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original research

The Interrelationships Among Sex Hormone Concentrations, Motoneuron Excitability, and Anterior Tibial Displacement in Women and Men

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Context: Sex hormone fluctuations have been implicated as a contributing factor to the high rates of noncontact injury to the anterior cruciate ligament in females.

Objective: To determine the strength of the relationships among variables of sex hormone concentrations, motoneuron excitability, and anterior tibial displacement (ATD) in women and men and to determine if these relationships differ between the sexes.

Design: Cohort study.

Setting: Sports medicine laboratory.

Patients or Other Participants: Twenty-eight regularly menstruating women (age = 22.4 ± 3.4 years) and 15 men (age = 22.3 ± 3.7 years) participated in the study.

Intervention(s): Fluctuations in sex hormones were determined for the participants. Female participants were tested every other day of their menstrual cycles, whereas male participants were tested every fourth day during the 28-day period.

Main Outcome Measure(s): We measured Hoffmann reflexes (maximum Hoffmann reflex [H_{max}] to maximum M-wave [M_{max}] ratio in the soleus), ATD under a 134-N load, and saliva concentrations of estrogen and progesterone. The independent variable was sex. Pearson product moment correlation coefficients were calculated for each participant by pairing measurements made on the same day. Two-tailed independent-samples *t* tests were used to determine the difference between the male and female correlations for each variable.

Results: Over the course of the study, the relationships between H_{max}:M_{max} and estrogen, H_{max}:M_{max} and progesterone, ATD and estrogen, and ATD and progesterone were not different between the sexes. However, the relationship between ATD and progesterone was different between the sexes (P = .036).

Conclusions: The observed correlations did not support our hypothesis that the relationships between sex hormone levels and reflex activity or between sex hormone levels and ATD would be different for women compared with men. If sex hormone concentrations significantly contribute to anterior cruciate ligament ruptures because of changes in laxity or in motoneuron excitability, their mechanism of action is likely multifactorial and complex.

Key Words: anterior cruciate ligament, Hoffmann reflex, knee laxity

Key Points

- Across the testing sessions, reflex responses were not directly or indirectly related to the hormonal changes.
- The relationships between sex hormone concentrations and anterior tibial displacement at the knee were weak.
- When taking into consideration the possible time lag effect between hormone concentration changes and a change in anterior tibial displacement or spinal reflexes, we found no differences between the men and women.

After years of research focusing on the multifaceted problem of noncontact anterior cruciate ligament (ACL) injuries, identifying a cause-and-effect relationship remains a challenge. Failure to identify 1 or more mechanisms responsible for the exponential difference in noncontact ACL injuries between women and men hinders the development of focused prevention and rehabilitation programs. Explanations for differences in the sex-related injury rate of noncontact ACL injuries historically have been grouped into 4 general categories based on either known or theorized differences between the sexes[1]: (1) anatomic,[2,3] (2) neuromuscular,[4,5] (3) hormonal,[6–18] and (4) biomechanical.[19–21] Of these categories, scientific investigation has shifted largely toward focusing on neuromuscular or hormonal factors or their combined contributions to this phenomenon.

Researchers have extensively studied sex hormones and their potential effect on function of the neuromuscular and musculoskeletal systems across the menstrual cycle in premenopausal women[6,22–26] and in postmenopausal women.[27] The role of sex hormones in the ACL injury-discrepancy issue can be categorized into 3 primary areas: (1) the effects of having sex hormone receptors on the ACL,[9,13,14,23,28–30] (2) the effects of estrogen concentrations on the biomechanical properties of the ACL and knee joint laxity,[8,10,12,31,32] and (3) the effects of sex hormones on injury rates.[18,33–36] Because estrogen receptors exist on the ACL,[14] interest in estrogen concentrations as a contributing factor in this issue remains high; however, the functional relevance of having receptors on the ACL remains unknown. The results of studies assessing the effects of estrogen on the mechanical properties of the ACL are contradictory, and some debate continues about whether a particular phase of the menstrual cycle places a woman at greater risk than a man for an ACL rupture.

Authors[20,37–39] of studies using traditional biomechanical approaches have shown functional movement differences between women and men, but the lack of an explanatory mechanism is troublesome. To our knowledge, no one has studied both neuromuscular and musculoskeletal changes concurrently with fluctuations in sex hormones.

The rationale for this study was 2-fold: (1) to explore the relationships between a neurologic measure and sex hormone concentrations, and (2) to attempt to confirm previous reports of how knee laxity relates to sex hormone concentrations. The interest in investigating a neurologic measure of motor control developed from the understanding that estrogen assists in the regulation of a variety of processes unrelated to reproduction and from the understanding of the diversity of locations where estrogen receptors are found. Specifically, because estrogen receptors are located in a variety of laminae in the spinal cord, the location of the synapse between alpha Ia afferents and motoneurons and the fluctuations in estrogen may affect the activity of alpha motoneurons and their contribution to the control of movement. Furthermore, during the past several years, estrogen and its interaction with a variety of neurotransmitters has led to the understanding that estrogen contributes to the regulation of a variety of neurologic functions in the brain and spinal cord. For example, estrogen has been implicated as a contributing factor for the higher rates of migraine headaches[40] and depression[41] and for the lower pain tolerance in women than in men.[42]

The purpose of our study was to determine the strength of the relationships between sex hormone concentrations and motoneuron excitability, between these concentrations and anterior tibial displacement (ATD) in women and men and, more importantly, to determine if these relationships differed between the sexes. Our hypothesis was that the relationships between sex hormone variables and motoneuron excitability and between these variables and knee joint laxity would be different between the sexes.

METHODS

Participants

Twenty-eight female participants (age = 22.4 ± 3.4 years) who reported regular menstrual cycles of 28 to 30 days with no more than 3 days of variation in cycle length during the past 3 cycles participated in this study. The number of participants that we included was based on an a priori power analysis of reflex and laxity data. Women were excluded if they reported the use of hormone-based birth control during the year before the study. To serve as a control group, 15 men (age = 22.3 ± 3.7 years) were enrolled in the study. We did not record height and weight data for the participants. Potential male and female participants were excluded if they reported a history of knee injury that necessitated the use of crutches. Each participant provided informed consent and enrolled in the study. The study was approved by the Institutional Review Board for the Protection of Human Subjects at Oregon State University.

After enrolling in the study, each participant reported to the Sports Medicine Laboratory for orientation. Each female participant provided a menstrual history and the date on which she expected to start her next menstrual cycle. Based on that date, a tentative testing date was established for the initial day of testing (day 2 of the cycle). Each female participant contacted the research team on the day that she started her cycle, and she was scheduled for testing the next day (within 24 hours of the onset of menstruation). Female participants were tested every second day until the start of their next cycles. A typical hormone profile of these participants is presented in Figure 1. The initial testing day for each male participant corresponded with the initial testing day for a female participant. The male participants were tested every fourth day for 28 days. On each day of testing, assessments of the motoneuron excitability, ATD, and sex hormone concentrations were obtained. The men were tested less frequently because testing every 2 days was not critical due to small inherent fluctuations of sex hormones.

Measurements

In an attempt to study both neuromuscular and musculoskeletal changes concurrently with fluctuations in sex hormones, we designed our investigation to measure motoneuron excitability, ATD, and hormonal concentrations in women and men over approximately 1 month. The dependent variable used to assess motoneuron excitability was the ratio between the maximum Hoffmann reflex (H_{max}) and the maximum M-wave (M_{max}). This commonly used neuromuscular measure quantifies the connectivity between the motor and sensory components of the neuromuscular system. More specifically, the H_{max} represents activation of motoneurons from all the Ia sensory fibers in a given muscle at a given state.[43] Similarly, the M_{max} represents activation of the motoneurons for a specific motoneuron pool.[43] Therefore, examining the ratio between these measures provides a window into the spinal connections between the sensory and motor fibers of a muscle. Because 2 researchers collected the reflex data, interrater reliability of this measure was established: intraclass correlation coefficient (ICC [2,1]) = 0.93. Additionally, intrarater reliability was established (ICC [2,1] = 0.99) at a value similar to the values reported by Palmieri et al.[44]

We measured ATD during an instrumented Lachman test at 134 N using a Compu-KT arthrometer system (MEDmetric Corp, San Diego, CA; intrarater reliability, ICC [2,1] = 0.85). Because of the large number of data collection sessions (more than 500), the ATD measures were taken by 3 investigators. However, each participant was tested by the same investigator for all of his or her measurements.

Sex hormone levels were assessed via saliva assays. Estrogen was assayed using the modified third-generation Double Antibody Estrogen assay (Diagnostic System Laboratories, Inc, Webster, TX) with a sensitivity of 1 pg/mL. Progesterone was measured by a routine radioimmunoassay technique with a sensitivity of 5 pg/mL. Although the role of progesterone in this ACL injury-discrepancy issue is not as suspect as the role of estrogen is, these hormones are known to interact with each other, so we included them.

Neuromuscular Assessment

Participants were placed in the prone position on a padded table to standardize head and neck position and ankle angle. Hoffmann reflex testing was conducted according to procedures outlined by Hugon.[45] To elicit

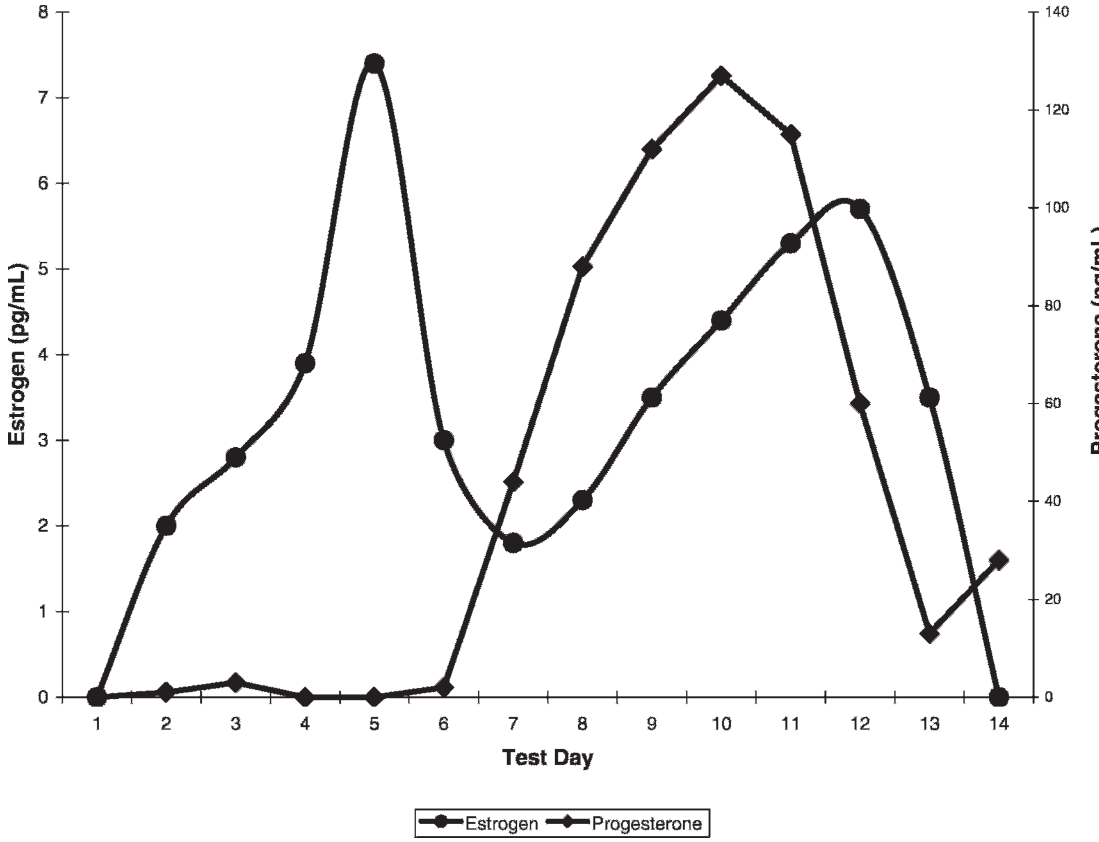

Figure 1. Typical sex hormone concentration profile of a female participant. The estrogen peak can be observed at test day 5 (day 11 of her menstrual cycle), with the following progesterone peak at test day 10 (day 21 of her menstrual cycle).

and record Hoffmann reflexes, electromyographic (EMG) electrodes and stimulating electrodes (BIOPAC Systems, Inc, Goleta, CA) were attached to the participant. All areas of skin where electrodes were placed were shaved and cleaned with alcohol before electrode placement. Surface EMG recording electrodes (Ag-AgCl) were placed over the soleus muscle belly. Electrodes were placed longitudinally on the skin with a 2-cm interelectrode distance. The location of the electrodes was marked with a permanent marker to enhance session-to-session reliability. The ground electrode was placed over the lateral malleolus. The EMG activity of the reflex contraction of the soleus muscle was collected and stored by a personal computer equipped with an MP100 data collection system (BIOPAC Systems, Inc). The EMG data were sampled at 2000 Hz. Because this type of EMG analysis differs from EMG analysis used in kinematics, filtering and conditioning were not employed. To elicit the reflex, a stimulating electrode (1 cm^2) was placed over the tibial nerve in the popliteal fossa for current delivery, and a dispersal pad (3 cm^2) was placed superior to the patella on the distal thigh. The H_{max} and M_{max} were measured through stimulation of the tibial nerve at intervals between 10 and 15 seconds. A percutaneous electric stimulus (1-millisecond duration pulse) was used to elicit the Hoffmann reflex and M-waves. Stimulus intensity was increased in small increments from the initial presentation of the Hoffmann reflex through the presence of the plateau of the maximal M-wave to provide mapping of a recruitment curve. Figures 2A and B show time course graphs of the H_{max}:M_{max} measurements of a typical female and a typical male participant.

Knee Joint Laxity Assessment

At each data collection session, ATD of the left knee was measured in millimeters using the KT2000 Knee Ligament Arthrometer (MEDmetric Corp) equipped with the Compu-KT software. The left knee was chosen based on convenience of testing. The data were processed by an analog-to-digital converter and were stored for subsequent analysis. For these measurements, we employed a standardized protocol that Harter et al[46] described. Each participant was instructed to lie supine on an examination table with both knees flexed to approximately 25° over a rigid plastic bolster and with the ankle positioned in a permanently mounted heel jig. Because the heels were to be placed in the same position for all data collections, we recorded the location of the knee bolster by placing reference lines on the examination table to standardize knee angle from day to day, thus ensuring consistent knee angle during the month-long data collections. We measured ATD 3 times at the 134-N load. This load was chosen because of its prevalent use reported in the literature. The mean of the 3 trials was used as the displacement value. Figures 3A and B show a time course graph of the ATD of a typical female and a typical male participant.

Hormone Concentration Assessment

The saliva samples were collected via passive drool into a clean collection vial. Participants provided a minimum of 2 mL of saliva by drooling down a straw into the vial. Samples were collected no sooner than 30 minutes after the last meal or teeth brushing and, following collection, were placed into a

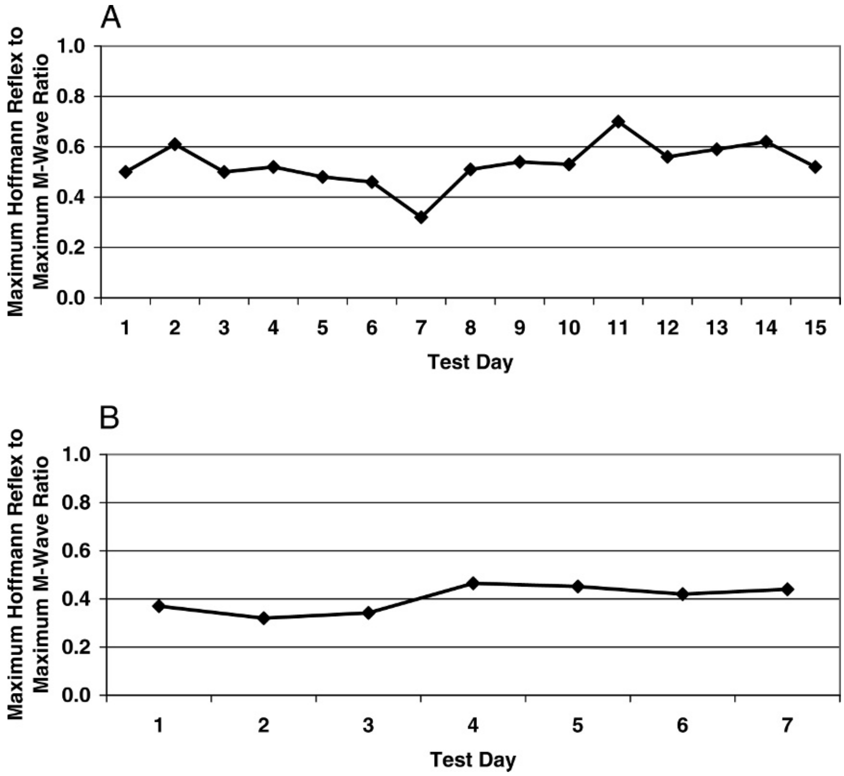

Figure 2. Reflex profiles of a typical female participant (A) and a typical male participant (B). Maximum Hoffmann reflex and M-waves were measured through stimulation of the tibial nerve. A percutaneous electric stimulus (1-millisecond duration pulse) was used to elicit the Hoffmann reflex and M-waves.

cooled centrifuge (4°C) and spun at 4000 *g* for 5 minutes before being frozen and stored at −70°C. Samples were transported to the Oregon Health Sciences University Primate Center for analysis. The primate center laboratory reported an intra-assay coefficient of variation for progesterone and estrogen that ranged from 5% to 8% and an interassay coefficient of variation that ranged from 8% to 12%.

Data Analysis

Data analysis was performed in several parts: (1) calculation of simple correlations between the hormone concentrations and the outcome variables and evaluation of potential differences between the average correlation of the variables for women and men, (2) exploratory analysis of lag correlations for selected variables, and (3) exploratory analysis of the relationships of variables based on the magnitude of day-to-day change in the hormone concentrations.

To evaluate the potential differences between the correlation of the variables for women and men, the established experimental unit was participant, so correlations between variables were calculated at the level of the participant. To complete this part of the analysis, 2-tailed independent-samples *t* tests were used to examine differences between the sex-grouped correlations. For example, to compare estrogen and ATD for each participant, we calculated the Pearson product moment correlation coefficient between the participant's daily measurements of estrogen and his or her daily measurements of ATD, pairing measurements within observation times. This procedure resulted in a correlation for each participant between the 2 variables. These correlations were then grouped by sex, and a *t* test (α = .05) was used to determine if a difference existed between the correlations.

Lag correlations were calculated because of our interest in evaluating a possible delayed effect of hormone concentration changes on joint laxity and motoneuron excitability, specifically ATD and H_{max}:M_{max}. For example, in each participant we calculated the correlation between the estrogen measurements taken on a given day and the ATD measurements taken 4 days after the estrogen measurements. This was accomplished by pairing the estrogen measurements for each participant ($estrogen_i$) with the lagged ATD measurement ($ATD_{i+4\ days}$). After this correlation was calculated for each participant, we grouped the correlations by sex and evaluated them with a *t* test, as described in the previous section. In addition, this procedure was applied to the data using an 8-day lag. The 4-day and 8-day lags were chosen because these time intervals matched in both sexes, with the women tested every 2 days starting on day 2 of menstruation and the men tested every 4 days.

Clearly, the rate at which hormone concentrations change across the menstrual cycle is not linear. To explore the potential effect of the magnitude of change in hormone concentrations on ATD and H_{max}:M_{max}, we calculated correlations between the magnitudes of change during a 4-day period to the variable of interest at the end of the 4-day period for each participant. For example, the difference between $estrogen_{day\ 1}$ and $estrogen_{day\ 4}$ was paired with the $ATD_{day\ 4}$. The magnitude of change also was calculated and correlated for an 8-day interval.

RESULTS

The daily means for all variables are presented in Table 1. Caution should be used in interpreting the hormone data in Table 1, because the hormone concentrations for each

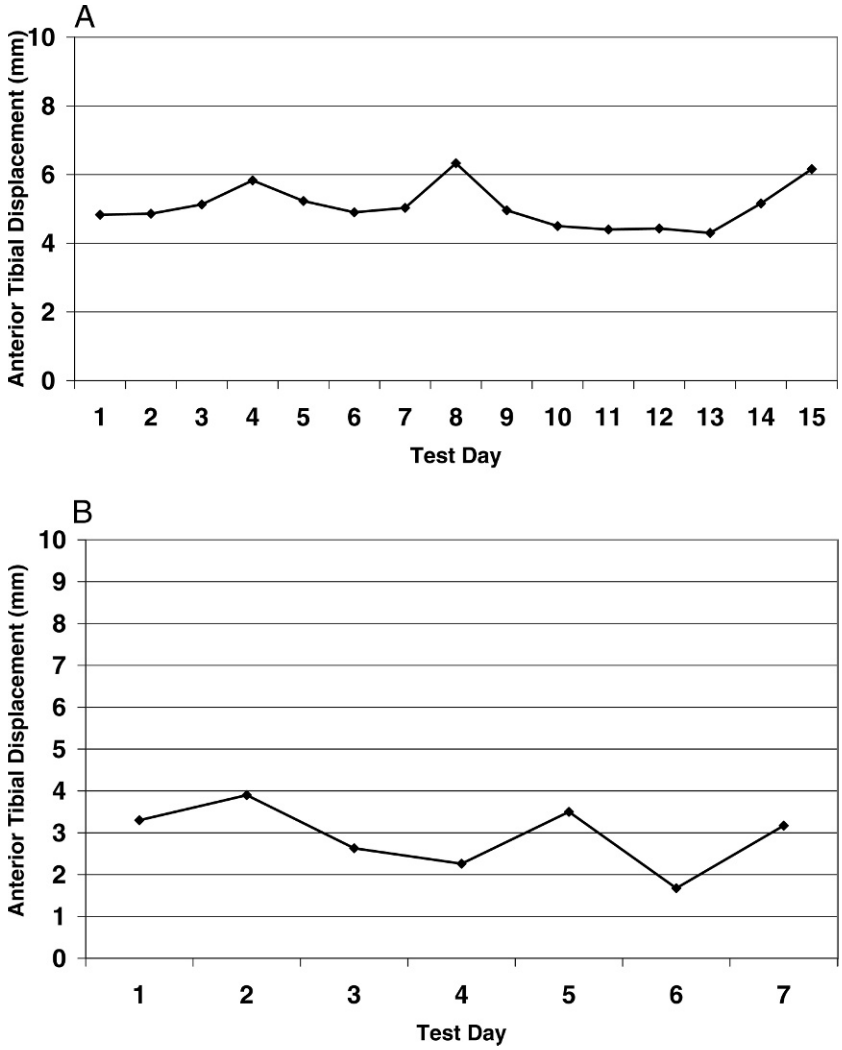

Figure 3. Representative mean plot of anterior displacement in a female participant (A) and a male participant (B) across serial testing with the Compu-KT arthrometer system at a load of 134 N.

participant did not rise and fall on the same days, resulting in large variations in the means. The correlations averaged across participants and grouped by sex are presented in Table 2. Additionally, Table 2 contains the results of the test of the differences for the correlations between the sexes. The average correlations ranged from −0.147 to 0.128 for the women and from −0.234 to −0.035 for the men. The tests of the differences between the correlations for the women and men indicated a difference in 1 of the calculated comparisons: ATD and progesterone ($P = .036$).

The results from the 4-day lag and 8-day lag correlations are presented in Tables 3a and 3b, respectively. The average correlation for the 4-day lag analysis for the variables in the women ranged from 0.059 to 0.153, and the average correlation for the 8-day lag analysis ranged from less than −0.001 to 0.143. Similarly for the men, the 4-day lag correlation average ranged from −0.400 to 0.043, and the 8-day lag correlation average ranged from −0.067 to 0.227. The test of the differences between the time-lagged correlations in the men and women showed no differences between the sexes (Tables 3a and 3b).

The means of the correlations in the analysis of the magnitude of change between consecutive data points are presented in Tables 4a and 4b. The mean correlation between the variables in the women ranged from −0.136 to 0.045 for the 4-day change and from −0.122 to 0.152 for the 8-day change. In the men, the average correlation for the 4-day change ranged from −0.156 to 0.077 and for the 8-day change ranged from −0.329 to 0.230. Again, the tests of the differences for the correlations in the men and women were not different between the sexes (Tables 4a and 4b).

DISCUSSION

Recently, several researchers have investigated the effects of sex hormones on ACL tissue[29,30,47,48] and ACL ruptures among women.[18,33–36] These authors addressed the presence of estrogen receptors on the ACL, the effect of estrogen on the strength of the ACL, the effect of estrogen on knee joint laxity, the risks of menstrual phase-dependent ACL injury, and neuromuscular implications of fluctuating estrogen concentrations. We undertook our study to determine the strength of the relationships between measures of sex hormone concentrations and motoneuron excitability and between these measures and ATD and to determine if these relationships differed between women and men.

Several aspects of knee joint laxity have been addressed in the literature, including the significance of the magnitude

Table 1. Daily Means (SD) of All Variables

Test Day	Maximum Hoffmann Reflex to Maximum M-Wave Ratio		Anterior Tibial Displacement (mm)		Estrogen (pg/mL)		Progesterone (pg/mL)	
	Women	Men	Women	Men	Women	Men	Women	Men
1	0.72 (1.13)	0.60 (0.19)	5.29 (1.90)	4.85 (1.33)	1.52 (1.30)	1.21 (1.67)	5.70 (5.18)	5.71 (5.19)
2	0.70 (0.15)	0.58 (0.20)	5.33 (2.08)	4.82 (1.45)	1.52 (1.49)	0.71 (1.20)	4.89 (5.65)	6.00 (4.26)
3	0.68 (0.16)	0.59 (0.22)	5.52 (2.23)	4.80 (1.39)	1.87 (1.31)	0.90 (1.37)	5.22 (7.27)	6.28 (4.37)
4	0.67 (0.16)	0.58 (0.22)	5.16 (2.14)	4.77 (1.77)	2.23 (1.29)	0.96 (1.37)	5.04 (4.96)	6.92 (5.15)
5	0.68 (0.17)	0.58 (0.21)	5.25 (2.26)	5.10 (1.63)	2.41 (1.83)	0.87 (1.36)	5.67 (6.37)	5.14 (4.82)
6	0.69 (0.16)	0.56 (0.20)	5.39 (2.21)	4.90 (1.77)	2.55 (1.94)	1.15 (2.06)	5.70 (7.86)	7.42 (5.65)
7	0.68 (0.17)	0.55 (0.21)	5.44 (1.95)	4.98 (1.83)	2.31 (1.62)	0.79 (1.45)	10.08 (11.47)	5.92 (4.80)
8	0.68 (0.17)		5.41 (1.98)		2.83 (2.22)		23.52 (35.11)	
9	0.67 (0.16)		5.62 (2.16)		2.96 (2.13)		39.33 (55.94)	
10	0.67 (0.16)		5.43 (2.07)		2.92 (2.13)		45.22 (58.08)	
11	0.68 (0.15)		5.72 (2.15)		3.60 (2.62)		57.74 (62.00)	
12	0.68 (0.15)		5.61 (1.78)		2.85 (2.19)		52.89 (65.28)	
13	0.68 (0.16)		5.57 (2.06)		2.47 (1.90)		28.64 (33.57)	
14	0.68 (0.15)		5.40 (2.06)		2.90 (2.10)		23.89 (28.59)	
15	0.69 (0.14)		5.44 (1.87)		2.88 (3.11)		10.38 (15.91)	

Table 2. Simple Correlations Across Participants and Grouped by Sex

	Correlation Mean				
	Women	Men	*t*	df	*P* Value
Anterior tibial displacement versus					
Estrogen concentration	−0.147	−0.114	−0.07	28	.947
Progesterone concentration	0.128	−0.172	2.17	37	.036[a]
Maximum Hoffmann reflex to maximum M-wave ratio versus					
Estrogen concentration	0.096	−0.035	0.26	28	.796
Progesterone concentration	−0.069	−0.234	1.65	37	.107

[a] $P < .05$.

of knee joint laxity,[32] laxity differences between the sexes,[32] and change in joint laxity across the menstrual cycle.[6,10,49] Our study was designed to determine if changes in ATD and motoneuron excitability corresponded with changes in hormone concentrations during the menstrual cycle. In a systematic review of anterior knee joint laxity and the menstrual cycle, Zazulak and colleagues[50] concluded that laxity may be related to hormonal changes. Their overarching conclusion was based on 3 studies that showed a connection between hormone concentrations and laxity. The authors' critique of these 3 studies concluded that 2 were performed early in this line of investigation and overall were controlled poorly. In our study, we found that, on average, the correlations between sex hormone concentrations and ATD were weak, with no differences between the correlations when men were compared with women except in the relationship between ATD and progesterone. These data are largely supported by previous findings in

Table 3a. Four-Day Lag Correlations of the Maximum Hoffmann Reflex to Maximum M-Wave Ratio and of Anterior Tibial Displacement

	Correlation Mean				
	Women	Men	*t*	df	*P* Value
Estrogen concentration versus					
Maximum Hoffmann reflex to maximum M-wave ratio	0.068	0.043	0.170	28	.866
Anterior tibial displacement	0.059	−0.043	0.508	28	.103
Progesterone concentration versus					
Maximum Hoffmann reflex to maximum M-wave ratio	0.086	−0.0173	1.870	35	.069
Anterior tibial displacement	0.153	−0.400	1.230	35	.192

Table 3b. Eight-Day Lag Correlations of the Maximum Hoffmann Reflex to Maximum M-Wave Ratio and of Anterior Tibial Displacement

	Correlation Mean				
	Women	Men	*t*	df	*P* Value
Estrogen concentration versus					
Maximum Hoffmann reflex to maximum M-wave ratio	−0.005	−0.018	0.957	28	.866
Anterior tibial displacement	−0.005	−0.017	0.072	28	.943
Progesterone concentration versus					
Maximum Hoffmann reflex to maximum M-wave ratio	0.143	0.227	−0.567	34	.574
Anterior tibial displacement	<-0.001	−0.067	0.414	34	.681

Table 4a. Means of the Correlations Calculated for the Magnitude of Change in 4-Day Hormone Concentrations

	Correlation Mean				
	Women	Men	*t*	df	*P* Value
Estrogen concentration change versus					
Maximum Hoffmann reflex to maximum M-wave ratio	−0.033	0.077	−0.737	29	0.467
Anterior tibial displacement value	−0.032	−0.089	0.426	29	0.673
Progesterone concentration change versus					
Maximum Hoffmann reflex to maximum M-wave ratio	−0.136	0.013	−0.912	35	0.368
Anterior tibial displacement value	0.045	−0.156	1.519	35	0.138

Table 4b. Means of the Correlations Calculated for the Magnitude of Change in 8-Day Hormone Concentrations

	Correlation Mean				
	Women	Men	*t*	df	*P* Value
Estrogen concentration change versus					
Maximum Hoffmann reflex to maximum M-wave ratio	−0.007	0.230	−1.373	29	.180
Anterior tibial displacement value	0.115	−0.120	1.127	29	.269
Progesterone concentration change versus					
Maximum Hoffmann reflex to maximum M-wave ratio	−0.122	−0.329	1.379	35	.177
Anterior tibial displacement value	0.152	−0.188	1.951	35	.059

which hormone fluctuations were not believed to influence knee laxity[6,12,51] but are in contrast to others.[8,10,32,52] Beynnon et al[31] evaluated the correlations between sex hormones and anterior-posterior knee laxity and stated that they found no "meaningful" correlations between hormone concentrations and knee joint laxity. In another study with similar methods to those of Beynnon et al[31] and us, Romani and colleagues[52] showed a negative correlation ($r = -0.70$) between estradiol concentration and ACL stiffness near ovulation when estrogen had peaked. However, during the luteal phase, when estrogen concentration is high for an extended period, they detected no relationship between estradiol and ACL stiffness. The authors could not adequately explain these interesting findings, ultimately concluding that the relationship may be a combined factor of receptor expression triggered by hormone levels.[53] Additionally, one of the clear differences between stiffness and laxity is that stiffness depends on the rate at which the force is applied to the ligament. Regarding the difference ($P = .036$) between the sexes in the correlations of ATD and progesterone, it is difficult to place much relevance in this finding because the mean correlations for both sexes were very weak (women = 0.128, men = −0.172). However, this finding does warrant additional consideration in future studies.

In clear contrast to our findings, Shultz et al[32] performed a study of serial arthrometer and hormone assessments and, based on a series of regression models, found data that supported the influence of sex hormones on the properties of the knee joint. They found that a combination of 3 hormones (testosterone, progesterone, and estrogen) with a time lag of 3 to 4.5 days explained up to 63% of the observed variance in knee laxity. The primary difference between their hormone data and ours is that they conducted an extensive analysis of the combined effect of the measured hormones. Their work was also somewhat exploratory in nature because they used several regression models to establish the greatest combined hormone effect at various time lags.

Neuromuscular variables have been assessed in the quest to understand the ACL injury discrepancies between women and men; however, to our knowledge, no authors have focused on mechanistic neurologic measures, such as the Hoffmann reflex. As early as the 1940s, the monosynaptic reflex was used as a tool for studying modulatory changes of the alpha motoneuron pool.[54] Alpha motoneurons are organized according to the muscles that they activate, and all of the motoneurons to a specific muscle constitute that muscle's "pool." We emphasize that, although the target motoneuron pool in our investigation was the soleus muscle rather than a muscle that directly controls the knee, the effect that hormones have on motoneuron excitability theoretically should not be limited to muscles acting on the knee. We did not intend to address local effects of estrogen on muscles acting at the knee or on the ACL, but we wanted to study the relationships of several variables from a multisystem perspective.

All the average correlations between the hormones and the H_{max}:M_{max} were near 0, suggesting that, as the hormones fluctuated, the reflex responses were not directly or indirectly proportional as a group. Because the issue of noncontact ACL injuries in women appears to be multifactorial, more attention should be given to the individual responses of the participants. Additionally, the use of a more mechanistic variable of spinal control, such as presynaptic inhibition, should be considered.

Since the early 1970s,[55,56] the effects of estrogen on neuron activity have been well established. Most investigations have involved the brain; however, estrogen receptors clearly exist in the brain and in the spinal cord, where they may have either a faciliatory or inhibitory effect. In a detailed comparative study, Shughrue et al[57] identified specific locations of both types of estrogen receptors (estrogen receptor-α and estrogen receptor-β) in the rat throughout the central nervous system. They reported that at least 1 type, and in many cases both types, of estrogen receptors were found in every laminae of the rat's spinal cord. Estrogen has been implicated as the cause of a higher incidence of migraine headaches,[58] depression,[41] and schizophrenia[40] and a lower pain tolerance[42] in women than in men. These conditions show estrogen's influence in both the brain and spinal cord. Information related to estrogen receptors of the spinal cord, specifically those located in laminae where the synapse of the monosynaptic loop is located, is of particular interest to our study.

The laboratory used to analyze hormone concentrations in the saliva of our participants also was used by Slauterbeck and colleagues[18] in their study of ACL injury rates. The ranges of estrogen concentrations and progesterone concentrations in our female participants were similar to the ranges that they reported.[18] The measurement of hormone concentrations in saliva has some attractive benefits and has been used in place of serum sampling for more than 20 years.[59] The primary benefit of saliva sampling over serum sampling is the ease of collection. We were concerned with our ability to recruit sufficient participants if we required venipuncture every other day for an entire month. Additionally, levels of estrogen in saliva have been shown to be a robust predictor of serum levels in other populations.[60]

Limitations

The limitations of our study are related mainly to the logistics of the labor-intensive data collection. We admit that the examiner's knowledge of where in the menstrual cycle each woman was at any given time had the potential to confound the results. We recommend that steps be taken to blind all examiners to the menstruation schedule of participants in future studies. Additionally, we would have preferred serum over saliva measurements of hormones but believed that the difficulty of repeated blood draws in the study group would have limited participation. One limitation related to our approach was the inability to determine any potential interaction effect of the hormones, as other researchers have reported.[32]

CONCLUSIONS

The solution to this complex puzzle remains elusive. With so many investigators reporting conflicting results, it will take a collection of well-controlled studies to finally answer the questions that we tried to address. It is possible, and perhaps even likely, that a hormonal factor is associated with this multifactorial problem, but the mechanism by which the hormones affect both the neural and muscular systems and connective tissue integrity need to be elucidated in future studies. Some other nervous system mediator may be interacting with the sex hormones to influence motor performance.

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