

Sex Differences, Hormone Fluctuations, Ankle Stability, and Dynamic Postural Control

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Context: Hormonal fluctuation as a risk factor in anterior cruciate ligament injury has been investigated with conflicting results. However, the influence of hormone fluctuations on ankle laxity and function has not been thoroughly examined.

Objective: To examine the potential hormone contributions to ankle laxity and dynamic postural control during the preovulatory and postovulatory phases of the menstrual cycle using an ankle arthrometer and the Star Excursion Balance Test in healthy women. The cohort group consisted of male control participants.

Design: Cohort study.

Setting: Research laboratory.

Patients or Other Participants: Twenty healthy women (age = 23.8 ± 6.50 years, height = 163.88 ± 8.28 cm, mass = 63.08 ± 12.38 kg) and 20 healthy men (age = 23.90 ± 4.15 years, height = 177.07 ± 7.60 cm, mass = 80.57 ± 12.20 kg).

Intervention(s): Ankle stability was assessed with anterior-posterior and inversion-eversion loading. Dynamic postural control was assessed with the posteromedial reaching distance of the Star Excursion Balance Test.

Main Outcome Measure(s): Female participants used

ovulation kits for 3 months to determine the time of ovulation; during their preovulatory and postovulatory phases, they were tested in the laboratory with an ankle arthrometer and the Star Excursion Balance Test. Male participants were tested on similar dates as controls. For each dependent variable, a time by side by sex repeated-measures analysis of variance was performed. Statistical significance was set a priori at $P < .05$.

Results: For anterior-posterior laxity, a side main effect was noted ($F_{1,38} = 10.93$, $P = .002$). For inversion-eversion laxity, a sex main effect was seen ($F_{1,38} = 10.75$, $P = .002$). For the posteromedial reaching task, a sex main effect was demonstrated ($F_{1,38} = 8.72$, $P = .005$). No influences of time on the dependent variables were evident.

Conclusions: Although women presented with more ankle inversion-eversion laxity and less dynamic postural control, hormonal fluctuations during the menstrual cycle (preovulatory compared with postovulatory) did not affect ankle laxity or dynamic postural control, 2 factors that are associated with ankle instability.

Key Words: Star Excursion Balance Test, ankle arthrometry, ankle instability

Key Points

- Anterior-posterior ankle laxity was greater on the dominant side than on the nondominant side in both women and men.
- Ankle inversion-eversion laxity was greater and dynamic postural control was less in women compared with men.
- However, ankle mechanical stability and dynamic postural control did not change in women before or after ovulation or in men between test sessions.

Lateral ankle sprains are one of the most common injuries among the physically active.^{1,2} Approximately 30% of those who suffer 1 ankle sprain develop chronic ankle instability (CAI); however, this number has been reported to be as high as 70%.^{3,4} Limited evidence suggests differences in ankle injury risk factors between men and women.^{5,6} For other lower extremity musculoskeletal injuries, such as anterior cruciate ligament (ACL) injury in the knee, intrinsic and extrinsic factors are proposed to explain the higher injury rate in females.^{7,8} One suggested intrinsic risk factor for ACL injury is the difference in hormones and hormonal fluctuations between men and women. Several groups^{9–12} have found that female hormones lead to increases in knee joint laxity. The underlying theory is that the cyclic rise and fall of estrogen, progesterone, and luteinizing hormone may make a female more susceptible to ACL injury because of alterations to the ligament during specific times of the menstrual cycle. Some authors^{13,14} have suggested that the follicular (days 1–7) or luteal (days 22–28) phase is responsible for

the increased injury rate in female athletes, whereas others^{15–17} have shown a relationship between higher ACL injury rates and higher estrogen levels during ovulation.

The theorized changes in ACL integrity in response to normal hormonal fluctuations in females may lead to increased laxity and decreased neuromuscular control of the knee and help to explain the increased rate of female ACL injuries.¹⁸ However, hormonal fluctuations in the female athlete may not provide a consistent explanatory model for higher ACL injury rates.⁴ In a recent International Olympic Committee current concepts statement, Renstrom et al¹⁹ noted that the likelihood of sustaining an ACL injury does not remain constant through the menstrual cycle. The heterogeneity in the findings of this body of literature supports the need for investigation into the influence of hormonal fluctuations on ligamentous laxity and subsequent injury risk.

With abundant ankle injuries and recurrences leading to CAI,²⁰ it is important to identify the prevalent factors in the de-

velopment of injury risk. Although different sets of risk factors for first-time ankle sprains have been identified for males⁵ and females,⁶ to our knowledge, the influence of hormonal differences (and associated fluctuations during the menstrual cycle) has not been addressed. Both mechanical and functional insufficiencies have been proposed to contribute to CAI.²⁰ If hormonal fluctuations potentially influence the risk of injury to the knee, they may also affect the risk of injury to the ankle. Mechanical instability of the ankle may be addressed on physical examination, with validated subjective scales or instrumented objective measures. Of the many outcome variables used to identify functional ankle instability, deficient dynamic postural control as assessed with the Star Excursion Balance Test (SEBT) has been reported consistently in patients with CAI.^{21–25} The SEBT is a lower extremity reaching task that consists of performing a series of single-limb squats while reaching maximally with the opposite limb in different directions. Previous authors^{26,27} have demonstrated that uninjured males and females perform differently in selected reaching directions of the SEBT. Determining whether these differences are influenced by normal hormonal fluctuations in women will help future researchers understand the potential contributions of hormonal fluctuations to altered neuromuscular control and potential ankle disability.

Although the intrinsic factor of hormonal fluctuation has been studied extensively in the context of ACL injury, we are aware of very few previous investigations devoted to its influence on ankle laxity.²⁸ This is probably because the discrepancy between male and female ACL injury rates is not seen in ankle injury rates. Therefore, it is possible that hormonal fluctuations and subsequent altered mechanical and functional instability may not contribute significantly to the risk of ankle injury. By investigating the effect of hormonal fluctuations on ankle laxity and dynamic postural control, 2 measures known to be associated with persistent ankle injury, we may better understand the risk factors and successful interventions for ankle instability in men and women.

Thus, the purpose of our study was to examine ankle laxity with an instrumented ankle arthrometer and dynamic postural testing with the SEBT in healthy women in the preovulatory and postovulatory phases of their menstrual cycles. A cohort group of healthy male participants was tested at similar times of the month to provide comparisons. We hypothesized that women would have greater laxity and better dynamic postural control than would men but that women would not demonstrate a difference in ankle laxity or dynamic postural control between the preovulatory and postovulatory phases.

METHODS

Participants

Twenty healthy women (age = 23.8 ± 6.50 years, height = 163.88 ± 8.28 cm, mass = 63.08 ± 12.38 kg) and 20 healthy men (age = 23.90 ± 4.15 years, height = 177.07 ± 7.60 cm, mass = 80.57 ± 12.20 kg) volunteered to participate. All were physically active (performing at least 30 minutes of activity 3 days per week) with no history of lower extremity injury or concussion in the last 12 months. The female volunteers had self-reported regular menstrual cycles and were not using oral contraceptives. This study was approved by the institutional review board, and all participants provided written consent before the study began.

Instrumentation

Measuring tapes were rigidly fixed to the floor at 45° angles to each other so we could assess reaching distances during the SEBT. This test has been used to quantify dynamic postural control differences in healthy males and females^{26,29} and in those with CAI^{21–24}; it has strong reliability, with intratester intraclass correlation values ranging from 0.78 to 0.96.^{30–32}

To assess ankle stability, we used a portable ankle arthrometer (Blue Bay Medical Inc, Navarre, FL), a device that is highly reliable and valid in assessing ankle ligamentous laxity, with intratester intraclass correlation values ranging from 0.82 to 0.97.^{33–36} The device consists of an adjustable plate that is secured to the plantar surface of the foot with an attached load-measuring handle, which distributes a load to the plate. A 6-degrees-of-freedom spatial kinematic linkage system that is connected to the footplate and to a tibial pad indicates the amount of displacement in the designated direction when the load is applied, supplying information on rotational and translational motion in the ankle complex. The relative motion between the footplate and the reference pad on the tibia was measured and sent via an analog-to-digital converter to an attached laptop computer, where a custom LabVIEW program (version 7.1; National Instruments Corp, Austin, TX) processed the information. Based on the manufacturer's recommendations, anterior-posterior (A-P) loading was performed first, followed by inversion-eversion (I-E) loading.

Procedures

Methods for defining and quantifying menstrual cycle phases vary in the literature. For financial and logistic convenience, our female participants used ovulation detection kits, which have been used successfully in previous investigations, to identify the expected fluctuations in hormonal levels.^{37,38} How menstrual cycle phases should be designated has been debated in the literature; we followed the work of Beynon et al³⁹ in defining the phases of interest as preovulatory and postovulatory.

Once a female volunteer was determined to have met the inclusion criteria, she reported to the laboratory for an introduction to the study. Based on her completion of a menstrual history questionnaire, we determined when she needed to administer the first ovulation kit. She was given instructions on how to use the ovulation detection kit (Answer Quick & Simple One-Step Ovulation Test; Church & Dwight Co, Inc, Princeton, NJ), according to the manufacturer's guidelines, and on keeping a daily ovulation journal for 2 consecutive months. At the end of the first month, she was asked to provide the results of the ovulation kit. Based on those results, we told her when to administer the second ovulation kit. Once we collected the information from both months, we asked her to report to the research laboratory approximately 5 days before (preovulatory phase) and 5 days after (postovulatory phase) the projected ovulation date for the third month. She continued to use the ovulation detection kit and record journal entries during the third month (laboratory data collection month) to confirm that the testing dates were correct. For all female participants, the ovulation detection kits and journals confirmed that the laboratory testing (ankle laxity and dynamic postural control) had occurred in the targeted phases during the third month of enrollment, and therefore all data were used for analysis.

The rationale for this method of data collection is supported by Beynon et al,³⁹ who chose to examine hormone levels be-

fore and after ovulation instead of looking at the 3 phases of the menstrual cycle. Their rationale was that the human menstrual cycle is controlled by 2 steroid hormones that are produced in the ovaries (ie, progesterone and estradiol) and secreted at different times during the course of a woman's monthly cycle. Estradiol secretion has 2 phases, with peaks at both a follicular (preovulatory) and a luteal (postovulatory) time period. Progesterone secretion is controlled by the corpus luteum and occurs only during the postovulatory period. Menses then begin with the failure of the corpus luteum and the rapid reduction in estradiol and progesterone levels.³⁹ Thus, assessing hormone levels before and after ovulation may be a more accurate way of characterizing the menstrual cycle and provide a more accurate way of determining when a female is going through each phase.

Male participants were physically active (performing at least 30 minutes of activity 3 days per week) and had no history of lower extremity injury or concussion within the previous 12 months. Men underwent the same laxity and dynamic postural control testing procedures as did women, with 2 testing sessions 10 days apart.

During the testing session, the participant's age, height, mass, and sex were recorded, and he or she completed an injury history questionnaire. The following tests were administered in a counterbalanced order: laxity measures (A-P and I-E) on the ankle arthrometer and dynamic postural testing on the SEBT (maximum distance [MAXD] in the posteromedial direction). Both forms of testing were performed on the dominant and nondominant sides, with the *dominant side* defined as the side the person would use to kick a ball. Although examining differences in limb dominance was not a primary purpose of our investigation, we thought that it was an important factor that could have clinical implications. Subsequently, the order by side was also counterbalanced.

For the ankle stability testing, the participant sat on a treatment table with the testing foot extended over the edge of the table and placed in the arthrometer. A strap was placed around the lower leg 1 cm above the malleoli, and the sole of the foot was secured on the footplate. Adjustments were made to the heel and dorsal clamps for comfort. The tibial pad was placed 5 cm above the ankle malleoli and secured with a strap on the lower leg. Each assessment began with the ankle positioned in 0° of plantar flexion (as confirmed by a goniometer placed over the talocrural joint), which was the measurement reference point.^{33,36} The A-P and I-E displacements were assessed according to the manufacturer's guidelines.

During each trial, participants were instructed to avoid contracting the calf muscles, and the investigators observed no such muscle contractions. Three trials each of A-P and I-E displacement were performed on each ankle. Total A-P motion was recorded in millimeters, and total I-E motion was recorded in degrees.

Dynamic postural control was assessed with the SEBT. The SEBT consists of 8 lines of measuring tapes that extend out from the center at 45° intervals. Recent investigators^{24,32} have demonstrated that the 8 reaching directions of the SEBT are redundant, and the entire task can be simplified by having the participant reach in the posteromedial direction only. Therefore, our participants performed only the posteromedial reach.

Each person began the test standing on 2 feet, with the foot of the testing leg in the center of the measuring tapes and the heel of the stance limb placed at the end of the tape. He or she then reached as far as possible in the posteromedial direction with the nontesting leg, lightly touched the line with the toe,

and then returned to a double-legged standing position. If the hands were removed from the hips or the support foot lifted off the floor at any time, that attempt was counted as unsuccessful and the trial was repeated. The investigator recorded the maximum reaching direction of a trial by placing a mark on the tape attached to the floor. Participants were allowed 4 practice trials,³² were given 2 minutes of rest, and then completed 5 successful trials. During each trial, the distance from the touch point to the stance leg at the center of the tapes was marked, measured, and recorded. The distance was normalized to the leg length of the stance leg (reach distance/leg length) to produce the dependent variable MAXD, which was reported as a percentage score.²⁹ A 5-minute rest was provided between performances on each testing limb.

Statistical Analysis

The means and standard deviations from the laxity and SEBT testing for each limb were calculated. For each dependent variable (A-P laxity, I-E laxity, MAXD), a separate sex (female, male) by time (preovulation, postovulation) by side (dominant, nondominant) repeated-measures analysis of variance was conducted. Statistical significance was set a priori at $P < .05$. When statistical significance was demonstrated, we performed a Tukey post hoc test. We used SPSS (version 15.0; SPSS Inc, Chicago, IL) for all statistical analyses. The Cohen d , using pooled standard deviations, and 95% confidence intervals (CIs) were calculated to determine effect size.

RESULTS

Ankle Laxity

For the A-P laxity measures, a main effect for side was noted ($F_{1,38} = 10.93$, $P = .002$). The dominant limb displayed greater A-P laxity (15.89 ± 3.28 mm) than did the nondominant side (14.49 ± 2.95 mm) ($d = 0.45$, 95% CI = -0.19 , 1.07). No main effects or interactions of sex or time on A-P laxity were evident (Table 1).

For the I-E laxity measures, a main effect for sex was present ($F_{1,38} = 10.75$, $P = .002$). Women had greater laxity ($63.49^\circ \pm 10.08^\circ$) than did men ($55.59^\circ \pm 8.34^\circ$) ($d = 0.85$, 95% CI = 0.19 , 1.48). No main effects or interactions of side or time were demonstrated (Table 2).

Star Excursion Balance Test

For the posteromedial reaching task, a main effect was noted for sex ($F_{1,38} = 8.72$, $P = .005$). Women presented with a smaller normalized reach distance ($81.7\% \pm 11.1\%$) than did men ($91.9 \pm 11.4\%$) ($d = 0.91$, 95% CI = 0.24 , 1.54). No main effects or interactions of side or time were seen (Table 3).

DISCUSSION

The purpose of our study was to determine whether hormonal fluctuations during the menstrual cycle in healthy women influenced selected factors linked to ankle instability: ankle laxity and dynamic postural control. Neither factor fluctuated in the women across the testing sessions before or after ovulation. Similarly, the healthy men, tested for comparison purposes, did not demonstrate variability across their 2 testing sessions in either the laxity or dynamic postural control mea-

Table 1. Anterior-Posterior Ankle Joint Laxity (mm) by Sex and Ovulatory Phase (Mean±SD [95% Confidence Interval])

Sex	Ovulatory Phase	Limb ^a	
		Dominant	Nondominant
Female	Preovulatory	16.52±2.79 (15.09, 17.19)	14.80±2.62 (13.52, 16.08)
Male	NA	15.07±3.49 (13.64, 16.50)	13.83±3.00 (12.56, 15.11)
Female	Postovulatory	16.31±3.25 (14.78, 17.84)	14.91±3.02 (13.50, 16.32)
Male	NA	15.66±3.49 (14.13, 17.19)	14.41±3.20 (12.99, 15.82)

Abbreviation: NA, not applicable.

^aMain effect for side ($F_{1,38}=10.93$, $P=.002$). Dominant>nondominant (Cohen $d=0.45$, 95% confidence interval= -0.19 , 1.07).

Table 2. Inversion-Eversion Ankle Joint Laxity (°) by Sex and Ovulatory Phase (Mean±SD [95% Confidence Interval])

Sex ^a	Ovulatory Phase	Limb	
		Dominant	Nondominant
Female	Preovulatory	62.24±10.07 (57.35, 67.12)	64.01±10.99 (59.53, 68.50)
Male	NA	56.99±11.48 (52.10, 61.87)	53.95±8.71 (49.46, 58.43)
Female	Postovulatory	63.54±6.12 (59.58, 67.50)	64.18±6.18 (60.59, 67.77)
Male	NA	55.79±10.75 (51.83, 59.75)	55.63±9.36 (52.04, 59.22)

Abbreviation: NA, not applicable.

^aMain effect for sex ($F_{1,38}=10.75$, $P=.002$). Women>men (Cohen $d=0.85$, 95% confidence interval= 0.19 , 1.48).

Table 3. Posteromedial Reach Distance (%) by Sex and Ovulatory Phase (Mean±SD [95% Confidence Interval])

Sex ^a	Ovulatory Phase	Limb	
		Dominant	Nondominant
Female	Preovulatory	80.96±11.52 (75.9, 86.0)	80.96±11.56 (75.9, 86.0)
Male	NA	91.24±10.72 (86.2, 96.3)	91.89±10.60 (86.9, 96.9)
Female	Postovulatory	81.96±10.53 (76.8, 87.2)	82.98±10.79 (77.8, 88.1)
Male	NA	91.97±12.39 (86.8, 97.2)	92.47±11.87 (87.3, 97.6)

Abbreviation: NA, not applicable.

^aMain effect for sex ($F_{1,38}=8.72$, $P=.005$). Men>women (Cohen $d=0.91$, 95% confidence interval= 0.24 , 1.54).

sure. Although some influences of limb dominance and differences between men and women on the measures were apparent, these measures did not fluctuate across the menstrual cycle.

Ankle Laxity

No influence of time on A-P or I-E or ankle laxity was demonstrated, meaning that the mechanical ankle stability of the women and men did not vary between the testing sessions. Therefore, the women did not experience any changes in ankle stability with menstrual hormone fluctuation. This result supports the findings of Beynnon et al²⁸ that ankle laxity did not fluctuate across the menstrual cycle. The potential effect of hormones on ligament laxity has been investigated in the ACL of the knee, with mixed outcomes,^{16,18,28} but investigations into how they may affect ankle stability and function have been limited. Our findings help support the concept that hormonal fluctuation may not have a significant influence on ankle ligament laxity during the preovulatory and postovulatory phases of a woman's menstrual cycle.²⁸ This result may affect research

efforts to determine whether hormonal fluctuation is a viable predictor of ankle injury.

Regarding sex differences in ankle stability, women presented with more I-E laxity than did men. This finding is consistent with the findings of Willems et al^{5,6} and Beynnon et al,²⁸ which suggested that females had more talocrural laxity than did males. Our female participants had approximately 8° more I-E laxity than did the men; this value was associated with a strong effect size and a 95% CI that did not cross zero. However, A-P laxity did not differ between women and men ($P=.26$). Although the average A-P laxity measure for women (15.63±2.92 cm) was greater than for men (14.74±3.30 cm), the result was associated with a low effect size and a 95% CI that crossed zero ($d=0.29$, 95% CI= -0.34 , 0.90). Therefore, I-E stability may be a differentiating factor to consider when examining ankle injury prediction models for women and men.

Limb dominance was a secondary factor that we examined. For A-P ankle laxity, a main effect for side was noted, with the dominant side displaying more laxity than the nondominant side. However, this result was associated with a lower ef-

fect size and a 95% CI that crossed zero, indicating that the magnitude of this significant result was small. Additionally, no difference was evident in I-E laxity between the dominant ($59.64^\circ \pm 10.23^\circ$) and nondominant sides ($59.44^\circ \pm 9.99^\circ$) ($P = .86$). This result was also associated with a low effect size and a large 95% CI that crossed zero ($d = 0.02$, 95% CI = $-0.60, 0.64$). In a systematic review, Beynnon et al⁴⁰ considered limb dominance a potential predictive factor for lateral ankle sprains and a risk factor for lower extremity injury because of the greater demand placed on the dominant limb during sport. Yet our finding is supported by the results of Beynnon et al⁴¹: Limb dominance was unrelated to the risk of ankle injury for male and female field hockey players. The authors noted that the literature regarding limb dominance and injury risk is divided. Although our results suggest that limb dominance has a small effect on ankle stability measures, inconsistency in the literature indicates that more studies may be needed to examine limb dominance as a risk factor for ankle sprains in order to draw a definitive conclusion.

Star Excursion Balance Test

No influence of time on MAXD in the SEBT was seen, so dynamic postural control did not fluctuate between the testing sessions. The SEBT is a dynamic balance reaching test with strong reliability,^{30–32} as shown by the lack of performance differences between the testing sessions. More importantly, this finding has potential clinical relevance related to understanding ankle injury. Because normal hormonal fluctuations did not affect dynamic postural control as measured by the SEBT, which is linked to the risk of ankle injury⁴² and associated with CAI,^{21–25} researchers and clinicians can be encouraged to focus on other factors that may contribute to ankle instability and subsequently limit dynamic postural control.

We did find a sex main effect for MAXD on the SEBT, with men demonstrating a larger value than did women. Although this result is not consistent with a recent investigation by Gribble et al,²⁶ the differences in the 2 studies may be explained by noting that Gribble et al²⁶ used the anterior, medial, and posterior reaches in the SEBT, whereas we used only the posteromedial reach direction. To our knowledge, no previous authors have compared healthy men and women on the posteromedial reach direction alone. Continued investigation into male and female differences on this task is warranted to determine the influence of sex on this measure of dynamic postural control.

Limitations

Multiple methods exist for tracking and quantifying stages of the menstrual cycle, but ovulation kits have been used in a previous investigation³⁷ of hormonal influences on knee stability, they are inexpensive, and they do not require specific personnel or resources. We recognize that serum tracking and profiling would have been a better option for determining the exact timing of ovulation; however, our resources were limited in this area. We believe that this method is appropriate because we tracked the participants' menstrual cycles via the home ovulation detection kits for 2 consecutive months and verified in the third consecutive month that appropriate markers of ovulation occurred.³⁸ Although the tracking depended on consistent input from the participants, we were diligent in our communications with them to encourage their full involvement.

Finally, multiple methods are available to identify menstrual cycle phase. As we discussed in the "Results" section, we used a 2-phase model that has been used successfully by previous authors²⁸ evaluating hormonal influences on joint stability. We believe that this was a simple yet valid method for addressing the primary purpose of this study. Future researchers may choose to use other types of categorizations.

CONCLUSIONS

Our primary purpose was to examine the influence of hormonal fluctuations during the menstrual cycle on 2 factors commonly associated with ankle injury. Both ankle mechanical stability and dynamic postural control remained consistent before and after ovulation in women; their male counterparts also demonstrated consistent results across the testing sessions. These findings indicate that those seeking ways to reduce ankle injury risk need not focus on hormonal factors that might influence ankle ligament integrity or contribute to dynamic postural control in the ankle joint. We did observe influences of sex and limb dominance on ankle stability and dynamic postural control. Perhaps this information will lead clinicians and researchers to focus on other interventions and factors that may be influencing ankle injuries.

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