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Hormonal Changes Throughout the Menstrual Cycle and Increased Anterior Cruciate Ligament Laxity in Females

Ned A. Heitz, MS, CSCS*; Patricia A. Eisenman, PhD†; Charles L. Beck, MD‡; James A. Walker, PhD‡

* Salt Lake City, UT; † The University of Utah, Salt Lake City, UT; ‡ The Orthopedic Specialty Hospital, Murray, UT

Objective: To determine whether women experience significantly greater anterior cruciate ligament (ACL) laxity in conjunction with estrogen and progesterone surges during a normal 28- to 30-day menstrual cycle.

Design and Setting: Serial estrogen and progesterone levels were measured via radioimmunoassay procedures to identify the follicular and luteal phases of a subject's menstrual cycle and to determine periods of peak hormonal surges. Concomitant ACL laxity measures were taken using a knee arthrometer. Hormone levels and ACL laxity were assessed on days 1, 10, 11, 12, 13, 20, 21, 22, and 23 of the menstrual cycle. Day 1 corresponds to the menstrual phase, when estrogen and progesterone levels are at their lowest. Days 10 through 13 correspond to peak estrogen surge (follicular phase), and days 20 through 23 correspond to peak progesterone surge (luteal phase).

Subjects: Seven active females between the ages of 21 and 32 years with at least one apparently healthy knee (no known knee anomalies) volunteered for participation in this study. Each subject stated that she experienced a normal (28- to 30-day) menstrual cycle and was not currently taking any type of hormone therapy (eg, birth control medication).

Measurements: Blood was drawn on days 1, 10, 11, 12, 13, 20, 21, 22, and 23 of each subject's menstrual cycle, and ACL laxity measurements were assessed immediately after the blood draws. Estrogen and progesterone levels were determined via radioimmunoassay procedures, and ACL laxity was determined using a knee arthrometer.

Results: A within-subjects, repeated-measures analysis of variance was applied to determine the presence or absence of significant differences in ACL laxity values over the course of a subject's menstrual cycle. We found a significant difference in ACL laxity when comparing baseline levels of estrogen with peak levels of estrogen. A significant increase in ACL laxity was also noted when comparing baseline levels of progesterone with peak levels of progesterone.

Conclusions: ACL laxity increased significantly throughout the menstrual cycle when comparing baseline with peak levels of estrogen and progesterone.

Key Words: estrogen, progesterone, knee arthrometer, radioimmunoassay

More females are participating in sports as a result of Title IX implementation; having become much more athletically active, females subsequently have sustained a significant percentage of total sports injuries.[1–4] Interestingly, it appears that female athletes have a disproportionately greater incidence of knee injuries than their male counterparts.[3] According to the NCAA Injury Surveillance Survey, females have an increased rate of knee injury and anterior cruciate ligament (ACL) rupture compared with males in basketball, soccer, and gymnastics.[5] The ACL is the most commonly disrupted ligament in the knee,[6] and its injury is occurring at an increasing rate in women's athletics.

The ACL extends posteriorly and laterally from the area anterior to the intercondylar eminence of the tibia to the posterior part of the medial surface of the lateral condyle of the femur. In general, the ACL prevents the tibia from moving anteriorly during weightbearing. It also stabilizes the tibia against abnormal internal rotation and serves as a secondary restraint against valgus and varus stress. The ACL works in conjunction with the thigh muscles, especially the hamstring muscle group, to stabilize the knee joint. It is most vulnerable to injury when the leg is partially flexed and in a weightbearing position, the tibia is externally rotated, and the knee is in a valgus position. This is an especially vulnerable position for females, who tend to demonstrate greater genu valgum than males due to a wider pelvis. Excessive genu valgum may predispose an individual to patellofemoral disorders and an increased ACL injury risk.

Typically, the ACL can sustain injury from a direct blow to the knee or from a single-plane force. The single-plane injury occurs when the lower leg is rotated while the foot is fixed to the playing surface, such as when an athlete suddenly decel-

Address correspondence to Ned A. Heitz, MS, CSCS, 319 East Hubbard Avenue, Salt Lake City, UT 84111. E-mail address: pcnheitz@ihc.com

erates, plants, and makes a sharp "cutting" motion, causing an isolated tear of the ACL.

Alarmingly, many female ACL injuries are the result of noncontact mechanisms: quick stopping and cutting motions in sports such as basketball, soccer, and gymnastics.[7] The unusual conditions under which the injuries are occurring are bringing more attention to female ACL injuries. The mechanism of the increased injury rate remains uncertain; however, possible explanations include sex differences in ligament or muscle strength, training history, anatomy, and conditioning techniques.

A hormonal influence on knee joint laxity has been suggested, along with these sex-related factors, as being associated with the increased ACL injury rate among female athletes.[7,8] The identification of estrogen and progesterone receptors in the fibroblasts of the human ACL suggests that pregnancy-related hormones may have an effect on the structure of this ligament.[9] Unlike her male counterpart, the menstruating athlete experiences cyclical changes in hormone levels throughout her reproductive years. The pregnancy-related hormones estrogen and progesterone fluctuate throughout the female menstrual cycle (Figure 1).

The duration of the menstrual cycle ranges from 24 to 35 days, averaging 28 days. Events occurring during the menstrual cycle can be divided into 3 phases: (1) the menstrual phase, (2) the follicular phase, and (3) the luteal phase.[10] The menstrual phase is caused by a sudden reduction in estrogen and progesterone and lasts for approximately the first 5 days of the cycle. The follicular phase lasts from days 6 to 13 in a 28-day cycle. It is during this phase that the developing follicle increases its secretion of estrogens.[11] Consequently, estrogens are the dominant ovarian hormones during this phase of the menstrual cycle. The luteal phase of the menstrual cycle is the longest in duration, lasting from days 15 to 28 in a 28-day cycle. It is the time between ovulation and the onset of the next menses. After ovulation, luteinizing hormone secretion stimulates the development of the corpus luteum. The corpus luteum then secretes increasing quantities of estrogens, progesterone, and relaxin.[11,12] If fertilization and implantation do not occur, the rising levels of these hormones will decrease to their initial, lowest levels when the cycle begins.[10]

Although it has been reported that estrogen and progesterone receptors have been identified on the ACL,[9] to date there is no published information available as to whether or not ACL laxity changes in response to peak estrogen and progesterone levels during the course of the menstrual cycle. The purpose of our study was to determine whether females experience significant differences in ACL laxity in conjunction with estrogen and progesterone surges during a normal 28- to 30-day menstrual cycle.

METHODS

Subjects

Seven female subjects ranging in age from 21 to 32 years (mean age = 26.9 ± 4.2 years, height = 170.5 ± 5.7 cm, weight = 62.7 ± 4.9 kg) participated in this study. Disqualification criteria included the presence of any of the following

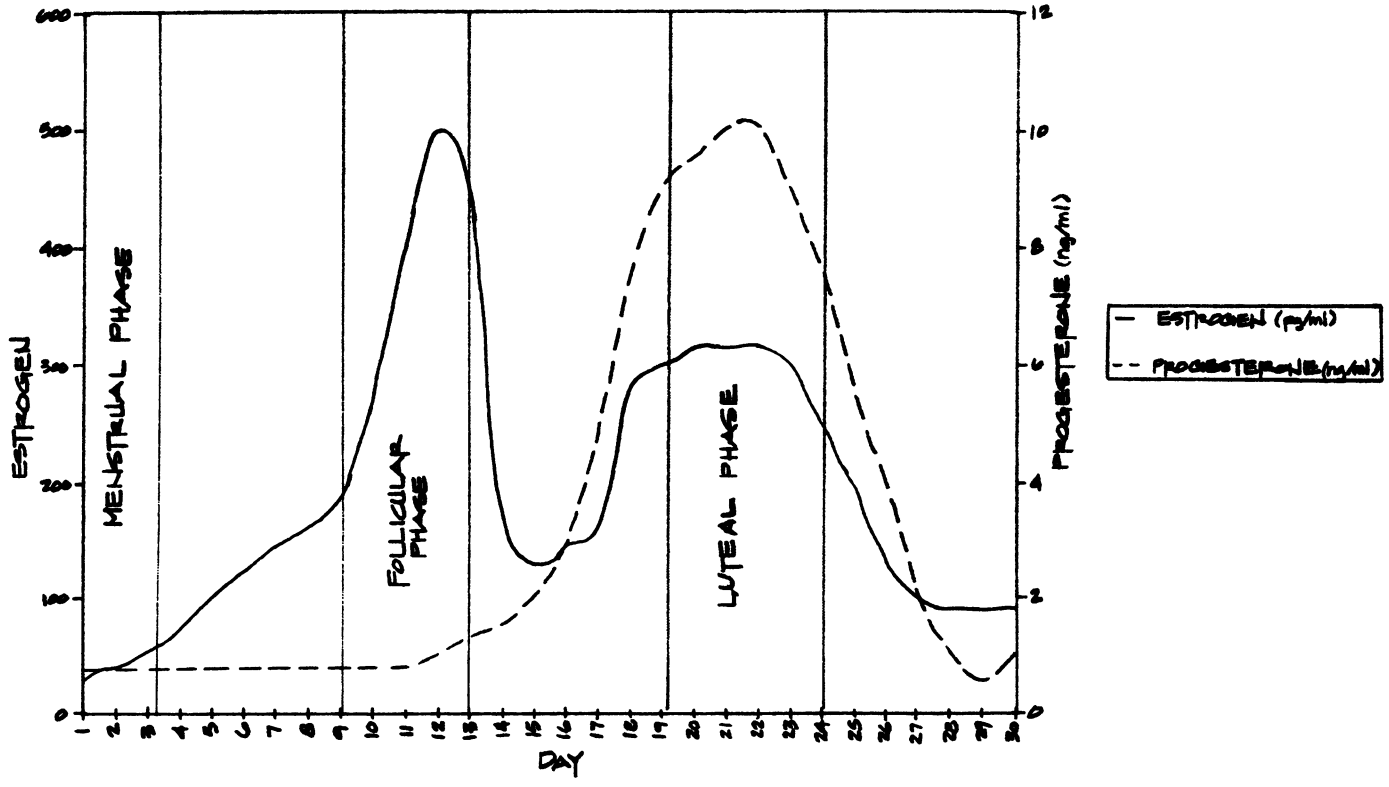

Figure 1. Estrogen and progesterone fluctuations throughout the menstrual, follicular, and luteal phases of the normal 28- to 30-day menstrual cycle.

conditions: (1) bilateral knee pathology, (2) use of hormone therapy, or (3) a 90-degree tubercle-sulcus angle of more than 10°. All participants stated that they experienced a normal (28- to 30-day) menstrual cycle. Each participant had at least one apparently healthy knee (no known knee anomalies), which was used for ACL laxity assessment during the course of the study. All participants stated that they were not currently taking any type of hormone therapy (eg, birth control medication) that might affect normal hormone level fluctuations. Because of the tendency of females to display a greater Q-angle than males, thus predisposing them to excessive genu valgum, we decided to eliminate participants with excessive genu valgum. To identify excessive genu valgum, a 90-degree tubercle-sulcus angle (Figure 2) was measured by palpating the transepicondylar axis of the femur and drawing a line perpendicular to that axis, which was compared with a line passing through the center of the patella and the tibial tuberosity. An angle of 0° has been defined as normal, whereas an angle of 10° or more is abnormal.[13] All participants had a measured 90-degree tubercle-sulcus angle of less than 10° (mean tubercle-sulcus angle = 5.1° ± 1.6°). Informed consent was received from each subject in accordance with institutional review board guidelines from the University of Utah, Salt Lake City, UT, and The Orthopedic Specialty Hospital, Murray, UT. The investigation was approved by the University of Utah Human Investigation Committee and The Orthopedic Specialty Hospital.

Instruments

A 21-gauge needle was used in conjunction with an 8.5-mL Vacutainer (Becton Dickinson Vacutainer Systems, Franklin Lakes, NJ) for all blood draws for the purpose of determining serial estrogen and progesterone levels. ACL laxity measurements were assessed with the KT-2000 knee arthrometer (MEDmetric, San Diego, CA), and values were plotted on an X-Y plotter for 67, 89, and 133 N of pull force.

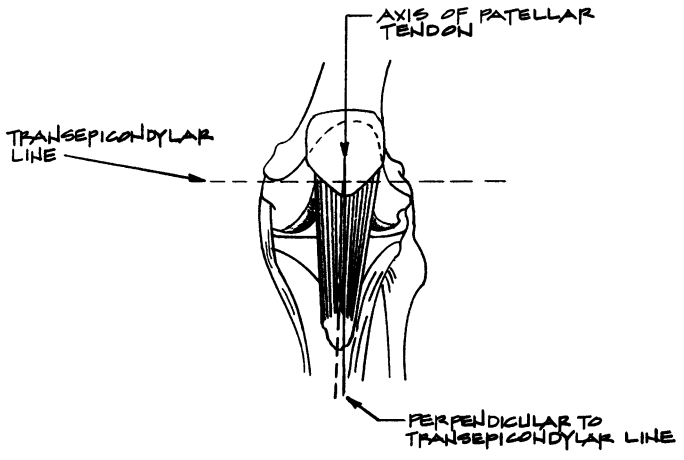

Figure 2. The 90-degree tubercle-sulcus angle is determined by palpating the transepicondylar axis of the femur and drawing a line perpendicular to that axis, which is compared with a line passing through the center of the patella and the tibial tuberosity.

Procedures

Each subject reported to The Orthopedic Specialty Hospital, Murray, UT on the day of her onset of menses. At this time, a blood sample and an ACL laxity measurement were taken. Blood samples were immediately taken to the Cottonwood Hospital Blood Laboratory, Murray, UT, for radioimmunoassay procedures. Estrogen and progesterone levels were recorded to the nearest pg/mL and ng/mL, respectively. Immediately after blood sampling, an ACL laxity measurement was taken using the KT-2000 knee arthrometer. Total tibial translation relative to the femur was plotted graphically on an X-Y plotter for 67, 89, and 133 N of force. Displacement was recorded to the nearest 0.5 mm for 133 N of force. This same procedure was completed on days 10, 11, 12, 13, 20, 21, 22, and 23 of the participant's menstrual cycle to determine peak levels of estrogen and progesterone during the follicular and luteal phases.

Statistical Analysis

A within-subjects, repeated-measures analysis of variance was used to determine the presence or absence of significant differences in knee laxity values over the course of the subject's menstrual cycle. Differences were considered statistically significant at an α level of 0.05 or less. All statistical analyses were performed using a personal computer and SPSS for Windows software (version 6.01, SPSS, Inc, Chicago, IL).

RESULTS

Means and standard deviations for estrogen and progesterone changes, as well as ACL laxity changes, throughout the subjects' menstrual cycles are listed in the Table. Phase I corresponds to day 1 of the menstrual cycle (menstrual phase) and represents baseline levels of estrogen and progesterone. Phase II corresponds to days 10 through 13 of the menstrual cycle (follicular phase), or peak estrogen surge. Phase III corresponds to days 20 through 23 of the menstrual cycle (luteal phase), or peak progesterone surge. We found a significant difference ($F_{1,6} = 3.56$, $P = .048$) in ACL laxity when comparing baseline levels of estrogen (Phase I) with peak levels of estrogen (Phase II). A significant difference ($F_{1,6} = 13.41$, $P = .006$) in ACL laxity was noted when comparing baseline levels of progesterone (Phase I) with peak levels of progesterone (Phase III) (Figure 3).

DISCUSSION

The purpose of our study was to determine whether women experience significant differences in ACL laxity in conjunction with estrogen and progesterone surges during a normal 28- to

Hormonal and ACL Laxity Changes Throughout The Menstrual Cycle (Mean ± SD)

Phase of Menstrual Cycle	Estrogen (pg/mL)	Progesterone (ng/mL)	ACL Laxity (mm)
Phase I (menstrual phase)	160.00 ± 66.24	.60 ± .40	5.6 ± 1.34
Phase II (follicular phase)	778.00 ± 255.43*	.64 ± .31	6.4 ± 1.64*
Phase III (luteal phase)	395.00 ± 134.57*	14.00 ± 5.44*	7.0 ± 1.66*

* Indicates significant changes compared with Phase I.

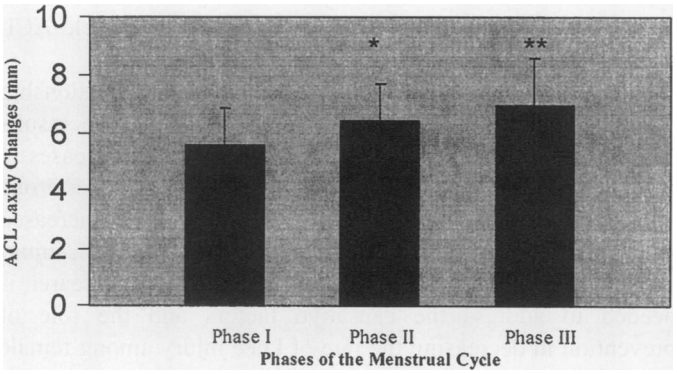

Figure 3. ACL laxity changes throughout phases I, II, and III.
***Significantly greater than Phase I (*P* = .048).**
****Significantly greater than Phase I (*P* = .06).**

30-day menstrual cycle. Estrogen and progesterone plasma levels were measured during the 3 phases of a single menstrual cycle for 7 women. The follicular and luteal phases of the menstrual cycle were identified to establish peak values for these hormones. ACL laxity measurements were then compared with the baseline and peak levels of estrogen and progesterone. If estrogen and progesterone influence ACL laxity, there should be a change in ACL laxity during the menstrual cycle.

Our results support the theory that hormonal changes experienced during the menstrual cycle may have an increased ACL laxity effect in women. In this study, the greatest ACL laxity was associated with the luteal phase. The literature shows that, during the course of a normal menstrual cycle, women tend to experience surging levels of estrogen and progesterone throughout the menstrual cycle.[11] Estrogen and progesterone are lowest during the menstrual phase of the cycle. During the follicular phase, estrogen levels rise dramatically as a result of rising levels of luteinizing hormone, while progesterone remains relatively low. During the luteal phase, progesterone levels increase as a result of the development of the corpus luteum.[10,11] The results of estrogen and progesterone assays obtained in our study agree with the above-mentioned hormone fluctuations experienced during the normal menstrual cycle (Table).

Some researchers have speculated that the increased joint laxity of females compared with males may contribute to the increased incidence of knee injuries among female athletes.[14,15] It has also been suggested that pregnancy-related hormones, specifically estrogen and progesterone, may have an effect in increasing joint laxity.[7] The pregnancy-related hormone relaxin is thought to be associated with ligamentous relaxation of the pubis and pelvis to accommodate the size of the fetus and fetal passage during birth. However, there is no definitive evidence for this.[16] Although it has been speculated that the hormones estrogen and progesterone may exhibit an increased ligamentous laxity effect, to date no research has examined comparisons between ACL laxity values and measured levels of estrogen, progesterone, or relaxin.

For a hormone to have an effect, a receptor must be present to accommodate that particular hormone. Recent evidence[9] reveals that estrogen and progesterone receptors are present in the ACL. More recently, these researchers used in vitro experimentation to establish a possible link between estrogen levels and fibroblast metabolism. Using rabbit ACLs, they found that collagen synthesis by the ACL fibroblasts significantly decreased with increasing local estradiol concentration in a dose-dependent manner and concluded that estrogen may have an effect on the structure of the ACL.[8] We found that ACL laxity increased with increasing levels of circulating estrogen associated with the follicular phase, which supports the theory that increased levels of estrogen may have an effect on the ACL. In another study,[17] researchers reported a greater number of ACL injuries during the ovulatory (follicular) phase of the menstrual cycle and concluded that hormone fluctuations need to be considered as a possible factor in increased incidence of ACL injuries among women. Their data were collected via an interviewer-administered questionnaire of 40 young females. The questionnaire investigated the time of injury as compared with the phase of the menstrual cycle. Our results agree with the suggestion that, if increased estrogen levels result in increased laxity, female athletes may be at increased risk for ACL injury during times when these hormones are at peak levels.

The differences found in our study, comparing ACL laxity values between baseline and peak levels of progesterone, support the evidence mentioned above[8] that fluctuating levels of hormones experienced during the menstrual cycle have an effect on female ACL laxity. Although significant differences were found between ACL laxity and peak levels of estrogen and progesterone, it should be noted that no measurements (hormone assays or ACL laxity measurements) were taken between the days of the follicular phase and the luteal phase (approximately days 15 to 20 of the menstrual cycle), and it is not known what changes, if any, occurred between the follicular and luteal phases of the menstrual cycle. Because no measurements were taken between the follicular and luteal phases, the difference in ACL laxity found during the luteal phase may actually have occurred earlier or may have been a delayed effect of estrogen. It is possible, as well, that increased

female ACL laxity could be a combined effect of estrogen and progesterone. Future research on this subject should include daily ACL laxity measurements throughout the entire menstrual cycle.

It is not known whether the differences in ACL laxity were the direct result of increased levels of circulating estrogen and progesterone. Changes in female ACL laxity may be due to conditions other than varying levels of estrogen and progesterone, specifically changing levels of relaxin. Relaxin has been found in the peritoneal fluid of nonpregnant, midluteal-phase females at greatly reduced levels, compared with levels found during pregnancy, and is thought to be secreted from the corpus luteum.[11,12] Our study did not measure varying levels of relaxin throughout the normal menstrual cycle. Future research should examine the possibility of relaxin receptors on the ACL. Also, future research should look at relaxin levels, as well as the combination of estrogen, progesterone, and relaxin, and the relationship to female ACL laxity.

A number of factors affect displacement of the knee joint: starting position of the joint (angle of knee flexion), external constraints on motion, applied force (load, direction, and point of application), muscle tone (quadriceps and hamstring relaxation), and ligament laxity.[18] Many investigators have observed that anterior laxity resulting from disruption of the ACL is best detected with the knee in 15° to 45° of flexion.[18–21] Because anterior-posterior laxity is altered by the angle of flexion of the knee, it is important that participants are placed in the same angle of flexion for each measurement.[18] The thigh support used in this study positioned the knee at approximately 35° of flexion. Both limbs of the participants were positioned in the same degree of rotation by means of a footrest, which constrained external rotation of the tibia. The base of the footrest is scaled, and the starting position of each subject was noted and maintained throughout subsequent ACL laxity measurements. Muscle tone was measured by manually assessing the participant's quadriceps and hamstrings and verbally instructing the subject to relax. Because this technique is fairly subjective in nature, it was impossible to ascertain whether true muscle relaxation was obtained. The test administrator for this investigation was experienced and had previously demonstrated acceptable intertester and intratester reliability, reducing the likelihood that the ACL laxity values obtained were compromised due to this phenomenon. An intraclass correlation for a single tester for 133 N of force for right leg anterior displacement has been reported to be 0.932.[22]

CONCLUSIONS

Although the subject sample size in our study was small, the results demonstrate a definite relationship between greater female ACL laxity and surging levels of estrogen and progesterone during a normal menstrual cycle. Because the women who participated in this study exhibited increased ACL laxity throughout the course of their menstrual cycle, it cannot be concluded that increased laxity increases the risk of ACL injury during these times of peak hormone levels. It is possible that increased ACL laxity may actually be the result of a protective mechanism designed to allow the ligament to elongate rather than rupture, in which case increased levels of circulating reproductive hormones may not be a contributing factor to increased female ACL ruptures. Because we do not yet know whether circulating hormones contribute to the increased incidence of ACL injuries, future research on this topic should focus on when during the menstrual cycle ACL injuries are occurring.

This is the first known study to examine the relationship between female sex hormones and ACL laxity. The results demonstrate that female ACL laxity significantly increases in conjunction with surging levels of estrogen and progesterone during the normal menstrual cycle. Due to the increased participation of women in sports and the increased ACL injury rate in female athletes compared with males, more research is needed to address the causative factors and the role of prevention in decreasing the rate of knee injury among female athletes. Our results, which indicate the importance of considering female sex hormones as one of several factors that may be contributing to the female ACL injury epidemic, provide sound justification for further research in this area.

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