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Lee A. Weidauer

Mary Beth Zwart

Jeffrey A. Clapper

Joe Albert

Matthew Vukovich

See next page for additional authors

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Authors

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Original Article

Neuromuscular performance changes throughout the menstrual cycle in physically active females

Lee Weidauer[1,2], Mary Beth Zwart[2], Jeffrey Clapper[3], Joe Albert[2], Matt Vukovich[2], Bonny Specker[1]

[1]Ethel Austin Martin Program in Human Nutrition, South Dakota State University, Brookings, United States of America; [2]Health and Nutritional Sciences Department, South Dakota State University, Brookings, United States of America; [3]Animal Science Department, South Dakota State University, Brookings, United States of America

Abstract

Objectives: To determine changes in neuromuscular performance throughout the menstrual cycle in females aged 18-25. **Methods**: Fifty physically active college females (25 on oral contraceptives (OC)) were recruited to participate. Data collection visits coincided with early-follicular (Fp), ovulatory (Op), and the mid-luteal (Lp) phases. Isokinetic peak torque at the knee (IPT) was measured at 60°/sec, 180°/sec, and 300°/sec. Grip force was measured using a handheld dynamometer. Plasma estradiol and progesterone confirmed menstrual cycle and serum relaxin was screened as a potential covariate. **Results**: Grip strength was lower during Fp (30.1±0.7kg) than during Op (31.5±0.7 kg, p=0.003) and Lp (32.6±0.7 kg, p<0.001). IPT at 60°/sec was lower during Fp (83±14 nM) than during the Op (86±15 nM, p=0.02). IPTs at 180°/sec and 300°/sec were lower during Fp than Op and Lp (180°:54±10 vs. 58±10 and 61±11 nM [both, p<0.001]; 300°: 43±9 vs. 46±9 and 47±9 nM [both<0.001]. The OC group-by-phase interaction was not significant for any of the outcomes. **Conclusions**: Results indicate that muscular performance is diminished during Fp and the lack of group-by-phase interaction indicates that this effect is not hormone-related. These data indicate that females may be at a greater risk of injury due to decreased strength during Fp than other phases of their cycle.

Keywords: ACL, Female, Grip, Isokinetic, Menstrual

Introduction

Lower extremity injuries are a serious concern in the military, athletic, and other physically active populations[1-3]. Anterior cruciate ligament (ACL) injuries are of particular interest because of the significant loss of training time and potential for long-term complications such as post-traumatic osteoarthritis[4]. When controlling for competitive level, female athletes appear to be at a greater risk for ACL injuries than male athletes[5]. Ireland et al. reported that female basketball players tear their ACL at almost three times the rate of men[6]. The discrepancy may be even larger in military training with one study reporting a relative risk of 9.74 per 1000 athlete-exposures for ACL injury for females compared to males[7]. These discrepancies necessitate further investigation into potential mechanisms for the greater incidence of ACL injuries in females with the goal of developing sex-specific prevention protocols.

While many mechanisms have been suggested, prior studies indicate that the increased risk is a function of musculoskeletal and hormonal risk factors[8-11]. Muscle force production is a key factor in reducing the risk of ACL injuries due to the stabilizing role of the quadriceps and hamstrings at the knee. Females have been reported to have weaker hamstrings than males[12] and a lower hamstring-to-quadriceps strength ratio[8] may increase their risk of non-contact knee injuries[13]. Additionally, greater knee joint laxity in females has been suggested as a potential reason for the disparity in injury rates between males and females[12,14]. Greater knee joint laxity would theoretically result in excessive motion

about the joint, thereby increasing the risk that forces on the ligament may exceed its yield point. However, several studies with small sample sizes and inconsistent methodology have investigated changes in knee joint laxity throughout the menstrual cycle with different studies indicating an association between knee joint laxity and menstrual cycle phases and some studies showing no association[15-18].

Fluctuations in female sex hormones, combined with the proposed relationship of these hormones to joint laxity[6,9], suggest that injury rates may fluctuate based on hormonal changes that occur throughout the menstrual cycle. Prior studies have reported that estrogen receptors are present in human ACL and skeletal muscle tissue and that fibroblast metabolism, myoblast growth, and collagen synthesis are all altered in the presence of estrogen[19-21]. The specific effects of estrogen on skeletal muscle have been studied extensively in post-menopausal women with one large meta-analysis reporting a beneficial effect of estrogen-based hormone replacement therapy on muscle strength[22]. While the underlying mechanism is not well understood, these studies suggest that as estrogen fluctuates throughout the menstrual cycle so might muscle force production and joint laxity. If this is the case, hormonal contraception could be associated with injuries by limiting the hormonal fluctuations and subsequent fluctuations in joint laxity and muscle function that occur during the cycle[23]. This is supported by previous studies investigating the association between ACL injury risk and contraceptive use that have indicated a protective effect of oral contraceptives on injury risk[24,25].

Relaxin is a hormone that is responsible for connective tissue changes late in pregnancy to accommodate childbirth. Due to its effect of reducing stiffness of tendons and ligaments, it is hypothesized that an increase in relaxin concentration could result in a decrease of force production as well as increased joint laxity. With the presence of relaxin specific binding sites on connective tissue in females, the mechanism exists for relaxin to act on these tissues[26,27]. This theoretical framework is supported by the results of a longitudinal investigation that found that an increase in serum relaxin concentration over 6 pg/mL was associated with a fourfold increase in ACL tears[28].

Previous studies have suggested that a potential relationship may exist between menstrual cycle phase and ACL injury risk; however, the results of these studies are inconclusive with injury risk being reported as highest during each of the three phases in at least one study[9,29-31]. While extensive progress has been made in the area of sex differences in the risk of ACL injuries, much of the data have been inconclusive. The purpose of this study was to investigate changes in muscular strength and stamina, and joint laxity throughout the menstrual cycle and to determine how oral contraceptive (OC) use alters these findings. We hypothesized that changes in isokinetic peak torque, grip strength, and joint laxity over the menstrual cycle phases would be greater in the non-OC group than the OC group due to greater variablitily in hormone concentrations.

Methods

Participant information

Physically active females aged 18 to 25 years old (n=50) were recruited from South Dakota State University through on-campus advertising and through meetings with classes in the Health and Nutritional Sciences Department. Twenty-five females who were taking OCs (monophasic) and twenty-five women that were not taking OC were recruited. Three participants in the non-OC group who did not ovulate, and one participant in the OC group who was missing hormone measurements, were excluded from all analyses. The South Dakota State University Institutional Review Board approved this protocol and informed consent was obtained from all participants prior to the study. On the day of the initial visit, participants completed a physical activity readiness screening (PAR-Q) and filled out a health history questionnaire that included the number of days that they perform at least 30 minutes of structured physical activity per week.

Inclusion and exclusion criteria

An initial questionnaire was administered to determine whether the menstrual cycle of potential participants was regular (every 21-35 days) and only those individuals with a regular menstrual cycle were invited to participate. In addition to cycles occurring every 21-35 days, menstrual regularity also included that participants had experienced menstrual bleeding in each of the past 3 months. For the OC group, only women who had been taking a monophasic OC for a minimum of 3 months prior to the study were invited to participate. Participants were excluded if they were using alternative contraception methods including vaginal rings, intrauterine devices, or injections. Participants were excluded if they had suffered any injury to their lower extremity in the past 6 months or had a previous ACL injury.

Visit schedule and bias reduction

Participants completed three visits that were designed to coincide with the early-follicular, ovulatory, and mid-luteal phases of the menstrual cycle (Table 1). All participants were asked to contact the study coordinator on the first day of their menstrual cycle to schedule an appointment for their first testing session. This visit took place within 72 hours of the onset of menses. Participants who were taking OCs had their subsequent visit on cycle day 14 to 16 and their final visit 1 week after their second visit. Participants who were not taking OCs were asked to take a home ovulation test every day beginning at day 7 which indicated when the luteinizing hormone (LH) surge occurred prior to ovulation. When a positive test occurred, the participants were asked to contact the study coordinator and set up a visit within 48 hours of the positive test. The final visit took place 7 days after the ovulation visit.

In order to decrease the effect of diurnal variability of hormone concentrations on our results, all study visits were scheduled in the morning and all follow-up visits were

Figure 1. Positioning of the participant for isokinetic testing on the Biodex System 4 ergometer.

scheduled within 2 hours of the time that the early-follicular phase visit was performed. Additionally, participants were asked to refrain from moderate or vigorous physical activity for 24 hours prior to the visit. Participants completed a 24-hour diet record using the ASA 24 (NIH, National Cancer Institute, Bethesda, MD) prior to each visit to ensure that dietary factors that may influence grip strength or isokinetic torque production were not different among visits. Finally, participants were asked to complete a menstrual symptom questionnaire at each visit to capture symptoms they may experience that could be attributed to their menstrual cycle. The instrument asked about musculoskeletal as well as psychological symptoms that may occur throughout the menstrual cycle.

Anthropometrics

Anthropometric measurements were taken in duplicate. Height without shoes was measured to the nearest 0.5 cm using a portable stadiometer (Seca Model 225). Weight was measured to the nearest 0.1 kg using a digital scale (Seca Model 770) with the participant wearing light clothing. Height and weight were screened as covariates due to the potential effect of body physique on muscle torque production.

Body composition

Dual-energy x-ray absorptiometry (DXA) scans were completed using a Hologic Discovery-A scanner (Apex Softward v.5.6, Hologic Inc., Bedford, MA). Our measurements of lean and fat mass are precise with percent coefficients of

variation of 0.4% and 1.3%, respectively. Lean and fat mass may be correlated with measures obtained from Biodex testing and therefore were considered as potential covariates.

Grip strength

Grip strength of the dominant side was measured using the digital Grip-D grip strength dynamometer (Takei Scientific Instruments, Niigata-City, Japan). We adjusted the apparatus to the proper hand size for each participant. The participant then held the dynamometer with their arm relaxed and extended downward and squeezed the instrument as hard as possible for three seconds. This test was repeated two more times and the highest reading of the three trials was recorded. Data from a previously performed reliability study in our lab indicates that no significant learning effect is present when three testing sessions are performed over the course of 30 days. The coefficient of variation for grip strength among the three visits was 4.4%.

Isokinetic testing

Isokinetic testing was performed using a Biodex System 4 (Biodex Medical Systems, Shirley, NY). Participants were seated with their shoulders and waist strapped to the chair (Figure 1). Following the manufacturer's recommendation, the dynamometer was oriented at 90° with a 0° tilt. The seat was set at 85° tilt with the axis of rotation at the knee set to pass through the lateral femoral condyle in a sagittal plane. The participant's distal lower leg was secured using Velcro strap positioned immediately proximal to the medial

Table 1. Data collected at each visit.

Procedure	Early Follicular	Ovulation	Mid Luteal
Informed Consent	X		
Health History Questionnaire	X		
Menstrual Symptom Questionnaires	X	X	X
Body Composition	X		
Anthropometrics	X		
Venipuncture	X	X	X
Isokinetic Testing	X	X	X
Laxity Testing	X	X	X
Fatigue Protocol	X	X	X

malleolus. Prior to beginning the testing, the participant was instructed to grab each of the side handles for support. Once the participant was properly positioned, they went through a series of 5 to 7 familiarization repetitions to get accustomed to the machine. The first 3 to 5 repetitions were done at very low effort with the final 2 repetitions being performed at 100% effort. Familiarization repetitions were performed at every testing session to ensure that participants were tested similarly across all visits. The performance trial consisted of 5 isokinetic knee extension and knee flexion repetitions at 60°/second, 5 repetitions at 180°/second, and 5 repetitions at 300°/second. Each series of repetitions were separated by a one-minute rest period. Standardized feedback was given to every participant reminding them to complete the testing with maximal efforts. This feedback was given during the flexion phase of repetition two. The Biodex has been reported to be a reliable tool for measuring isokinetic torque production up to 300°/second (ICC=0.99, CV<2%)[32]. In addition to peak torque measurements, isokinetic testing was used to determine the knee flexor-to-knee extensor ratio. Data from a previously performed reliability study in our lab indicate that no effect of study visit order when three testing sessions were performed over the course of 30 days. The coefficients of variation among the three study visits at 60°, 180°, and 300°/sec were 6.8%, 5.8% and 6.2%, respectively (Supplemental Table 1).

Fatigue protocol

A fatigue protocol was utilized to examine whether or not individuals fatigued at a different rate at different phases of the menstrual cycle. The fatigue test was performed a minimum of 30 minutes after the initial isokinetic testing and consisted of participants performing a number of repeated maximal leg extension and flexion movements at 180° per second using the Biodex. The participant was considered "fatigued" when they performed three consecutive repetitions in which their peak torque in both extension and flexion were less than 50% of the peak torque measured during the non-fatigued isokinetic testing.

Knee joint laxity

Knee joint laxity was measured using the KT 1000 knee arthrometer with the participant lying in a comfortable supine position. An adjustable thigh support platform was placed under both legs just proximal to the popliteal space. A foot support platform was placed under both feet with the patient's feet located in a position that allows the knee to be in a neutral position. All of these tests were done prior to (rested) and immediately following the Biodex testing (fatigued) to assess the effect of thigh fatigue on knee joint laxity. The same certified athletic trainer performed all knee joint laxity measurements. A reliability study was performed prior to the start of the study and a percent coefficient of variation of 4.3% was calculated for this examiner.

Blood collection procedure

Blood was collected by a trained phlebotomist prior to any physical measurements taking place. The participant was placed in a seated position with their feet flat on the floor and their arm lying on the collection table. The phlebotomist used a BD Vacutainer blood collection kit (Becton, Dickinson and Company, Franklin Lakes, NJ) to collect the sample from a vein in the antecubital region. A total of 17 ml of blood was collected into a 10ml serum separation tube and a 7 ml tube with K3 EDTA additive.

Estradiol-17

Plasma concentrations of estradiol (E2) were determined in duplicate by radioimmunoassay (RIA). Estradiol-17 (E8875; Sigma Life Science, St. Louis, MO) was the standard and radioiodinated E2 (#07138228; MP Biomedicals, Solon, OH) was the tracer. Antisera (GDN#244 anti-estradiol-17 6-BSA; Fort Collins, CO) was used at a dilution of 1:425,000. Sera (250-L) was extracted with a 4-mL volume of methyl tert-butyl ether. Recovery of [^{125}I]estradiol-17 added to plasma before extraction averaged 96±2%. Inhibition curves of increasing amounts of sample were parallel to standard curves. Intra- and inter-assay coefficients of variation were

Table 2. Participant characteristics.

	Non-OC (n=22)[a]	OC (n=24)[b]	P-Value
Height (cm)	167.0 ± 6.5	169.9 ± 7.0	NS
Lean Mass (kg)	45.3 ± 4.8	45.1 ± 5.9	NS
Fat Mass (kg)	18.5 ± 5.6	20.6 ± 6.8	NS
Age (years)	20 ± 1	20 ± 1	NS
Phys. Act. (day/wk)	5.0 ± 1.5	4.6 ± 1.5	NS

Data are presented as mean ± SD. [a]3 participants did not ovulate and were excluded from all analyses. [b]1 participant did not have complete data and was excluded from all analyses.
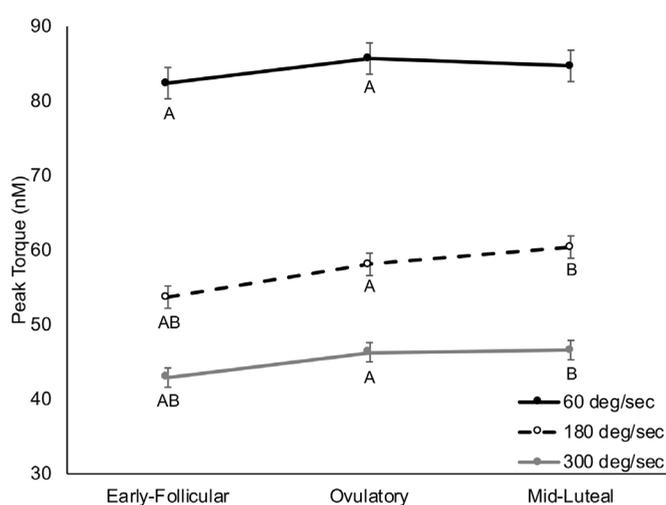
Figure 2. Marginal mean peak torque at 60°, 180°, and 300° per second (marginal mean ± SE). Data are marginal means after adjusting for contraception group (not significant), lean mass, and fat mass. Data were compared within different test speeds using pairwise comparisons using a Bonferroni adjustment.

12.8% and 12.6%, respectively. Sensitivity of the assay was 0.4 pg/tube.

Progesterone

Plasma concentrations of progesterone were determined in duplicate by RIA. Progesterone (P0130; Sigma Life Science; St. Louis, MO) was the standard and radioiodinated progesterone (#07-170126; MP Biomedicals, Solon, OH) was used as the tracer. Antisera (#111.2C7.3; Enzo Life Sciences, Farmingdale, NY) was used at a dilution of 1:700,000. Inhibition curves of increasing amounts of sample were parallel to standard curves. Intra-assay coefficient of variation was 8.0%. Sensitivity of the assay was 0.10 ng/tube.

Relaxin

Plasma concentrations of relaxin were determined in duplicate by RIA using the Relaxin 2 kit (RK-035-62,

Phoenix Pharmaceutical, Inc, Burlington, CA) according to the manufacturer's directions. Inhibition curves of increasing amounts of sample were parallel to standard curves. Intra-assay coefficient of variation was 5.9%. Sensitivity of the assay was 7.8 pg/tube.

Statistical analysis

Student's t-tests were used to compare the baseline characteristics of the OC and non-OC groups. Linear mixed effects models were used to determine the effect of menstrual cycle phase on dependent variables while adjusting for covariates. Dependent variables included grip strength, isokinetic peak torque, repetitions to fatigue, and knee joint laxity. Independent variables that were tested included the log of estrogen and relaxin concentrations, menstrual cycle phase, and OC use. Covariates that were screened included menstrual symptoms, lean mass, fat mass, age, height, physical activity days, caloric intake,

Table 3. Menstrual symptoms by phase and group.

	Follicular		Ovulation		Luteal	
	NOC	OC	NOC	OC	NOC	OC
I feel more irritable than normal*	33%	21%	5%	0%	10%	4%
I am experiencing menstrual cramps*	38%	54%	0%	8%	0%	4%
I am feeling more depressed than normal	10%	4%	0%	0%	0%	0%
I am experiencing more stomach pain than normal*	33%	21%	5%	0%	10%	13%
I get tired more easily*	57%	33%	10%	4%	10%	21%
I am taking a prescription for menstrual pain	0%	4%	0%	4%	0%	4%
I am feeling weak or dizzy	0%	8%	0%	0%	10%	0%
I am feeling tense or nervous	5%	13%	5%	8%	19%	8%
I am experiencing diarrhea	14%	0%	0%	0%	5%	0%
I am experiencing backaches or lower back pain*	33%	33%	19%	8%	19%	8%
I am taking OTC medication for pain*	10%	13%	0%	0%	0%	0%
I am experiencing pain in my breasts	5%	13%	0%	0%	10%	0%
I am using heat to treat my abdominal or back pain	0%	8%	0%	4%	0%	0%
I am experiencing constipation	0%	0%	5%	0%	5%	4%
I am experiencing muscle spasms in my legs	0%	0%	0%	0%	0%	0%
I am feeling more bloated than normal*	53%	38%	5%	0%	19%	17%
I am feeling more nauseous than normal	14%	4%	0%	0%	5%	8%
I am having more headaches than normal	19%	8%	0%	4%	10%	8%

*Data shown are the percentage of participants who responded "Yes" to each statement. NOC = No oral contraception. OC = Oral contraception. Chi² analysis revealed no between-group differences for any of the variables at any phase. *Indicates differences in the proportion of menstrual symptoms experienced among phases (chi², p<0.05).*

macronutrient intake, and caffeine intake. To determine whether or not changes throughout the menstrual cycle were different between the OC and non-OC groups, a group-by-phase interaction was used. Final models included OC use, menstrual cycle phase, lean mass, and fat mass. Marginal means were calculated and post-hoc comparisons were performed using a Bonferroni adjustment with a=0.05. All analyses were performed using STATA 15 (*StataCorp. 2017. Stata Statistical Software: Release 15*. College Station, TX: StataCorp LLC).

Results

Participant characteristics

Participant characteristics by OC group are given in Table 2. Age, height, lean mass, fat mass, and physical activity days per week at each visit did not differ between groups. No differences between groups were observed in the proportion of participants experiencing menstrual symptoms at any of the visits; however, a greater proportion of participants experienced menstrual symptoms during the early-follicular phase than the ovulatory or luteal phases in 7 out of 18 categories (Table 3). Total caloric intake, protein intake, carbohydrate intake, and caffeine intake 24 hours prior to each visit were not different among visits (Table 4).

Grip strength

Grip strength was greater at the ovulation (31.5±4.1 kg) and mid-luteal visits (32.5±4.9 kg) than at the early-follicular visit (30.0±4.4 kg). No group-by-phase interaction was observed, indicating similar menstrual phase differences for non-OC and OC users. Grip strength was not different between the OC and non-OC group at any visit (Supplemental Table 2).

Isokinetic testing, fatigue, and knee joint laxity

Extension peak torque at 60o/sec was 4% lower during the early-follicular visit (82.4±2.1 nM) than the ovulation visit (85.7±2.1 nM; p<0.05), but was not different at the mid-luteal phase visit (84.7±2.1 nM) (Figure 2). Extension peak torque at 180° was 8-11% lower at the early-follicular phase (53.7±1.5 nM) than the ovulatory and mid-luteal phases (58.1±1.5 nM and 60.4±1.5 nM, respectively; both, p<0.05). Similarly, extension peak torque at 300°/sec was 7-8% lower at the early-follicular phase (42.9±1.3 nM) than the ovulatory and mid-luteal phases (46.3±1.3 nM and 46.6±1.3 nM, respectively; both p<0.05).

Flexion peak torque at 60°/sec was 8% lower at the early-follicular visit than the ovulation visit (39.5±1.5 nM and 43.0±1.4 nM, respectively; p<0.05). Flexion peak torque at 180o/sec also was 13-14% lower at the early-

Table 4. Dietary intakes, flexor to extensor ratio and knee joint laxity by cycle phase.

	Early-Follicular	Ovulatory	Mid-Luteal
Caloric Intake (Kcal)	2180 ± 1247	1942 ± 751	1827 ± 574
Protein (g)	82 ± 41	69 ± 31	76 ± 27
Carbohydrate (g)	283 ± 171	256 ±89	226 ± 76
Fat (g)	85 ± 60	60 ± 25	73 ± 27
Caffeine (g)	44 ± 86	50 ± 88	54 ± 91
FE Ratio 60°/s (%)	49.1 ± 11.3	50.4 ± 10.4	49.3 ± 8.3
FE Ratio 180°/s (%)	54.0 ± 11.3	56.7 ± 10.2	54.4 ± 9.8
FE Ratio 300°/s (%)	67.5 ± 14.0	68.3 ± 14.7	66.6 ± 12.5
Repetitions to Fatigue	40.6 ± 8.4	40.0 ± 8.7	39.3 ± 8.6
Rested Joint Laxity (mm)	6.7 ± 2.7	6.4 ± 2.3	6.1 ± 2.1
Fatigued Joint Laxity (mm)	6.9 ± 2.2	6.4 ± 2.3	6.4 ± 1.9
Data given as means ± SD. No significant differences among visits were observed. FE Ratio = Knee flexor-to-knee extensor ratio.			

Table 5. Hormone concentrations by menstrual cycle phase.

	Non-OC (n=22)[a]	OC (n=24)[b]	P-Value
Early-Follicular Phase			
Estradiol (pg/ml)	67 (22-110)	40 (18-81)	0.001
Progesterone (ng/ml)	0.05 (0.04-0.13)	0.04 (0.03-0.23)	NS
Relaxin (pg/ml)	24 (8-74)	22 (10-67)	NS
Ovulatory Phase			
Estradiol (pg/ml)	121 (60-330)	33 (13-109)	<0.001
Progesterone (ng/ml)	0.32 (0.03-12.26)	0.04 (0.01-0.32)	<0.001
Relaxin (pg/ml)	22 (20-47)	22 (7-67)	NS
Mid-Luteal Phase			
Estradiol (pg/ml)	148 (60-298)	29 (15-67)	<0.001
Progesterone (ng/ml)	1.04 (0.03-12.21)	0.04 (0.03-0.42)	<0.001
Relaxin (pg/ml)	27 (11-90)	24 (8-60)	NS
[a] 3 participants did not ovulate and were excluded from all analyses. [b] 1 participant did not have complete data from all analyses. All data were log-normal distributed, therefore data are presented as geometric means (95% CI).			

follicular visit (28.5±1.0 nM) compared to the ovulation and luteal phase visits (32.6±1.0 nM and 33.0±1.0 nM, respectively; both, p<0.05). Results were similar for flexion peak torque at 300°/sec with 9-10% lower peak torque being observed at the early-follicular phase visit (28.4±1.2 nM) compared with the ovulation and luteal phase visits (31.7±1.2 nM and 31.1±1.2 nM, respectively; both p<0.05).

The group-by-phase interaction was not significant for any of the extension or flexion peak torque measures, and extension and flexion peak torque values were not different between the OC and non-OC group at any visit (Supplemental Table 2).

Knee flexor-to-knee extensor ratio at 60°, 180° and 300°o/second; repetitions to fatigue at 180o/second;

and both rested and fatigued joint laxity did not change throughout the menstrual cycle (Table 4).

Hormone concentrations

Hormone concentrations for the non-OC and OC groups at each visit are given in Table 5. During the early-follicular phase, participants in the non-OC group had higher estradiol concentrations than the OC group, (p<0.001) and progesterone concentrations did not differ. At both the ovulatory and mid-luteal phase visits, estradiol and progesterone concentrations were both higher in the non-OC group than the OC group (p<0.001 for all comparisons). Relaxin concentrations did not differ between non-OC and OC groups at any visit. Significant group-by-phase interactions were observed for estradiol and progesterone

with concentrations of both hormones becoming elevated in the non-OC group after the early-follicular phase.

Discussion

The purpose of this study was to investigate changes in muscular strength and stamina, measured by isokinetic testing of the knee flexors and knee extensors, throughout the menstrual cycle and to determine whether or not changes were different between OC and non-OC users. The key finding from the current study is that flexion and extension isokinetic peak torques were lower in the early-follicular phase than the ovulatory and mid-luteal phases during the 180° and 300°/sec testing. Additionally, during the 60°/sec testing, peak torque was greater during the ovulatory phase than the early-follicular phase. Particularly troubling in the area of ACL injury prevention is the finding of 4-11% lower flexion peak torque during the early-follicular phase of the cycle. Given the role of the hamstrings in protecting against anterior tibial translation, a decrease in hamstring strength could place women in the early-follicular phase of their cycle at a greater risk of suffering an ACL injury. The relationship between ACL injury and hamstring strength has been shown previously in a matched case-control study that found lower hamstring strength in females who went on to tear their ACL[33]. In addition to decreased lower extremity strength during the early-follicular phase, grip strength also was lower during the early-follicular phase than the ovulatory and mid-luteal phases. This is important because it indicates a whole body effect rather than an isolated effect or learning effect. The absence of a learning effect also is supported by previous work that indicated a high level of test-retest reliability[34] and mechanical reliability[32] with isokinetic measurements using the Biodex System 4 and our results finding no change in three measurements taken longitudinally over a 30-day period (see Supplemental Table 1).

The findings from the present study are similar to findings from a previous study that reported lower peak torque production during leg extension and flexion during the early-follicular phase compared to the ovulatory phase in female soccer players[35]. In the study by Dos Santos et al., no differences were observed in males tested at the same time intervals, and the authors suggested that the lower peak torque during the early-follicular phase may be due to hormonal effects that are specific to females. On the other hand, a study similar to ours reported no phase-specific differences in force, velocity, or power output during half squats on a Smith machine[36]. One explanation for why these findings could differ from ours could be that the nature of isokinetic exercise requires greater muscle recruitment than an isotonic movements such as a squat and therefore may be more sensitive to small physiological changes in neuromuscular performance.

Previous studies investigating injury risk throughout the menstrual cycle have reported that risk of ACL injuries are greater early in the menstrual cycle[37] and in particular prior to ovulation[38,39]. Decreased muscle strength could potentially increase the risk of suffering an ACL injury and our findings of lower peak torque in the early-follicular phase may indicate a potential risk factor for ACL injuries. However, it is important to note that other studies have reported ACL injury risk to be greatest during the ovulatory phase which is when the greatest torque production was observed in the current study[9,40].

Significant group-by-phase interactions were observed for estradiol and progesterone with concentrations of both hormones being elevated in the non-OC group after the early-follicular phase. However, these hormonal fluctuations did not appear to affect any of the outcome measures in this study because no significant group-by-phase interactions were observed. This finding is in agreement with a previous study indicating no influence of reproductive hormones on skeletal muscle contractility[41]. The finding of no difference between the OC and non-OC groups in the study by de Jonge et al. and the present study are somewhat surprising given previously published work that reported a protective effect of oral contraception on ACL injury risk[24,25]. However, it is important to consider that hormonal fluctuations are not the only physiological change throughout the menstual cycle.

In the absence of a hormone-related effect, one potential explanation for lower skeletal muscle strength during the follicular cycle is low iron concentrations. While this was not measured in the present study, the negative effect of low iron concentrations and the benefits of iron supplementation in female athetes has been well documented in both submaximal and maximal exercise[42]. It has previously been reported that as many as 30% of all female collegiate athletes are iron deficient[43] and therefore any small decrease in iron concentration due to menstrual bleeding could have significant implications on neuromuscular performance. Furthermore, one study indicated that iron concentrations were lower during the early follicular phase than the ovulatory phase[44]. If iron concentrations are lower during the early follicular phase than the ovulatory or luteal phases, then theoretically a decrease in maximal exercise performance may be expected. Future studies should be performed to determine if menstrual phase changes in musculoskeletal performance can be attenuated by using supplemental iron.

Limitations

A limitation of this study is the reliance on each participant completing maximal contractions during muscle testing. We addressed this limitation by using the peak torque measurement from a total of five repetitions rather than the average peak torque. We also compared the coefficient of variation among repetitions to determine if any participants had a within-test variability that was outside the norm for the entire sample. An additional limitation is that the study only utilized a single menstrual cycle and each participant began the study during the early-follicular phase. While this may introduce the chance of a learning effect, this is not likely based on findings from a reliability trial that showed no

increases in isokinetic peak torque or grip strength among three visits performed over 30 days (see Supplemental Table 1). Additionally, the percent difference among measurements in the present study was greater than the coefficient of variation previously observed in the reliability study for all measurements except the 60o/second isokinetic peak torque where the percent difference between the early-follicular and ovulation phases was smaller than the percent CV.

Conclusion

In conclusion, grip strength and peak torque were lower during the early-follicular phase than the ovulatory and mid-luteal phases. A surpising finding was that changes in peak torque and grip strength throughout the cycle were similar regardless of whether or not the female was taking oral contraceptives. Given the role of oral contraceptives in stabilizing hormone concentrations throughout the cycle, the results of this study indicate that cycle phase dependent differences in strength may not be explained by sex hormone fluctuations. Future studies investigating the role of other hormones or non-hormonal factors as it relates to ACL risk factors throughout the menstrual cycle are necessary to determine appropriate strategies for attenuating the strength deficits observed in the present study.

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Supplemental Table 1. Reliability of grip strength and isokinetic peak torque.

	Baseline Mean[1]	CV[2]	r[3]	p-value[4]
Grip Strength	44.7 ± 15.9 kg	4.4	0.04	0.96
Ext. Peak Torque 60°/sec	150.1 ± 55.2 Nm	6.8	0.08	0.89
Ext. Peak Torque 180°/sec	103.3 ± 31.2 Nm	5.8	0.09	0.83
Ext. Peak Torque 300°/sec	80.8 ± 22.2 Nm	6.2	0.12	0.78
Joint Laxity	5.8 ± 1.8 mm	4.3	0.01	0.99

[1]Data are means ± SD at the baseline visit. [2]Percent coefficient of variation. [3]Correlation-coefficient for the relationship between outcome measures and visit. [4]P-value for the correlation between outcome measures and visit number.

Supplemental Table 2.

	Early-Follicular		Ovulatory		Mid-Luteal	
	Non-OC	OC	Non-OC	OC	Non-OC	OC
Grip Strength (kg)	30.2 ± 4.2	29.9 ± 4.7	31.9 ± 3.7	31.1 ± 4.5	32.9 ± 5.0	32.9 ± 5.0
Extension Peak Torque						
60°/sec (Nm)	80.6 ± 16.1	84.1 ± 13.2	84.7 ± 15.8	86.7 ± 13.9	83.9 ± 16.5	85.4 ± 12.2
180°/sec (Nm)	51.8 ± 9.9	55.4 ± 9.4	55.6 ± 10.7	60.4 ± 10.2	58.9 ± 11.5	61.8 ± 9.8
300°/sec (Nm)	41.2 ± 9.5	44.5 ± 7.6	44.1 ± 9.1	48.3 ± 8.9	45.5 ± 9.3	47.6 ± 8.1
Flexion Peak Torque						
60°/sec (Nm)	37.5 ± 6.7	42.0 ± 11.2	43.9 ± 15.9	42.0 ± 7.5	40.6 ± 9.3	42.2 ± 8.5
180°/sec (Nm)	26.8 ± 7.9	30.7 ± 6.8	30.3 ± 7.7	34.9 ± 6.8	30.7 ± 6.9	34.2 ± 6.9
300°/sec (Nm)	27.4 ± 7.7	30.2 ± 8.0	28.0 ± 7.4	34.9 ± 9.7	29.0 ± 7.6	33.1 ± 7.0