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Neuromuscular and biomechanical characteristics do not vary across the menstrual cycle

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Abstract Research examining the menstrual cycle and its relationship to ACL injury has focused on determining the incidence of ACL injury during the different phases of the menstrual cycle and assessing the changes in neuromuscular and biomechanical characteristics between these phases. Conflicting results warrant further investigation to determine if neuromuscular and biomechanical characteristics respond in a similar pattern to the fluctuating estradiol and progesterone. The purpose of this study was to determine if changes in the levels of estradiol and progesterone significantly altered fine motor coordination, postural stability, knee strength, and knee joint kinematics and kinetics between the menses, post-ovulatory, and mid-luteal phases of the menstrual cycle. Ten healthy and physically active females (Age: 21.4 ± 1.4 years, Height: 1.67 ± 0.06 m, Mass: 59.9 ± 7.4 kg), who did not use oral contraceptives, were recruited from the local university population. Single-leg postural stability, fine motor coordination, knee strength, knee biomechanics, and serum estradiol and progesterone were assessed at the menses, post-ovulatory, and mid-luteal phases of the menstrual cycle. Levels of estradiol were significantly higher during the post-ovulatory ($P = 0.016$) and mid-luteal phases ($P < 0.001$) compared to the menses phase. Levels of progesterone were significantly lower during the menses ($P < 0.001$) and post-ovulatory phases ($P < 0.001$) compared to the mid-luteal phase. No significant differences existed between phases of the menstrual cycle for fine motor coordination ($P = 0.474$), postural stability ($P = 0.707$), hamstring – quadriceps strength ratio at $60°\mathrm{s}^{-1}$ ($P = 0.748$) or $180°\mathrm{s}^{-1}$ ($P = 0.789$), knee flexion excursion ($P = 0.6$), knee valgus excursion ($P = 0.899$), peak proximal tibial anterior shear force ($P = 0.797$), flexion moment at peak proximal tibial anterior shear force ($P = 0.698$), or valgus moment at peak proximal tibial anterior shear force ($P = 0.924$). The results of the current study suggest neuromuscular and biomechanical characteristics are not influenced by estradiol and progesterone fluctuations. All neuromuscular and biomechanical characteristics remained invariable between testing sessions despite concentration changes in estradiol and progesterone.

Keywords Hormones · Knee · Female · Anterior cruciate ligament · Menstrual cycle

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Introduction

Research examining the menstrual cycle and its relationship to ACL injury has focused on two areas: incidence of ACL injury during the different phases of the menstrual cycle [19, 24, 27] and changes in neuromuscular and biomechanical characteristics between phases as a result of fluctuating hormonal levels. Specifically, previous research has suggested a higher incidence of ACL injury in female athletes during particular phases of the menstrual cycle. Approximately 22–40% of ACL injuries were documented to have occurred between 2 days pre-menses and 1–4 days post-menses (21% of total days for 28 day cycle) [19, 24, 27]. Additional research identifying variations in strength [7, 12], postural stability [12], knee joint laxity [11, 12, 23, 25], proprioception [9, 12], and performance [9] between phases has demonstrated conflicting results. Although these findings may have merit related to risk of noncontact ACL injury, the mechanism of increased injury rates during certain phases of the menstrual cycle and variability of neuromuscular and biomechanical characteristics between phases warrants further investigation.

Given the potential for greater ACL injury risk during certain phases of the menstrual cycle [19, 24, 27] and established differences in strength [1, 13, 16], proprioception [21], electromyographic activation patterns [17], and other biomechanical factors [3, 4, 6, 13, 16–18] between male and female athletes, it is possible that neuromuscular and biomechanical characteristics may be influenced by the fluctuating sex hormones between the phases of the menstrual cycle. The objective of this study was to identify differences in neuromuscular and biomechanical characteristics between the menses, post-ovulatory, and mid-luteal phases of the menstrual cycle. Specifically, we wanted to determine if fluctuating hormone levels of estradiol and progesterone significantly modulated fine motor coordination, postural stability, knee strength, and knee joint kinematics and kinetics. We hypothesized the specific neuromuscular and biomechanical characteristics would differ at the post-ovulatory and mid-luteal phases compared to the menses phase.

Methods

Subjects

Ten physically active females (Age: 21.4 ± 1.4 years, Height: 1.67 ± 0.06 m, Mass: 59.9 ± 7.4 kg) were recruited from the local university population. All subjects were eumenorrheic and ovulatory. Subjects provided written informed consent prior to participation in accordance with the University Institutional Review Board. Subjects attended six neuromuscular testing sessions over a 2-month-period (three sessions per month to coincide with the menses, post-ovulatory, and mid-luteal phases of the menstrual cycle). The first month of testing was used to familiarize the subjects with the required tasks and the second month to collect data for this study. All testing was performed on the dominant limb, which was defined by the preferred leg used to kick a ball.

Procedures

Subject screening

Potential subjects were screened for histories of injury, nutritional practices, menstrual dysfunction, thyroid dysfunction, and physical activity. Thyroid status was documented in all subjects using a serum level of triiodothyronine (T3), thyroxine (T4), and thyroid stimulating hormone (TSH). Screening blood samples were evaluated by an independent laboratory (University of Pittsburgh Clinical Laboratory System, Inc, Pittsburgh, PA, USA). A mid-luteal (approximately day 21) serum progesterone level (10 ng/mL) was used to document eumenorrhea. Subjects with a mid-luteal progesterone level less than 10 ng/ml, history of serious knee injury or other lower extremity injury within the prior 6 months, previous or current eating disorder, or previous or current menstrual dysfunction were excluded from participating.

Menstrual phase assessment

Subjects reported for neuromuscular testing and blood assay testing during each of the three phases of the menstrual cycle during a two-month-period (month 1: testing familiarization; month 2: data collection and analysis). Phases of the menstrual cycle were defined based on the first day of menses. Subjects reported for initial neuromuscular testing at day three of the menstrual cycle (menses). Subjects collected daily urine samples with an over-the-counter ovulation detection kit (First Response, Ovulation Prediction Kit) starting at day 10 and continuing until a positive test was elicited (post-ovulatory). Within 24–36 h of a positive ovulation test, subjects reported for a second neuromuscular testing session. A third testing session was conducted 7 days (mid-luteal) following a positive ovulation test (approximately day 21–23 of the menstrual cycle). The days of testing were selected to determine if changes in hormonal levels would contribute to corresponding variations in neuromuscular characteristics across the menstrual cycle. Specifically, the determined time points coincided with low levels of estradiol and progesterone (menses), elevated estradiol and low progesterone (post-ovulatory), and elevated estradiol and progesterone (mid-luteal). Blood samples were collected by a certified phlebotomist at the completion of each neuromuscular testing session to assess hormone levels and analyzed for serum levels of estradiol and progesterone. Estradiol and progesterone levels were measured in duplicate in each specimen using a radioimmunoassay (Diagnostic Products Corporation, Los Angeles, CA, USA). All samples were batched and analyzed in the same run to reduce variability. Within assay variability was approximately 7% at an estradiol concentration of 60 pg/ml and 6% at a progesterone concentration of 1.6 nM/L.

Postural stability testing protocol

Postural stability was assessed using a Kistler force plate (Kistler Corporation, Amherst, NY, USA) at a sampling frequency of 100 Hz. Each subject was asked to complete a single-leg standing balance test (barefooted) under an eyes-open condition Three, 10 s trials were collected. During testing, subjects were instructed to keep a straight knee position with feet shoulder width apart and hands on hips. Subjects were directed to focus on an eye-level target located approximately 2 m in front and instructed to regain balance as quickly as possible and continue the trial if they touched down on the force plate. If the subject touched down off the force plate, the trial was discarded and replaced with a subsequent trial. Test–retest reliability of balance data previously collected in our laboratory yielded an Intraclass Correlation Coefficient (ICC) of 0.775–0.8567 and Standard Error of Measurement (SEM) of 0.187–0.328 N.

Fine motor control

Fine motor control was assessed using the Crawford Small Parts Dexterity Test. This test was a timed assessment of the subject's hand-eye coordination and fine motor dexterity. Using a wooden board with separate wells for pins, collars, and screws, the subjects performed two sets of activities for which the total time to complete each test was recorded. The two tests were performed as follows: (1) subjects inserted small pins into close fitting holes in a plate and then placed small collars over the protruding pins using tweezers and (2) subjects placed small screws into threaded holes in the plate and tightened with a screwdriver until striking the metal tray beneath. A composite fine motor coordination score was calculated by summing the times to complete each task. Test–retest reliability of strength data previously collected in our laboratory yielded an ICC of 0.7920–0.8158 and SEM of 0.59–0.74 min.

Strength assessment

Isokinetic knee strength data of the dominant limb were collected with the Biodex System 3 Multi-Joint Testing and Rehabilitation System (Biodex Medical Inc., Shirley, NY, USA). Torque values were automatically adjusted for gravity by the Biodex Advantage Software v.3.0 (Biodex Medical Inc., Shirley, NY, USA).

Subjects were seated in the Biodex chair and secured using thigh, pelvic, and torso straps to minimize accessory and compensatory movements during the knee strength testing. The lateral femoral epicondyle of the dominant limb was aligned with the dynamometer's axis of rotation. Subject setup was standardized and recorded to allow for consistent positioning across the repeated testing sessions. Two submaximal and two maximal practice repetitions were provided to ensure unrestricted movement, comfort, and familiarization. Subjects performed reciprocal quadriceps and hamstring concentric contractions at $60°s^{-1}$ (five repetitions) and $180°s^{-1}$ (ten repetitions). Average peak torque to body weight (BW) was recorded for each direction with the ratio of hamstrings to quadriceps strength calculated for each speed. Test–retest reliability of strength data previously collected in our laboratory yielded an ICC of 0.809–0.9112 and SEM of 0.07–0.15 BW.

Functional assessment

Raw coordinate and force data were collected using the Peak Motus 3D Motion Analysis System (Software Version 6.03, ViconPeak, Inc., Centennial, CO, USA) interfaced with six high-speed optical cameras (Pulnix Industrial Product Division, Sunnyvale, CA, USA) and a force plate (Kistler Instrument, Corp., Amherst, NY, USA). Coordinate and force data were collected at 120 and 1,200 Hz, respectively. Linear and circumferential anthropometric measurements of the dominant leg were recorded for each subject. Spherical reflective markers (diameter 0.025 m) were placed at designated anatomical landmarks as described by Vaughn et al. [26]. Subjects were given a verbal description and visual demonstration of the single-leg stop jump tasks. Each subject performed a series of practice trials until proper technique was demonstrated and followed with five test trials. The technique for the stop jump task consisted of the following: (1) an initial starting point measured as 40% of the subject's height from the middle of the force plates, (2) a forward jump to the force plates with a single leg landing, and (3) a vertical jump for maximal height following. The practice trials and test trials were standardized across all testing sessions.

Data reduction

Ground reaction force data from the postural stability test were exported to a custom Matlab program (Release 12, The MathWorks, Natick, MA, USA) to calculate the standard deviation of the force data in the anterioposterior, mediolateral, and vertical directions. A composite postural stability score was then calculated based on the resultant standard deviation force of the three force directions.

Raw analog data from the force plates were used to calculate the ground reaction force data for each jump trial. The raw coordinate data were filtered using an optimized cut-off frequency. The ground reaction forces were filtered using a 4th order Butterworth filter with a cut-off frequency of 100 Hz. Anatomical joint angles were calculated in order to examine clinically applicable angles. Total excursion variables were calculated as the total range of motion between initial contact and peak knee flexion and initial contact and peak knee valgus. An inverse dynamics procedure was used to calculate the joint kinetic data during the stop-jump tasks. The joint resultant moment and forces calculated using this procedure were the estimated external moments and forces and were based on the ground reaction forces and segment inertial forces. Joint resultant forces were normalized to body weight and joint resultant moments were normalized to a product of body weight and height. All of the kinematic and kinetic calculations were performed in the Kinecalc module of the Peak Motus software package. Joint kinematic data, joint kinetic data, and ground reaction force data from the functional assessment were exported to a custom Matlab program for identification of the variables of interest: proximal tibial anterior shear force, knee flexion/extension moment, knee valgus/varus moment, knee flexion excursion, and knee valgus excursion were analyzed. Data were averaged across five trials.

Statistical analysis

SPSS 11.5 (SPSS, Inc., Chicago, IL, USA) was used for all statistical procedures. Separate one-way repeated measures ANOVA were used to determine differences in the dependent variables of postural stability, fine motor coordination, knee strength, and knee kinematic and kinetic data. A Bonferroni post hoc analysis was performed when applicable. Statistical significance was determined a priori at $P < 0.05$ for all procedures.

Results

Estradiol and progesterone levels and patterns were consistent with those expected between the phases of the menstrual cycle identified in the current study. Levels of estradiol were significantly higher during the post-ovulatory ($P = 0.016$) and mid-luteal phases ($P < 0.001$) compared to the menses phase. Levels of progesterone were significantly lower during the menses ($P < 0.001$) and post-ovulatory phases ($P < 0.001$) compared to the mid-luteal phase. Estradiol and progesterone data are provided in Table 1.

No significant differences existed between phases of the menstrual cycle for fine motor coordination ($P = 0.474$), postural stability ($P = 0.707$), hamstring–quadriceps strength ratio at $60°\mathrm{s}^{-1}$ ($P = 0.748$) or $180°\mathrm{s}^{-1}$ ($P = 0.789$), knee flexion excursion ($P = 0.6$), knee valgus excursion ($P = 0.899$), peak proximal tibial anterior shear force ($P = 0.797$), flexion moment at peak proximal tibial anterior shear force ($P = 0.698$), or valgus moment at peak proximal tibial anterior shear force ($P = 0.924$). Dependent variable data are provided in Table 1.

Discussion

The objective of this study was to identify differences in neuromuscular and biomechanical characteristics between the menses, post-ovulatory, and mid-luteal phases of the menstrual cycle. Contrary to our hypotheses, no significant differences were demonstrated between phases of the menstrual cycle for any of the dependent variables. The results of this study suggest neuromuscular and biomechanical characteristics are not influenced by the variation in hormonal levels between phases of the menstrual cycle.

Normal levels of estradiol and progesterone were identified within each of the appropriate phases of the menstrual cycle and were consistent with other research [2, 9, 22]. Based on the assumption that not all subjects would present with the average 28 day cycle, testing was timed to ovulation to adjust for individual variation in cycle length. Additionally, ovulation was confirmed in all subjects using a screening mid-luteal

Table 1 Dependent variables across the three phases of the menstrual cycle

Variable	Menses	Post-ovulatory	Mid-luteal
Estradiol (pg/ml)	41.4 ± 5.4	136.6 ± 84.1*	122.0 ± 33.6*
Progesterone (ng/ml)	0.56 ± 0.14**	2.75 ± 1.06**	12.1 ± 3.89
Fine motor coordination (min)	13.47 ± 1.9	12.96 ± 1.95	13.26 ± 1.85
Postural stability (N)	3.31 ± 0.84	3.16 ± 0.81	3.35 ± 1.03
Hamstrings:quadriceps strength ratio (60°s^{-1})	0.508 ± 0.05	0.512 ± 0.053	0.518 ± 0.067
Hamstrings:quadriceps strength ratio (180°s^{-1})	0.56 ± 0.064	0.572 ± 0.067	0.579 ± 0.082
Knee flexion excursion (°)	30.2 ± 12.6	32.2 ± 8.1	29.1 ± 11.4
Knee valgus excursion (°)	–4.4 ± 7.5	–5.2 ± 6.9	–5.8 ± 5.1
Peak proximal tibial anterior shear force (NM/BW)	1.25 ± 0.18	1.33 ± 0.4	1.3 ± 0.3
Flexion moment at peak anterior tibial shear force (NM/((BW × HT))	0.154 ± 0.03	0.174 ± 0.064	0.167 ± 0.052
Valgus Moment at Peak Anterior Tibial Shear Force (NM/((BW × HT))	0.053 ± 0.033	0.051 ± 0.028	0.048 ± 0.037

* Significantly higher than menses phase

** Significantly lower than mid-luteal phase

progesterone threshold level of 10 ng/ml and subjects with confounding factors, such as luteal insufficiency and thyroid conditions, were excluded from participating. One subject was withdrawn from the study as a result of an anovulatory cycle (no positive ovulation between days 10–17, despite a documented mid-luteal serum progesterone level greater than 10 ng/mL to confirm eumenorrhea.

No difference in postural stability was demonstrated between phases of the menstrual cycle. This finding is supported by Hertel et al. [12] and is in contrast to Friden et al. [8] and Friden et al. [10] who reported impaired postural stability during the mid-luteal phase and suggested this as a possible cause for the higher incidence of ACL injury in the luteal phase. The findings of Friden et al. [8] and Friden et al. [10], may have included other confounding hormonal factors as the subjects included premenstrual symptoms. Given the sensitivity of the postural stability measure, this variable was expected to have the most likelihood of being influenced by the menstrual cycle.

Fine motor coordination was measured to quantify concentration and neuromuscular coordination on a smaller scale than that of gross biomechanical tasks. This test is typically used to assess finger coordination following neurological pathology, however tracking of fine motor coordination may indicate significant changes in dextrity as a result of hormonal fluctuations. No significant differences were demonstrated for fine motor coordination and we are unaware of any previously published research assessing this variable throughout the menstrual cycle. Posthuma et al. [20] reported better coordination in the late luteal phase compared to the follicular phase of non-premenstrual symptomatic subjects with worse coordination in females with pre-menstrual symptoms. Like the postural stability measurement, other confounding hormonal factors may have contributed to the diminished coordination in the late luteal phase data for the premenstrual symptomatic subjects. Further research is needed to verify the sensitivity of this measure to recognize hormonal-related differences particularly in asymptomatic females.

Quadriceps and hamstrings strength deficits in female athletes have been postulated as potential contributor to ACL injuries as the lower extremity is unable to eccentrically control the lowering of the body resulting in injurious knee positions [16]. These dangerous landing positions and loading conditions are often the result of inefficient neuromuscular control and the inability to effectively dissipate dangerous landing forces with excessive resultant forces being transmitted across the knee [3, 17]. Lephart et al. [16] contended that strength differences may contribute to various biomechanical differences such as an abrupt stiffening of the knee upon landing. Appropriate quadriceps strength controls the deceleration of the knee over a greater range of motion during functional tasks. No significant strength differences were demonstrated for isokinetic testing at 60 or 180°s^{-1}. The strength testing results in the current study are consistent with Elliot et al. [5], Friden et al. [7], Janse de Jonge et al. [14], and Hertel et al. [12].

It was hypothesized that the fluctuating hormonal levels would influence competitive performance by altering the neuromuscular processes associated with dynamic tasks [15]. The single-leg stop jump task was utilized as it was believed that this functional activity best replicates the mechanism responsible for increased ACL injury risk. That is, the combined sharp deceleration and quick change in direction results in a forceful knee valgus moment that is typically associated with non-contact ACL injuries. Knee joint kinematics and kinetics did not vary between phases of the menstrual cycle. No research that we are aware of has examined knee joint biomechanics between the phases of the menstrual cycle, however Friden et al. [9] demonstrated improved coordination during the post-ovulatory phase, while Friden et al. [7] reported no variance in performance of a single-leg hop test. The biomechanical results of the current study are further supported by the current strength data.

Limitations to the current study include a small sample size and the resulting low power (0.1–0.16) associated with each variable. Based on the effect size differences for each variable (0.04–0.20), it is unlikely that significant differences would have been demonstrated with a larger sample. A post hoc sample size estimate based on the current data with a statistical significance level of 0.05 and power of 0.90 resulted in 2–11 subjects for all variables with the exception of postural stability (36 subjects), hamstrings:quadriceps strength ratio at 60°s^{-1} (14 subjects), and valgus moment at peak proximal tibial anterior shear force (19 subjects). Subjects were tested three times throughout the menstrual cycle at time points deliberately chosen to coincide with varying levels of estradiol and progesterone. Despite testing the subjects at the identified time points relative to ovulation, additional testing between the menses and post-ovulatory phases, early luteal phase, and late luteal phase may account for intersubject variability in fluctuating hormonal patterns or delayed response patterns [23].

The results of the current study suggest that neuromuscular and biomechanical characteristics are not influenced by estradiol and progesterone fluctuations. All measured characteristics remained unchanged between testing sessions despite concentration changes in estradiol and progesterone. The subjects studied in the current project possessed normal menstrual cycles in length and ovulation, and the results should not be extended to females with menstrual disorders or unrecognized menstrual dysfunction. The current study also measured changes in peripheral variables and future research should consider central factors as well as investigate similar hypotheses in females with known menstrual disorders.

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