The Effect of Menstrual-Cycle Phase on Hamstring Extensibility and Muscle Stiffness

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Context: Preventing noncontact ACL injuries has been a major focus of athletic trainers and researchers. One factor that may influence female noncontact ACL injury is the fluctuating concentrations of hormones in the body. Objective: To determine whether muscle properties change across the menstrual cycle. Design: Repeated measures. Testing was performed within 3 d after the onset of menses and ovulation. Repeated-measures ANOVAs were used to determine changes in variables across the menstrual cycle, and Pearson correlations were used to determine relationships between variables. Participants: 8 women with normal menstrual cycles. Main Outcome Measures: Active hamstring stiffness and hamstring extensibility. Results: Hamstring extensibility ($P = .003$) increased at the ovulation testing session but hamstring muscle stiffness ($P = .66$) did not. Conclusions: The results indicate that hamstring muscle stiffness did not change across the menstrual cycle and hamstring extensibility increased at ovulation, when estrogen concentration increases.

Keywords: active hamstring stiffness, flexibility, estrogen, anterior cruciate ligament

In the research and sports rehabilitation community, substantial focus has been placed on preventing ACL injuries to save health care dollars in both the short and long term. It is estimated that the short-term cost associated with an ACL incident is $11,500 per injury, with approximately $2 billion per year being spent in the United States.¹ Long-term consequences of ACL injuries are becoming more apparent, with 79% of patients who undergo ACL-reconstruction surgery having visual change on X-rays that indicate osteoarthritis.² Development of osteoarthritis as a result of injury may result in increased incidence of knee-replacement surgery and long-term quality-of-life consequences, which emphasizes the importance of injury prevention.

Greater muscle stiffness can equate to an increased ability to prevent joint distraction or movement that is necessary to prevent injuries.³ Greater tissue

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compliance potentially means a greater chance for injury. Females demonstrate less stiffness than males in the muscles that surround and support the knee, including the hamstrings and quadriceps. A variety of measures have been used to assess musculotendinous stiffness, including passive methods, oscillatory methods, and functional measures. A common method of assessing musculotendinous stiffness is via oscillation as described in several research studies. Briefly, this method estimates stiffness from the damped frequency of oscillation after a perturbation to the joint.

One possible explanation of reported gender differences in ACL injury rates is the different absolute levels of reproductive hormones in men and women, as well as changes in these concentrations across the menstrual cycle in women. Previous research has demonstrated that the risk of sustaining a noncontact ACL injury is not constant across the menstrual cycle. In addition, estrogen receptors have been found in a variety of tissues including ligament and muscle. Shultz et al found that over the menstrual cycle, 63% of the variance in anterior tibial translation when an anterior load was applied to the tibia was explained by hormonal concentration of testosterone, estrogen, and progesterone when a time delay was accounted for. This supports the notion that tissue adaptations occur throughout the menstrual cycle and tissue properties may change in response to the fluctuating hormone levels. However, the relative contributions of the passive capsuloligament restraints and dynamic (muscle) components to knee stability have not been quantified. This means that changes in anterior tibial translation across the menstrual cycle might be caused by the effect of other tissues in addition to the ligament itself.

Muscle properties have been found to be influenced by reproductive hormones in a manner consistent with greater risk of ACL injury. Previous research has found that muscle stiffness and neuromuscular activation patterns change across the menstrual cycle in ways that may increase the risk of injury. This could be problematic in the hamstrings, which are theorized to prevent anterior tibial translation of the tibia on the femur and play a major role in knee stability. If joint stability and the ability to respond to external loading are compromised, the risk of injury may increase. Therefore, the primary purpose of this research was to determine whether hamstring muscle properties change across the menstrual cycle at time points similar to when anterior knee laxity has been observed to change. Our hypothesis was that active hamstring stiffness would decrease and hamstring extensibility would increase from postmenses (when estrogen concentration is low) to postovulation (when estrogen concentration is greatest). A secondary purpose of our project was to determine whether there are any correlations between the selected muscle properties and whether these relationships change as a result of hormonal influences.

**Methods**

**Subjects**

Sixteen premenopausal women were recruited from the university community to participate in this study. To participate, the following criteria were met: (1) no history of lower extremity injury within the past 3 months that limited physical
activity for more than 48 hours; (2) no history of ACL injury; (3) no history of taking oral contraceptives or any hormone-altering drug including implants and patches within the past 6 months; (4) self-reported normal menstrual cycle for the past 6 months; (5) no history of a diagnosed neurological disorder or visual impairment; (6) physically active, participating in a minimum of 30 minutes of activity 3 times a week; and (7) have a positive urine-based ovulation test. During the testing period subjects were asked to maintain their level of activity and avoid changes in type of activity. They read and signed an informed-consent form approved by the university’s institutional review board before data collection.

**Determination of Testing Times**

Participants were tested at 2 intervals at points unique to each individual’s menstrual cycle. The menses testing session occurred within 3 days after the self-reported onset of menses, and the ovulation testing session occurred within 3 days after ovulation. These time points were chosen because knee-laxity values are lowest on day 3 after menses and estrogen levels typically peak appreciably within 3 days after ovulation, so the tissues were expected to experience the greatest influence of estrogen. Subjects were given a commercial urine-based ovulation kit (Earth’s Magic Inc, Cary, NC), instructed on proper use, and told to contact the investigator when the test strips indicated positive ovulation.

**Measurements**

All testing was performed on the subject’s dominant leg, which was defined as the leg used to kick a ball for maximal distance. To avoid potential changes in stiffness or flexibility, the participants were asked to abstain from even slight changes in physical activity or stretching for 3 days before each testing session.

Before testing, subjects performed a 5-minute warm-up on a stationary bike at a pace equivalent to 25% of individual perceived maximal exertion. Each subject then performed a standardized stretching routine to prepare the hamstrings, quadriceps, and gastrocnemius for testing.

After the warm-up subjects were prepared for surface electromyography (EMG) electrode placement, which involved shaving, abrading, and cleaning the areas with isopropyl alcohol to reduce electrical impedance. A telemetered surface EMG system (T42L-8T0 Telemetry, Konigsberg, Inc, Pasadena, CA) was used to assess muscle activation throughout testing. The system includes an 8-channel differential preamplified transmitter and receiver (input impedance 200 kΩ, CMRR > 70 dB, SNR > 40 dB). The EMG signal was amplified by a factor of 10,000 over a bandwidth of 0.01 to 500 Hz and passed via an A/D converter (National Instruments, San Antonio, TX) at 1000 Hz to the storage computer. Bipolar Ag-AgCl surface electrodes (Medicotest, Rolling Meadows, IL) measuring 10 mm in diameter with a center-to-center distance of 2.0 cm were placed in parallel alignment over the area of greatest muscle bulk in the muscle bellies of the vastus lateralis, biceps femoris, and lateral gastrocnemius. A reference electrode was placed on the tibial tuberosity. All electrode placements were confirmed and checked for cross talk by manual muscle tests.
Active Hamstring Stiffness. Following the protocol set forth by Blackburn et al., the Biodex System 3 dynamometer chair (Biodex Medical Systems, Inc., Shirley, NY) was lowered so the participant could lie flat in a prone position. An ankle orthotic designed to maintain the foot in a neutral position was attached to the foot with an elastic wrap on the test limb. A triaxial accelerometer (PCB Piezotronics, Depew, NY) was adhered to the heel of the orthotic using a small amount of wax and was used to obtain the tangential acceleration of the shank segment. The participant’s test leg was suspended off of the chair with the knee resting on an attachment arm. A strap was placed around the participant’s pelvic area to maintain the hips in a horizontal position. The leg remained stationary on the attachment arm as the chair was lifted until the hips were positioned in 30° of flexion (indicated by a handheld goniometer). Ten percent of the participant’s body weight was added to the distal shank using cuff weights. The participant’s shank was passively placed parallel to the ground. In this position, its geometric relationship to the flexion angle of the hip ensured that the knee was also in 30° of flexion. The subject was asked to hold her leg in this position using an isometric hamstring contraction. During assessment, the investigator applied a brief downward force over the posterior aspect of the ankle orthotic. The perturbation forces the knee into extension, lengthening the hamstrings and initiating damped oscillatory knee flexion/extension. Subjects were instructed to maintain pre-perturbation muscle-contraction levels and to not intervene with the perturbation. To monitor hamstring isolation, EMG from the vastus lateralis and lateral gastrocnemius was observed. Any trials in which excessive vastus lateralis or lateral gastrocnemius activity was observed were discarded and repeated. The perturbation was applied at a random time after hamstring contraction to reduce the likelihood of subject anticipation.

Active knee-flexor stiffness was assessed by modeling the knee as a single-degree-of-freedom mass spring system. In this model, active knee-flexor stiffness was determined as a function of oscillatory motion of the lower limb after a perturbation using the equation \( k_a = \frac{4\pi^2 m r^2 f^2}{f^2} \), where \( k_a \) is active stiffness, \( m \) is the total mass of the lower leg (shank, foot segment, and 10% body mass), \( r \) is the distance from the lateral femoral condyle to the lateral malleolus, and \( f \) is the damped frequency of oscillation (per millisecond). Mass of the shank and foot segment was estimated using standard anthropometric approximations. The damped frequency of oscillations was defined as the inverse of the time interval between the first 2 recorded oscillations. This measure has been reported to have moderate to high reliability (ICC2,1 = .70; SEM = 28.83 N-m/rad).

Hamstring Extensibility. Hamstring flexibility was determined by measuring maximal passive hip flexion with the participant supine. The tester moved the participant’s hip into flexion while maintaining the knee in full extension. When the participant verbally indicated the end of range of motion (ROM), an assistant held the leg in place while the hip-flexion ROM was assessed using a handheld goniometer. Extensibility was defined as the angle formed by the longitudinal axis of the femur and a line parallel to the ground. One investigator took all ROM
measurements. The intrasession reliability for the investigator for all ROM measurements was excellent and shows that the measure was reliable and precise across the 3 trials in each testing session (menses ICC$_{3,1} = .97$, SEM = 1.20°; ovulation ICC$_{3,1} = .98$, SEM = 1.37°).

### Data Reduction and Statistical Analysis

Data were reduced using a customized software program (MatLab version 7.0; MathWorks Inc, Natick, MA). Statistical analyses were performed using SPSS version 14.0 statistical software (SPSS, Inc, Chicago, IL). Separate repeated-measures analyses of variance (ANOVAs) were performed for each dependent variable (hamstring extensibility and active hamstring stiffness), and the within-subject factor was menstrual-cycle phase with 2 levels (postmenses and postovulation). Bivariate correlation coefficients were calculated between extensibility and active stiffness within each time point (menses and ovulation), as well as the change scores between each test. Change scores were calculated by subtracting the ovulation session values from the menses session values. Statistical significance was established a priori at $\alpha \leq .05$.

### Results

Of the 16 subjects tested at menses, 8 (height = 161.0 ± 12 cm, mass = 65.7 ± 6.3 kg, shank length = 40.0 ± 3.0 cm) returned for the second testing session at ovulation and 8 were excluded because they never produced a positive ovulation test. Statistical analyses were performed on the 8 subjects tested at both time periods who had positive ovulation tests. Statistical analysis revealed that hamstring extensibility increased from menses (95%CI 81.7–96.9°) to ovulation (95%CI 89.7–104.7°; $N = 8$, $F_{1,7} = 1.26$; Figure 1). No significant difference was observed between the menses test period (95%CI 100.6–182.6) and ovulatory test period (95%CI 94.8–170.5) for active stiffness ($N = 8$, $F_{1,7} = 0.22$; Figure 2). Correlation analyses did not reveal significant correlations between the dependent variables within each test phase or change scores between phases (Table 1).

### Discussion

We hypothesized that a significant decrease in active muscle stiffness and a significant increase in muscle extensibility would occur within 3 days postovulation in women with a normal menstrual cycle. These hypotheses were made based on 3 previous findings: Females have less muscle stiffness than males$^{3,4}$; these differences are not fully explained by neuromuscular or anthropometric differences in men and women$^{3,4}$ although some research has found that gender differences are eliminated when controlling for anthropometrics; and estrogen has a weakening effect on collagen.$^{28,29}$ It is commonly speculated that differences in sex hormones, neuromuscular characteristics, and anthropometric characteristics contribute to less stiffness and more extensibility in women. However, research has demonstrated less stiffness in females even when anthropometric differences were standardized,$^{3,30}$ and it was suggested that these differences are likely the result of
**Figure 1** — Hamstring extensibility was observed to be greater in the postovulatory phase. Values are expressed in degrees as means and standard deviations. *Significantly different between phases.

**Active Hamstring Stiffness**

**Figure 2** — Active hamstring muscle stiffness did not change between the 2 phases of the menstrual cycle. Values are expressed in degrees as means and standard deviations.
sex differences in tendon viscoelastic properties and muscle architecture. However, recent research has failed to support changes in neuromuscular and biomechanical characteristics such as kinematics, externally applied moments, and fine motor coordination at different points in the menstrual cycle. It is important to point out that these studies suffer from the same limitation as ours in that an assumption is made that testing on a particular day or days of the menstrual cycle will yield the same hormone profile in all women. Therefore, the natural variance in hormone profiles among women may negate observable differences.

We did not observe significant changes in active stiffness across the menstrual cycle. Previous research using similar testing procedures found negative correlations between active stiffness and extensibility, indicating that as extensibility increases active stiffness should decrease. As such, we expected that the significant increase in extensibility would be accompanied by a decrease in active stiffness. One potential explanation as to why this did not occur might be the variance associated with extensibility and active stiffness. Active stiffness had larger standard deviation values and was associated with overlapping confidence intervals, and extensibility had smaller standard deviations. The increased variability of the active stiffness could also explain why we did not see statistically significant differences in active stiffness but did with hamstring extensibility. Although not statistically significant, active stiffness decreased at ovulation by about 9% with an effect size (ES) of 0.19. The values we observed are similar to those reported by Blackburn et al and Granata et al. Previous research investigating leg-stiffness changes over the menstrual cycle revealed significant changes. The effect size and percent change for leg-stiffness differences between menstrual-cycle phases were similar to those in the current study (day 7 of menstrual cycle vs ovulation: ES = 0.31, change = 5%; day 1 of menstrual cycle vs ovulation: ES = 0.65, change = 8.8%). However, the previous research had 11 subjects and, as a result, better statistical power. We performed a post hoc sample-size calculation and found that to obtain significance with an a level of .05 we would need to increase our sample size to 19 to 21 subjects. Thus, a major limitation of this study is that we were underpowered. Our results should be interpreted with caution because of the low sample size, and future studies should increase sample size.

Eiling et al used a hopping stiffness protocol to examine lower extremity stiffness during 3 phases of the menstrual cycle and found that stiffness decreased at ovulation. Several methodological differences may partially explain the discrepancies

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Correlations were calculated between active stiffness and extensibility scores during the postmenses and postovulation tests. Correlations were also calculated for the change scores between phases. No significant correlations were observed.
between our results and those of Eiling et al. First, Eiling et al assessed vertical leg stiffness using a hopping protocol. Although similar to our protocol in that they are both measures of stiffness, the relationship between the 2 is unknown. Second, they used adolescent netball players who tended to be elite in talent, whereas we used a general college population. Third, their testing protocol was performed on a day calculated as the day of ovulation instead of within 3 days after a positive ovulation. Fourth, gender differences in stiffness are amplified at higher joint loads. Because the demand for muscle activity was much higher in the Eiling et al study, menstrual-cycle-phase-dependent changes in stiffness may have been more apparent. The differences could also be amplified because the hopping task requires participants to engage all the muscles in the lower extremity instead of just the hamstrings. Finally, Eiling et al monitored each subject’s menstrual cycle for 3 months before testing to verify menstrual-cycle regularity, whereas we relied on self-report of menstrual-cycle normality within certain parameters.

Increased extensibility at ovulation may result from an influence of reproductive hormones on musculotendinous tissue. Estrogen may act on the muscle itself, affecting the mechanical properties of the hamstring muscles. Although estrogen receptors have been found in muscle, no research has indicated that these receptors influence the mechanical properties of skeletal muscles in response to stretching, and further research is needed before conclusions can be drawn about this mechanism. Estrogen may also affect muscle via influences on other body tissues. For example, estrogen has been found to alter pain modulation whereby high levels of estrogen were associated with activation of endogenous opioid neurotransmission during painful stimulus. Therefore, our results might indicate changes in stretch tolerance. Previous research has found that repeated stretching can influence how a person perceives a sensation and that this change is related to an increase in stretch tolerance. The greater estrogen concentration at ovulation may increase the hamstrings’ stretch tolerance, thus explaining the increase in hamstring extensibility without a significant decrease in stiffness. Muscles can provide joint stability via active (active stiffness), as well as passive (flexibility), restraints. Increased hamstring flexibility at ovulation may represent a decrease in the passive stability provided by the hamstrings because they are the primary restraints to anterior tibial translation. However, it is not clear whether increases in flexibility are related to decreased joint stability. This topic requires further investigation. We observed an 8% increase in hamstring extensibility during ovulation. In addition, we observed a large effect size of 0.88 (menses – ovulation/pooled SD) associated with hamstring extensibility, and the 95% confidence intervals associated with extensibility display minimal overlap, indicating a distinct distribution for each point in the menstrual cycle. Our extensibility values are similar to previously reported values for females using the straight-leg raise. The investigator who assessed extensibility had high reliability with the measurement but was not blind to the phase of each subject, which may have led to unintentional bias.

There are limitations with the current study design. First, we did not measure serum hormone levels. Instead we attempted to create individualized testing sessions around each subject’s menstrual cycle through the use of ovulation kits and extrapolate that information to the average female hormonal profile. However, research has shown that the day of ovulation may or may not coincide with the
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stradiol peak, and because we did not measure actual hormone levels we cannot verify that we tested all women at the desired time point in the menstrual cycle (ie, after the estrogen peak and before appreciable progesterone rise). Furthermore, it is possible that there is a delay between when hormone concentrations change and when soft tissues are affected, so changes in muscle properties may have occurred outside the window examined. A second major limitation of this study is the lack of statistical power and sample size, which limits the study’s generalizability to larger populations. We initially set out to collect data on 16 subjects, but we encountered difficulty in obtaining positive ovulation tests for half the subjects, so they were removed from the study because they did not meet the criteria of having a normal menstrual cycle. However, we feel that this inclusion criterion strengthens the results of this study even though it resulted in our losing 50% of our subjects. It is important to test at standardized points in the menstrual cycle and to test individuals with similar menstrual status to increase the chances of achieving desired hormonal-profile differences. Future research should try to increase sample size. In addition, the order in which testing sessions occurred with respect to the menstrual cycle was consistent, with all subjects being tested initially at menses. Future research should analyze serum hormone levels, use a larger sample size, collect over multiple days, and counterbalance testing order across the menstrual cycle to determine whether hormone concentration does affect muscle properties. In addition, our measurement of active stiffness is only an estimate; the ratio of change in force to change in length was not assessed directly. However, this method has been used in numerous previous investigations and has been shown to be a valid and reliable technique. Finally, we did not investigate the reliability of hamstring-flexibility measures in separate sessions 14 days apart. We examined the reliability and precision of the measures within each testing session. It is possible that participants were more comfortable and relaxed with the examiner during the second test session, resulting in greater extensibility.

Clinical Significance and Conclusion

We observed no change in hamstring muscle stiffness across the menstrual cycle, but our results should be interpreted with caution because of our small sample size. We found that hamstring extensibility was greater at ovulation than at menses. Ovulation is when estrogen concentration is theorized to be greatest. Clinically, an increase in ROM is not necessarily a negative consequence. Athletic trainers attempt to improve ROM and extensibility as a means of injury prevention. Future research should attempt to determine whether increased muscle flexibility is linked to passive joint stability. This study had limitations that should be addressed by future research by increasing the sample size, testing at more time periods, and including serum hormone levels to verify concentration levels.

References