each trial, and subjects were allowed to watch a real-time display of their force output during each trial.

**MEPs.** The subject remained seated as described in the previous section. The transcranial magnetic stimulation figure-of-8 coil was placed at a 45° angle contralateral to the cross of the 2 reference lines drawn on the swim cap. This was done to achieve optimal stimulation of the correct area of the central sulcus, according to previous mapping of the homunculus. Magnetic stimuli were delivered with the MagStim stimulator and were synchronized with the recording of EMG activity on a nearby computer via a 16-bit data-acquisition system (MP150, Biopac Systems Inc, Santa Barbara, CA) to allow for immediate measurement of MEP amplitude and latency. Subjects received 2 stimulations 10 seconds apart at both 1.7 and 2.0 T to acclimate to the machine. Each subject then received 3 stimulations 10 seconds apart at 2.0 T, which is the maximal capacity of the device. This intensity was found to be the most effective at producing consistent MEPs in the testing position during pilot testing. The mean amplitude and latency of the MEP at maximum stimulation intensity were used for data analysis. If there was difficulty eliciting an MEP, the coil was repositioned to maximally stimulate the appropriate area of the central sulcus. In most cases, 3 to 5 stimulations were required to ensure correct placement of the coil before MEP measurement occurred. Once the optimal location of the stimulating coil was determined, the inner diameters of the 2 openings in the coil were traced onto the swim cap. Each subject kept this swim cap on during the functional-exercise protocol to standardize the placement and orientation of the stimulating coil. The stimuli were delivered with the targeted muscles at rest, and the responses from both the vastus lateralis and the biceps femoris were recorded simultaneously.

**Functional-Exercise Protocol.** Subjects participated in a general lower extremity exercise protocol that combined treadmill walking with body-weight-resisted lower extremity exercise. They started walking on the treadmill at a self-selected pace, with an incline of 0% with an increase of 0.5%/min. After 5 minutes of walking, subjects stepped off the treadmill and immediately performed 10 alternating step-ups (5 with the right leg and 5 with the left leg) followed by 10 body squats (Figure 2). These exercises were repeated until each subject completed 1 minute of the activity. Subjects repeated this combined 6-minute exercise bout 5 times for a total of 30 minutes of activity. A rating of perceived exertion (RPE) was used to measure exertion at the end of each bout of walking (Figure 1). The baseline measurements were repeated after the completion of the functional-exercise protocol in the same order as the baseline testing. Strength and transcranial magnetic stimulation measurements were completed no more than 10 minutes after completion of the exercise protocol.

**Data Processing**

**Torque Data.** Knee-extensor and knee-flexor MVIC were measured by taking the mean torque over the most consistent 2-second time epoch including the maximum torque output. These measurements were normalized to body mass (Nm/kg).

**EMG.** Raw EMG signal was processed using a 10-sample moving average window root-mean-square algorithm. Mean root-mean-square amplitude was calculated for a 2-second time epoch at the point of maximal torque production (Figure 3). EMG data were band-pass filtered (10–500 Hz) and notch filtered (59.5–60.5 Hz) to limit the contribution of 60-Hz noise.

**MEPs.** MEP latency was measured in milliseconds from the onset of stimulation to the most obvious

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Figure 1 — Subject preparation for measurement of motor-evoked potential.
initial deflection of the MEP. The peak-to-peak value of the MEP was quantified as the amplitude (Figure 4), and transmission length was calculated by subtracting shank length (the distance from the lateral joint line of the knee to the floor) from the subject’s overall height. MEP velocity was calculated as follows: (subject height – shank length)/latency.

**Statistical Analysis**

All statistical tests were performed using SPSS statistical software (version 17.0, SPSS Inc, Chicago, IL). We performed 2-tailed independent-samples t tests to compare preexercise values for all variables between males and females. We also performed an analysis of covariance (ANCOVA) for each dependent variable using the preexercise scores as the covariate. Five subjects were excluded from quadriceps and hamstring activation and normalized torque analysis due to a data-collection error (error in collection of torque data). Two additional subjects were excluded from quadriceps MEP analysis due to corrupted baseline EMG data. Since not all subjects had a measurable hamstring MEP, we performed an exploratory comparison for the 23 subjects who exhibited measureable hamstring MEP during concurrent quadriceps MEP testing. Two-tailed independent-samples t tests were performed to compare RPE values at all time points between males and females. In addition, individual 2-tailed independent-samples t test were performed to compare RPE after warm-up with RPE measures at the end of exercise, as well as 5 minutes postexercise. Tests were considered statistically significant if the P value was .05 or less.

**Results**

**Preexercise Testing**

During maximal isometric testing, males exhibited significantly greater knee-extension torques ($t_{27} = 2.209$, $P = .036$) than females. In our exploratory analysis of

![Figure 2 — Body-weight squats and step-ups completed between bouts of treadmill walking during the exercise protocol.](image-url)
Figure 3 — Quadriceps and hamstrings EMG activation and maximum voluntary isometric contraction torque for a single isometric knee-extension trial.

Figure 4 — Quadriceps and hamstrings latency and amplitude for motor-evoked potential.
hamstring MEP, males exhibited significantly higher hamstring MEP amplitudes ($t_{27} = 3.021, P = .007$).

**Postexercise Testing**

After exercise, when controlling for preexercise values we observed significant gender differences in quadriceps MEP amplitudes. Specifically, males exhibited greater quadriceps MEP amplitude after exercise than females did (males $-34.2\%$, females $-63.6\%$; $F_{1,31} = 6.492; P = .016$). After exercise, females exhibited increased knee-extension torque (1.5%) and decreased vastus lateralis activation ($-13.5\%$) while males exhibited decreased knee-extension torque ($-8.5\%$) and increased vastus lateralis activation (1.0%); however, none of these differences were statistically significant (Table 2).

**RPE Values**

There were no significant differences for RPE at any time point between genders (Figure 5). After exercise, the mean RPE value corresponded to a “hard” level of exertion and was significantly greater than the level of exertion reported after the warm-up protocol (warm-up $= 7.7 \pm 1.4$, 30 min $= 14.9 \pm 1.7$, $P < .001$). RPE was also measured 5 minutes after completion of the exercise protocol and was found to be significantly greater than the RPE reported immediately after the warm-up period (warm-up $= 7.7 \pm 1.4$, postexercise $= 13.1 \pm 1.3$).

**Discussion**

The primary findings of this study were that males had significantly greater knee-extension torque and hamstring MEP amplitude than females before exercise. After exercise, males exhibited greater quadriceps MEP amplitude than females. This indicates an adaptive response to fatigue that is different between males and females.

Preservation of lower extremity force-generating capacity throughout athletic activity may be beneficial in reducing injury risk and improving functional performance. However, previous research has shown that asymmetry in force-generating capacity in a rested state may represent a significant risk factor for ACL injury. Females have consistently been shown to have greater knee-extension torque relative to knee-flexion torque than males (increased quadriceps-to-hamstring ratio). It has been suggested that greater relative knee-extension torque may lead to increased anterior tibial translation with less resistance from the hamstrings and therefore may result in greater strain on the ACL.

This investigation shows that while females have lower absolute values for knee-extension torque at baseline and postexercise, males experience an 8.5% decrease in torque while females were able to not only preserve quadriceps function but also, in many cases, improve the level of torque output from baseline. This preservation may be advantageous in terms of functional ability and muscle endurance; however, when considered in the context of

| Table 2 Quadriceps and Hamstrings Muscle Activation and Motor-Evoked Potentials Across Genders and Exercise Conditions |
|-------------------------------------------------|-------------------------------------------------|---------------------------------|---------------------------------|
| Females | Males | **Mean** | **Mean** |
| **MEP amplitude (mV)** | **% Δ** | **MEP latency (ms)** | **% Δ** | **MEP velocity (m/s)** | **% Δ** | **Torque (Nm/kg)** | **% Δ** | **Activation (mV)** | **% Δ** |
| **quadriceps** | 88.7 ± 102.9 | 32.3 ± 34.9† | −63.6 | 193.3 ± 192.6 | 127.2 ± 112.7 | −34.2 | 32 |
| **hamstring** | 89.3 ± 77.6* | 37.0 ± 31.9 | −58.6 | 223.5 ± 134.0 | 186.8 ± 155.0 | −16.4 | 23 |
| **quadriceps** | 23.4 ± 1.9 | 26.3 ± 1.7 | 12.4 | 24.0 ± 1.8 | 25.7 ± 1.8 | 7.1 | 32 |
| **hamstring** | 25.0 ± 1.9 | 26.3 ± 1.9 | 5.2 | 25.1 ± 1.5 | 26.5 ± 1.7 | 5.6 | 23 |
| **quadriceps** | 52.9 ± 4.7 | 47.2 ± 3.6 | −10.8 | 53.6 ± 3.5 | 47.4 ± 10.6 | −11.6 | 32 |
| **hamstring** | 49.4 ± 3.0 | 46.9 ± 3.6 | −5.1 | 51.2 ± 3.1 | 48.4 ± 3.6 | −5.5 | 23 |
| **quadriceps** | 1.95 ± 0.53* | 1.98 ± 0.67 | 1.5 | 2.47 ± 0.68 | 2.26 ± 0.67 | −8.5 | 29 |
| **hamstring** | 1.41 ± 0.43 | 1.33 ± 0.41 | −5.7 | 1.62 ± 0.32 | 1.56 ± 0.55 | −3.7 | 29 |
| **quadriceps** | 153.6 ± 77.6 | 132.8 ± 66.9 | −13.5 | 227.9 ± 115.9 | 227.5 ± 200.4 | 1.0 | 29 |
| **hamstring** | 195.3 ± 122.6 | 163.6 ± 117.9 | −16.2 | 275.2 ± 176.3 | 261.4 ± 110.6 | −5.0 | 29 |

Abbreviations: MEP, motor-evoked potential.

*Significant preexercise difference between groups ($P < .05$). †Significant postexercise difference between groups ($P < .05$).
a 5.7% decrease in knee-flexion torque, females may be exposing themselves to increased ACL strain during functional activity due to the increase in quadriceps dominance of lower extremity function. 

Quadriceps-dominant function in females compared with males has been consistently reported; however, the relative effects of gender and exercise on MEPs and force-generating capacity have not been directly investigated. It has been reported that in females type I (fatigue-resistant) muscle fibers tend make up a greater percentage of total muscle cross-sectional area within the quadriceps, which may help explain the propensity toward quadriceps preservation during exercise. In this investigation, females were able to maintain knee-extension torque despite experiencing a large decrease (63.6%) in quadriceps MEP amplitude. This has been related to decreases in both motor-cortex and motor-neuron excitability. While this may seem counterintuitive, when considered with the previous reports regarding quadriceps muscle histology, lower levels of excitability may be sufficient to maintain force-generating capacity in the slow-twitch type I muscle fibers that dominate females’ quadriceps.

The results of the current study are a combination of central drive (via measures of cortical excitability using transcranial magnetic stimulation) and peripheral muscle function (via measures of strength and activation) that describe different patterns of fatigue between males and females. We observed a nonsignificant reduction in quadriceps torque after exercise in males as opposed to a slight increase for females. Female participants in the current study also had, on average, reduced knee-extension strength but similar knee-flexion strength. These observations may be explained by type I muscle-fiber dominance in the quadriceps muscles of females. This gender difference in muscle-fiber type has not been reported in the hamstrings. Furthermore, there have been several reports of reduced fatigability in females of both the upper and the lower extremity musculature. The lack of quadriceps fatigue in concert with the decrease in knee-flexion torque may create a quadriceps-dominant asymmetry in females, thereby potentially increasing strain on the ACL.

The current study had a reduced sample size due to issues with data collection, which may make it difficult to
find statistically significant differences. Despite this fact, the sample sizes analyzed for knee-extension torque (n = 29) and quadriceps MEP amplitude (n = 32) are consistent with previous investigations in this area. Second, while our fatigue protocol has been pilot tested and used in previous investigations in our laboratory, the lack of objective physiological data during and after exercise limits our ability to quantify the intensity and duration of the fatigue experienced in this study. Finally, due to the dynamic nature of our exercise protocol, it is unclear whether movement of the swim cap occurred during testing; however, we attempted to limit this movement by marking the cap to allow realignment to anatomical landmarks if movement did occur.

Conclusions

Females experienced greater central neuromuscular changes manifested as more pronounced reduction in quadriceps MEP amplitude despite preservation of knee-extension torque-generating capabilities. Reduced central neural drive of the quadriceps coupled with knee-extension torque preservation after exercise may create an environment of increased risk of knee injury in females.

References

Gender and Exercise Effects of Neuromuscular Function


