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The Menstrual Cycle, Sex Hormones, and Anterior Cruciate Ligament Injury

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Objective: To determine if anterior cruciate ligament (ACL) injuries in female athletes occur randomly or correlate with a specific phase of the menstrual cycle.

Design and Setting: Female athletes who sustained ACL injuries reported the days of their menstrual cycles and provided saliva samples for sex-hormone determination. Salivary sex-hormone profiles were assessed to confirm the self-reported menstrual histories.

Subjects: A total of 38 female athletes (20 college, 15 high school, 1 middle school, 2 recreational) with recent ACL injuries participated in the study over a 3-year period.

Measurements: Athletes with recent ACL injuries completed a questionnaire defining the injury, the last menstrual cycle, prior knee injury, school, and type of birth control used (if any). Each subject provided a 30-cc saliva sample within 72 hours of injury. Saliva samples were placed into sealed containers and frozen at −20°C. We obtained 13 additional control samples from uninjured females to test the correlation between saliva and serum sex-hormone levels. Progesterone and estrogen were assayed by radioimmunoassay. Physical examination, magnetic resonance imaging, or surgery confirmed the injury in all subjects.

Results: The correlations between saliva and serum estrogen and progesterone were 0.73 ($\alpha = .01$) and 0.72 ($\alpha = .01$), respectively. Ten of 27 athletes who reported their cycle day at time of injury sustained an ACL injury immediately before or 1 to 2 days after the onset of menses. We rejected the null hypothesis that such high frequency was due to random chance.

Conclusions: A significantly greater number of ACL injuries occurred on days 1 and 2 of the menstrual cycle. Salivary sex-hormone levels correlated with the reported cycle day.

Key Words: estrogen, progesterone, remodeling, female athlete, athletic injury

Females injure their anterior cruciate ligaments (ACLs) more frequently than males participating in similar athletic activities.[1–3] The cause of this sex discrepancy is likely multifactorial.[4–6] In addition to their increased susceptibility to injury, women are at risk for increased knee laxity, ACL graft rupture, and other less successful outcomes after ACL reconstruction compared with males.[7] Although other authors have shown that functional outcomes after ACL reconstruction may be similar in men and women,[8,9] females' knees have demonstrated significantly more laxity (measured as individual mean postoperative manual maximum differences) after both hamstring and bone-patellar tendon-bone reconstructions.[7,8] One possible explanation for these observations is sex-specific differences in ligament remodeling.

Tissue remodeling occurs through a continuous cycle of protein synthesis and degradation.[10–14] In this process, old or damaged structures are degraded and replaced with newly synthesized molecules.[10–14] The balance between the degradative and biosynthetic arms of this process is controlled by the relative activities of matrix metalloproteinases and tissue inhibitors of metalloproteinases.[10–12,14] The expression of some of these proteins is regulated by steroid hormones.[15–18] For example, estrogen-dependent collagenase production and progesterone-dependent inhibition of collagenase have been observed in pig pubic ligaments.[15] Additionally, increasing the concentration of estrogen in an ACL tissue-culture model resulted in decreased fibroblast and procollagen production.[19] We, therefore, hypothesized that the type of hormone or the nature of exposure to it could affect the remodeling capabilities of the ACL and thereby alter its mechanical properties.

One prediction of this hypothesis is that ACL injury would be more likely at a certain time or times during the menstrual cycle. Although one group of investigators[20] identified a trend toward an increase in injuries in the ovulatory phase and a decrease during the follicular phase, they did not conclusively identify a difference in injury rates as a function of the menstrual cycle. Other researchers have implicated different cycle phases for increased incidence of ACL injury.[3,21,22] However, these studies were limited by their reliance on athletes' histories provided to the medical staff regarding when the injuries occurred relative to the menstrual cycle. To overcome this limitation, serum, urine, or saliva could be examined to determine the sex-hormone profile and thereby verify the cycle day of the athletes at the time of injury. Salivary levels of sex hormones determined with supersensitive assays correlate well with those measured in serum.[23] Saliva samples are easy to obtain in an athletic setting because little advanced preparation and no equipment are required. Our objectives were to determine if menstrual histories provided at the time of ACL injury could be confirmed by measuring salivary estrogen (as estradiol [E_2]) and progesterone (P) levels and then to determine if ACL injuries occurred randomly or clustered in a specific phase of the menstrual cycle.

METHODS

Thirty-eight athletes with ACL injuries participated in the study. All study participants completed questionnaires defining their ACL injury, last menstrual period, prior knee injury, school, and type of birth control used (if any). Each athlete provided a 30-mL saliva sample within 72 hours of injury. Saliva samples were stored in a sealed container and frozen at −20°C. The samples were then shipped on dry ice to the Oregon Regional Primate Research Center (Beaverton, OR) for analysis. Physical examination, magnetic resonance imaging, or surgery confirmed the injury in all subjects.

One of the 38 athletes reported having a hysterectomy; her data were excluded due to inadequate information about hormone-replacement therapy. Consequently, study analyses included data from 37 patients: 21 provided both saliva samples and menstrual histories, 10 provided only saliva samples, and 6 provided only menstrual histories.

Because the previous literature indicated mixed results regarding the correlation between saliva and serum sex-hormone levels,[23–28] we first performed an analysis to establish whether such a correlation existed in our sample. Thirteen control samples from uninjured females were obtained to test the correlation between saliva and serum sex-hormone levels. Salivary progesterone was assayed by routine radioimmunoassay, and E_2 was assayed with a modification of the 3rd Generation Double Antibody Estradiol assay (Diagnostic System Laboratories, Webster, TX) at the Oregon Regional Primate Research Center.

To test our null hypothesis that the ACL injuries were not correlated with the athletes' menstrual cycles, we performed a Monte Carlo simulation to generate multiple pseudocontrol groups. If the ACL injuries were not correlated with the menstrual cycle, the injuries would occur randomly throughout the cycle. That is, the probability of injury would follow a uniform distribution over the menstrual cycle. Using Stata computer software (version 6, Stata Corp, College Station, TX), we simulated 50 hypothetical control groups, each with 100 subjects. The timing of an ACL injury assigned to each hypothetical subject was determined by a uniform distribution to ensure that the probability of injury in each 2-day interval was the same for all intervals. We then compared the injured group with each computer-generated group. We tested whether the probability of injury was the same for each 2-day interval between the injured athletes' group and a group of 100 hypothetical subjects.

RESULTS

The correlations between saliva and serum estrogen and progesterone were 0.73 (α = .01) and 0.72 (α = .01), respectively. The correlation between the self-reported last menstrual period at the time of injury and the actual salivary and progesterone levels was 95% for the 21 athletes who provided this information (Table). Because salivary sex-hormone levels confirmed self-reported menstrual history in all but one case, we were able to use the measured sex-hormone levels to place athletes who did not provide menstrual histories in the appropriate phase of the menstrual cycle at the time of injury. Among all 37 athletes for whom data were analyzed, 25 injured their ACLs during the follicular phase, 1 during the ovulatory phase, and 11 during the luteal phase of the menstrual cycle. Six athletes injured their ACLs while on oral contraceptives, of whom 5 sustained their injury during the follicular phase and 1 during the luteal phase. Only 1 of the athletes on oral contraceptives reported her cycle day at the time of injury (day 2 of menses). Interestingly, the E_2 and P concentrations were very low in this athlete (E_2 = 0.73 pg/mL, P = 58 pg/mL) and 3 others on oral contraceptives (E_2 range, 0.16–0.20 pg/mL; P range, 15–29 pg/mL), suggesting that they were all injured around the time of menses.

Predicted Phase in Cycle versus Reported Cycle Day

Subject No	Estradiol Concentration (pg/mL)	Progesterone Concentration (pg/mL)	Predicted Phase	Reported Day
1	6.28	169	ovulatory	9
2	0.92	0.73	follicular	6
3	1.08	149	luteal	19
4	2.66	149	luteal	15
5	1.69	127	luteal	24
6	1.15	75	luteal*	39
7	2.28	137	luteal	17
8	3.38	98	follicular	11
9	1.43	62	follicular	9
10	0.91	48	follicular	1
11	0.48	77	follicular	1
12	3.17	207	luteal	14
13	2.10	51	follicular	1
14	2.71	61	follicular	13
15	0.73	58	follicular	2
16	3.91	99	follicular†	24
17	1.18	125	luteal‡	4
18	1.06	178	luteal	30
19	1.02	62	follicular	11
20	0.62	64	follicular	1
21	1.39	45	follicular	14

*Low estradiol and low progesterone levels indicate that this patient was in the late luteal phase at the time of injury; these levels would also be consistent with the early follicular phase in an oligomenorrhoeic woman.
†High estradiol and low progesterone levels indicate that this patient was still in the follicular phase on day 24 of a long (oligomenorrhoeic) cycle.
‡This is the only athlete whose reported menstrual-cycle day conflicted with measured estradiol and progesterone levels.

Among the 27 athletes who self-reported their menstrual histories, 10 sustained injuries during the few days before and the first 2 days after the onset of menses (Figure 1). The frequency of observed injury for days 1–2 of the menstrual cycle was significant at α = .05 for all 50 comparisons between the injured athletes and our computer-simulated subject groups. This implies that the high frequency of the ACL injury observed in this interval was not due to random chance. For days 25–26, 27–28, and 7–8, the athletes' incidence of ACL injury was lower than the probability determined by a uniform distribution at α = .10 in 33, 34, and 36 comparisons, respectively, out of 50 total simulations. For the other intervals, fewer than 10 comparisons out of 50 were significant at α = .10,

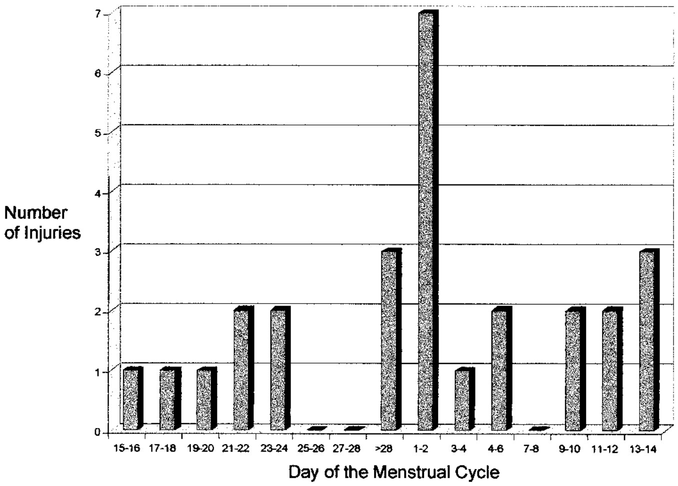

Figure 1. Distribution of anterior cruciate ligament injuries among 2-day intervals during the menstrual cycle. Note that 10 of 27 athletes were injured just before or during the 2 days after the beginning of the menstrual cycle. Using a Monte Carlo simulation to define a control group, we found a significant difference in the frequency of observed injury for days 1 and 2 of the menstrual cycle (α = .05) for all 50 comparisons between the injured athletes and our computer-simulated subjects.

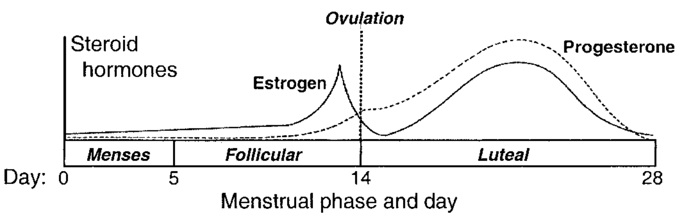

Figure 2. Changes in concentrations of estrogen and progesterone during the menstrual cycle. Estrogen concentration rises during the follicular phase, reaches a peak just before ovulation, and then drops sharply. It rises again and, along with progesterone, reaches a broad peak during the luteal phase. Estrogen and progesterone concentrations are both low during menses.

indicating that during these intervals, ACL injuries were likely to be uncorrelated with the menstrual phases.

Therefore, a significantly greater number of ACL injuries occurred on days 1 and 2 of the menstrual cycle. Salivary sex-hormone levels correlated with self-reported cycle day.

DISCUSSION

This is the first study to confirm self-reported menstrual histories with salivary sex-hormone profiles at the time of ACL injury. We found that 26 of 37 athletes tore their ACLs during the follicular phase of the menstrual cycle. Among athletes who self-reported their menstrual histories, 10 of these 27 injuries occurred during the few days before and the 2 days after the onset of menses (Figure 1). The levels of E_2 and P are both low at this time (Figure 2). This hormonal condition contrasts with the follicular phase during which P is low and E_2 peaks sharply before ovulation, and it follows the midluteal phase during which E_2 and P are both elevated for several days. These results are consistent with a report of no correlation between ACL injury and the general category of "luteal" phase,[3] as well as 2 other reports indicating that injury is more likely during the late luteal and early follicular phases of the cycle.[21,22]

We did not obtain information on the typical lengths of the athletes' menstrual cycles. Although the "normal" menstrual cycle lasts 28 days, 3 of our 37 athletes (8% of our sample) sustained injuries after day 28. The probability that ACL injuries occurred during the prolonged menstrual interval (>28 days) was no different from the probability determined by a uniform distribution. That is, we could not reject the null hypothesis that the injuries occurring during this time were due to random chance.

We chose to use computer-simulated subjects because there was no well-defined control group. To qualify to be in a legitimate experimental control group, subjects would have needed the same menstrual cycles as the injured females but different distributions of injury among the cycles' phases. Therefore, instead of performing an experimental-control group comparison, we tested a simpler null hypothesis that the ACL injuries occurred randomly in each day of the menstrual cycle. Each computer-generated subject had an equal chance (0.0357) of injury in each day of her menstrual cycle. That is, these subjects had the same menstrual cycles as the injured subjects, but the probability of injury was different. We rejected our null hypothesis and found that ACL injuries occurred most frequently during the early menstrual cycle.

Past studies of the correlation between salivary and serum sex-hormone measurement have yielded conflicting results.[24–28] However, more recent supersensitive, double-antibody techniques for measuring sex hormones in saliva have shown good correlation between saliva and serum levels.[23] One group has even recommended using saliva to obtain hormone profiles in patients with difficult venous access.[24] Using saliva to obtain sex-hormone profiles of athletes fits well in the athletic arena because little planning or equipment is needed to obtain and store the saliva. A simple ziplock-type bag works well to hold the saliva and can be placed immediately on ice and transferred to a freezer soon thereafter or upon return from a road trip.

Our findings depend heavily on the accuracy with which we determined the day of the menstrual cycle at the time of injury. Such determinations are complicated by problems in obtaining blood from injured athletes in an athletic setting and the fact that a single measurement of one hormone cannot unequivocally define the day of the menstrual cycle. However, we successfully overcame these problems by measuring both E_2 and P in saliva. This approach is effective because elevated (at or near the typical highest concentration) E_2 is characteristic of the follicular phase (with an E_2 spike occurring at ovulation), elevated P is characteristic of the luteal phase, and low E_2 and P are characteristic of menses. Menstrual phases defined in this way correlated well (>95%) with the self-reported menstrual histories (see Table), thereby confirming the accuracy of our athletes' recollections of their menstrual cycles. We, therefore, have confidence that all but 1 or 2 of our athletes accurately recalled the dates of their last menstrual periods before ACL injury.

Although we do not know why ACL injuries occur around the time of menses, our current research is focused on characterizing sex differences in ACL tissue remodeling. Cyclic changes in E_2 and P may alter expression of genes encoding tissue-remodeling enzymes and proteins, which, in turn, could favor either net tissue degradation or repair at specific times during the menstrual cycle. If a molecular basis for sex differences in ACL injury is found, treatments may be instituted to decrease the injury rates in females.

In conclusion, ACL injuries occurred most frequently on days 1 and 2 of menses, suggesting that ACL injury is not random but occurs more often around the time of menses, when circulating sex-hormone levels are low and after a time when both E_2 and P were elevated. Additionally, salivary sex-hormone profiling correlates well with serum profiling, and in this athletic population, adequately identified menstrual cycle phase at the time of injury. This is, therefore, an effective technique to identify correlations between injury and hormone patterns in athletes.

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COMMENTARY

Susan E. Kirk

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It is well accepted that females involved in athletic activities have a substantially greater risk of sustaining anterior cruciate ligament (ACL) injuries compared with their male counterparts. With the continued growth of women's sports, such an injury no longer brings merely the worry of the end of a scholastic career but also the potential loss of scholarship or professional income. Most investigators have suspected that the reason for sexual disparity in the incidence of ACL injury is multifactorial; however, several recent studies have attempted to explore the link between one obvious difference between men and women: sex steroids. In this issue of the *Journal of Athletic Training*, Slauterbeck et al[1] have attempted to link the frequency of ACL injury to menstrual cycle phase and have used the novel method of salivary estrogen (estradiol) and progesterone measurements to attempt to confirm subjects' self-reported cycle phases at the time of injury.

The authors note that a flaw in the design of previously reported studies has been the lack of hormonal confirmation of menstrual phase. Their study design attempts to correct this problem in a noninvasive manner by using salivary assays for the major female sex steroids, estradiol and progesterone. Those involved in the care or training of athletes can appreciate the difficulty in trying to obtain serum samples at the time of injury in a reliable and convenient manner. Slauterbeck et al have shown a moderate correlation between the results of their salivary and serum assays in a separate group of subjects. However, with this method, they have not been able to overcome the difficulty of assigning women to a particular phase of the menstrual cycle with a single sample.

Although the pattern of menses is typically consistent from cycle to cycle, with a preovulatory surge of estrogen in the late follicular phase and then a second, more gradual increase in both estrogen and progesterone during the luteal phase, the overlap of hormone levels is such that one cannot determine the day of the cycle by a single measurement.[2] Review of Figure 2 in the article by Slauterbeck et al demonstrates that an equally elevated level of estrogen can be seen in both the late follicular and the midluteal phases. The authors have attempted to overcome this ambiguity by using paired samples of estrogen and progesterone, but as their figure demonstrates, midrange estrogen and progesterone are seen both immediately after the ovulatory surge and in both the early and the late luteal phases. In addition, the authors allowed the sex steroid samples to be collected up to 72 hours postinjury. Figure 2 demonstrates a substantial change (either increase or decrease) in hormone levels over this time at most phases of the cycle. It is quite possible that the 72-hour window allowed collection of samples just after ovulation had occurred, a period when levels of both estrogen and progesterone are similar to those seen in the early follicular phase.

An additional complicating factor is the considerable variability among women, as well as in individual women from cycle to cycle, with regard to both cycle length and hormonal peaks.[3] For the purpose of data analysis, the authors have assumed that a normal cycle is 28 days, but most references cite a range of 24 to 32 days. Moreover, only 13% of normal women have cycles that are consistently within this range over 1 year.[3] Therefore, one cannot accurately assign a woman to an exact position in the menstrual cycle on the basis of a single hormonal measurement.

The authors relied on subjects to identify which phase of the cycle they believed themselves to be in at the time their injury occurred. Obstetricians know how unreliable the self-reported date of the last menstrual period is when attempting to determine gestational age. This area may also lead to incorrect interpretation of results. A strict description of the beginning of menses is required to eliminate subject error in dating the onset of menses. A typically used and reliable method is the date when feminine protection (sanitary napkins or tampons) is required. However, even with more rigid guidelines, self-reporting of menstrual history has been shown to be an unreliable measure of cycle parameters.[4] In this study, 27% of the subjects were not able to provide any menstrual history at all.

In vivo and in vitro models have demonstrated that estrogen does affect fibroblast and procollagen production and subsequent tissue remodeling, but it is risky to interpret the results obtained in different species and with different ligaments and then apply them to normal women injured during an athletic activity. Therefore, the exact impact of the sex steroids on ACL injury remains an unanswered question, as the authors acknowledge. Salivary assays are a potentially useful tool for convenient and noninvasive collection of samples that will allow a more complete investigation of this area. However, in addition to the large volume of saliva required per sample for this assay (30 mL), not all investigators have found this method to demonstrate acceptable reliability.[5] Therefore, it is important that other methods be developed to allow the convenient and noninvasive collection of hormonal specimens, potentially immediately at the time of injury or more frequently during 1 or several menstrual cycles. An additional method using capillary blood obtained with a lancet device shows promise.[5] More studies must be performed in valid populations to confirm both methods as acceptable replacements for serum estradiol and progesterone measurements. Only then will we begin to gain a more complete understanding of the role of female hormones in injuries sustained during athletic performance.

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AUTHORS' RESPONSE

Dr Kirk is absolutely correct that similarities in hormone levels at different phases of the menstrual cycle make it difficult to define unequivocally the phase of the cycle from measurements on a single sample. Fortunately, that was not the major intent of our hormone measurements. Single measurements *can* be used to disprove inaccurate recollections of menstrual history. For example, if a patient reports that she is on day 5 (follicular phase) of her cycle but has an elevated level of progesterone indicative of the luteal phase, the single hormone measurement would disprove the recalled menstrual history. Accordingly, in our study the hormone measurements were not used as the sole indicator of menstrual phase but to verify reported menstrual histories. Our hormone data conflicted with the recalled history for only 1 patient, supporting the view that the menstrual histories our patients reported were largely accurate.

We believe that, as athletes, this patient population may be more aware of their physical state than others. In the example of obstetric patients Dr Kirk cited, the patients were being asked to recall a date that was 4 to 8 weeks (or more) in the past. It is easy to imagine that such recollections would be less accurate than those of athletes who menstruated perhaps only days, or at most 4 weeks, before injury. Indeed, the patients we identified as being most at risk were actually menstruating at the time of injury.

We agree with Dr Kirk that there is room for improvement in the methods used to assess hormone concentrations in an athletic setting. However, the ultrasensitive radioimmunoassay can accurately determine hormone levels in saliva specimens, such as those our athletes provided. Thus, we believe such measurements are sufficiently reliable, when used in combination with self-reported menstrual history from injured athletes, to support the conclusions of this study.