

The Effects of Oral Contraceptive Use on Muscle Stiffness Across the Menstrual Cycle

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Objective: To determine the effect of oral contraceptives (OC) on hamstring neuromechanics and lower extremity stiffness across the menstrual cycle (MC).

Design: Causal comparative.

Setting: Research laboratory.

Participants: Thirty, healthy, normally menstruating female volunteers who were using OC (OC group, n = 15) or not (non-OC group, n = 15).

Assessment of Risk Factors: Stiffness and hamstring neuromechanics were assessed at 2 points of the MC corresponding to low (menses) and high (ovulation) hormone concentrations. Menses testing took place 3 to 5 days after the onset of menses (or pills 3-5 for the OC group). Ovulation test session occurred 2 to 4 days after ovulation identified using a commercial ovulation kit (or pills 15-17 in the OC group).

Main Outcome Measures: Lower extremity stiffness and hamstring neuromechanics [stiffness, electromechanical delay, rate of force production (RFP), time to 50% peak force (T50%)] and blood plasma concentrations of estradiol- β -17, free testosterone, and progesterone.

Results: Estradiol- β -17, free testosterone, and progesterone increased at ovulation in the non-OC group and remained constant in the OC group. No changes were observed across the MC or between the groups in other variables ($P > 0.05$).

Conclusions: Although previous literature suggests a prophylactic effect of OC use with respect to musculoskeletal injury risk, our results indicate that OC use does not affect muscle properties in manners thought to reduce ACL injury risk.

Key Words: hormone, estrogen, menstrual cycle, anterior cruciate ligament

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INTRODUCTION

Female athletes' risk of anterior cruciate ligament (ACL) injury is greater than male athletes.¹ One of the contributing factors for this disproportionate injury rate includes gender differences in hormone concentrations.² In the most recent ACL consensus statement, researchers agreed that estrogen receptors have been identified on the ACL³ and muscle,⁴ ACL injuries are greater during the preovulatory phase of the menstrual cycle (MC),⁵ and hormones can cause an interaction between ACL tissue and mechanical stresses.⁶ Researchers noted a lack of understanding of how hormones influence skeletal muscle and how this may affect joint stability.

In support of this theory, alterations in lower extremity stiffness,⁷ hamstring neuromuscular control,⁸ extensibility,⁹ and strength¹⁰ have been reported to change across the MC. For example, Eiling et al⁷ observed a decrease in lower extremity stiffness at ovulation, which was accompanied by appreciable increases in estrogen. The authors suggested that decreased stiffness may compromise dynamic joint stability, making the knee more susceptible to injury. However, this area is controversial, with others reporting equivocal results in similar variables.^{9,11-13} Research is needed to determine if cyclic alterations in hormonal profiles influence skeletal muscle to a degree in which dynamic stability could be compromised across the MC.

Monophasic varieties of oral contraceptives (OC) provide a constant estrogen dosage across the MC, thus attenuating the cyclical estrogen peak noted at ovulation. Oral contraceptives have been theorized to mitigate the influence of MC phase and associated hormonal fluctuations on ACL injury with mixed results.¹⁴⁻¹⁶ No mechanism has been identified as to why this protective effect may occur, but OC users have demonstrated altered tissue properties when compared with eumenorrheic controls.^{17,18} Specifically, OC users have less Achilles tendon strain¹⁷ and greater ACL laxity.¹⁸

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The hamstrings are a muscle group of interest because they are capable of limiting the load on the ACL by resisting anterior tibial translation (ATT).^{19–21} Stiffness refers to the ratio of change of force to change in length ($k = \Delta\text{force}/\Delta\text{length}$), and stiffer hamstrings provide greater resistance to lengthening induced by ATT compared with less stiff hamstrings.²² As ATT places tensile loading on the ACL,²³ heightened hamstring stiffness likely limits ACL loading. Lesser hamstring^{24,25} and lower extremity²⁶ stiffness have been demonstrated in women than in men, potentially contributing to the greater female ACL injury risk.

Temporal aspects of hamstring neuromechanical function may also be crucial to knee joint stability. Anterior tibial shear force must be countered by adequate posterior tibial shear force provided by the hamstrings to limit ACL loading. Delay in hamstring force production would permit a greater amount of ATT to occur, thus increasing ACL loading. Individuals with greater hamstring stiffness are capable of attaining a relative level of maximal force in a shorter period compared with those with more compliant hamstrings.²⁷ Therefore, a decrease in hamstring stiffness at ovulation associated with an increase in estradiol may compromise neuromechanical function, thus potentially increasing ACL injury risk. The purpose of this investigation was to compare hamstring neuromechanics and leg stiffness between OC and non-OC females across the MC. We hypothesized that stiffness would decrease at ovulation relative to menses, but this change would be smaller in OC users because of the constant estrogen environment created by OC.

METHODS

Subjects

Thirty physically active women volunteered for this investigation. Subjects were assigned to groups consisting of eumenorrheic women (non-OC) or those using monophasic OCs. Additional eligibility criteria included (1) 20 minutes of physical activity 3 times per week (minimum), (2) 18 to 25 years of age, (3) no lower extremity injury or surgery in the past 6 months, (4) no history of pregnancy, and (5) self-reported regular MC for the past 6 months. Subjects in the OC group were required to have used a monophasic OC continuously for 6 months, and non-OC subjects were required to not have used any OC within the previous 6 months. All subjects were informed of the study procedures and read and signed an informed consent document approved by the Institutional Review Board at the University of North Carolina at Chapel Hill before participation. Fifteen subjects per group were determined sufficient to detect clinically relevant changes (8%–10% difference, 80% power, and an alpha level error of 5%) in lower extremity⁷ and hamstring stiffness²⁷ using previously published data.

Experimental Procedures

Each subject completed a familiarization session in addition to testing sessions completed at menses and ovulation. The principal investigator was blind to group assignment and phase. In the non-OC group, testing occurred 3 to 5 days after the self-reported menstruation, which corresponds with

low levels of estrogen, and within 2 to 4 days after ovulation, which corresponds with higher levels of estrogen. Ovulation was identified using a urine-based ovulation kit (Earth's Magic, Inc, Cary, North Carolina). The OC group testing occurred within the days associated with pill numbers 3 to 5 (placebo pills, menses) and 15 to 17 (ovulation) and is similar to previously reported periods.^{12,18} During each session, 5 to 7 mL of blood was obtained from an antecubital vein via a 23-gauge needle and syringe. Blood was immediately transferred to a Vacutainer tube containing EDTA as an anti-coagulant. Blood sample tubes were then centrifuged at 3000g at 4°C until plasma was separated, which was then stored at -80°C until hormonal analysis. Plasma specimens were analyzed for estradiol- β -17, free testosterone, and progesterone concentrations using a solid-phase single antibody radioimmunoassay procedure (assay sensitivity: estradiol- β -17 = 1.4 pg/mL, free testosterone = 0.15 pg/mL, progesterone = 0.02 ng/mL) (Siemens Medical, Los Angeles, California). All assay samples were processed in duplicate, and quality control procedures were used.²⁸ The resulting assay between and within coefficients of variation were less than 10% for all unknowns and standards. Total estrogens are comprised primarily of estrone, estriol, estradiol- β -17, and their conjugates, with estradiol- β -17 being the major component, which is why it was analyzed only in this study.²⁹

Subjects performed 3 separate assessments in a counter-balanced order with 3 practice trials allowed before data collection: (1) lower extremity stiffness, (2) hamstring stiffness, and (3) hamstring neuromechanical function. The intrasession reliability (intraclass correlation coefficient_(3,1)) ranged from 0.85 (time to 50% peak force [T50%]) to 0.92 (rate of force production [RFP]) except for electromechanical delay (EMD), which was 0.56, indicating that most variables had good precision and reliability. Lower extremity stiffness was assessed by having subjects perform a double-leg hopping task on a force plate (model 4060-NC; Bertec, Corp, Columbus, Ohio). Subjects assumed a standardized position without shoes and hands placed on the hips and were instructed to keep pace with a metronome (2.2 Hz) as they hopped continuously for 1 minute.^{7,26,30} The vertical ground reaction force (GRFv) was used to calculate lower extremity stiffness. The GRFv represents acceleration of the total body center of mass (COM) in the vertical dimension and was divided by mass to derive acceleration ($a = F/m$). Acceleration was then double integrated to derive vertical displacement of the COM. Simple linear regression was used to characterize the relationship between the GRFv and COM displacement, and the slope of the resulting regression equation was used to represent lower extremity stiffness ($k = \Delta\text{GRFv}/\Delta\text{COM}$ vertical position) (Figure 1). This value represents the stiffness of the musculoskeletal structures of the lower extremity acting as a single spring. Ten acceptable ground impacts were selected for analysis based on the published criteria.²⁶ The hopping frequency was required to fall within $\pm 5\%$ of 2.2 Hz, and a minimum correlation coefficient of $r = 0.80$ for the relationship between GRFv and COM displacement was required to ensure linearity. The slope values (ie, stiffness) for the 10 selected trials were averaged and used for statistical analyses.

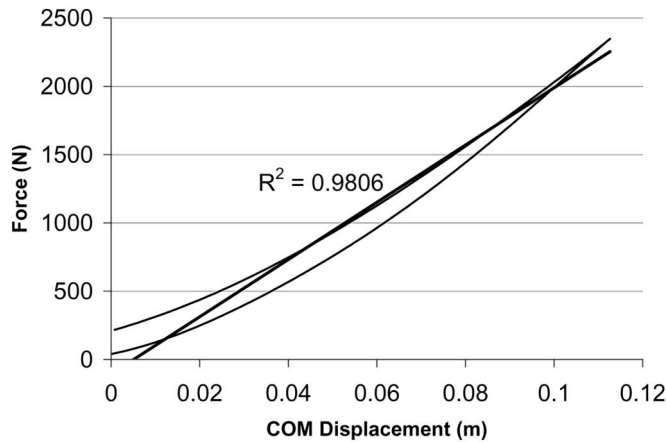


FIGURE 1. Lower extremity stiffness assessment. The regression slope of the force-displacement curve estimates lower extremity stiffness.

Hamstring stiffness was calculated from the damping effect imposed by the hamstrings on oscillatory knee flexion/extension induced by perturbation.^{9,24,25} A preamplified electromyography electrode (DeSys, Inc, Boston, Massachusetts; amplification factor = 10 000 (20-450 Hz) or interelectrode distance = 10 mm; CMMR @ 60 Hz > 80 dB; input impedance > 1015//0.2 X/pF) was positioned parallel to the biceps femoris hamstring muscle 50% of the distance between the joint line and greater trochanter. Electrode placement was verified via manual muscle testing. Electromagnetic motion analysis (Ascension Technologies, Inc, Burlington, Vermont) was used to assess knee flexion with a sampling rate of 100 Hz. Subject setup has been previously reported.²⁵ Briefly, a right-handed global reference system was defined with the positive x axis (anterior), positive y axis (left), and positive z axis (superior), and knee flexion was defined as Euler angles with y axis corresponding to flexion and extension.

Subjects were positioned prone with the right thigh supported in 30 degrees of flexion (Figure 2). A load equal to 10% body mass was secured near the ankle of the right leg. The investigator passively aligned the shank segment with the horizontal, and the subject was required to maintain this position via isometric hamstring contraction, placing the knee in approximately 30 degrees of flexion. A brief manual perturbation was applied to the calcaneus, forcing the knee into extension and initiating damped oscillatory flexion/extension.^{25,27} The principal investigator provided all perturbations to all subjects for consistency, and subjects were instructed to not interfere with the perturbation and to return the shank to the start position after the perturbation. An accelerometer (PCB Piezotronics, Depew, New York) was secured to a rigid splint placed on the subject's foot and captured the damped oscillatory motion of the shank segment (Figure 3). Accelerometer data were low pass filtered at 10 Hz (fourth order, zero-phase-lag Butterworth), and the time instances of the first 2 oscillatory peaks in the tangential acceleration profile (t_1 and t_2) were used to calculate the damped frequency of oscillation (f) using the following equation:

$$f = \frac{1}{(t_2 - t_1)}$$

Hamstring stiffness was calculated using the following equation, where k = stiffness, m = system mass (mass of shank and foot segment³¹ + applied mass), and f = damped frequency of oscillation.

$$k = 4\pi^2mf^2$$

Subjects performed 5 trials with 1 minute of rest between the trials to reduce the likelihood of fatigue, and

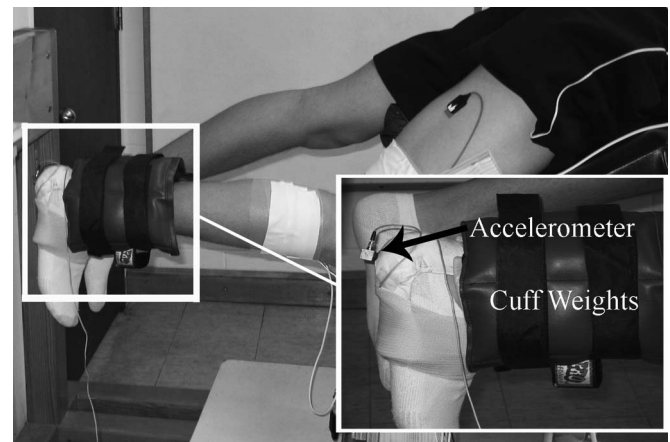


FIGURE 2. Experimental setup for hamstring stiffness assessments. The subject was positioned prone with their hip and knee flexed to 30 degrees. An accelerometer was fixed to a splint secured at the ankle. Ten percent body mass was attached at the ankle using cuff weights.

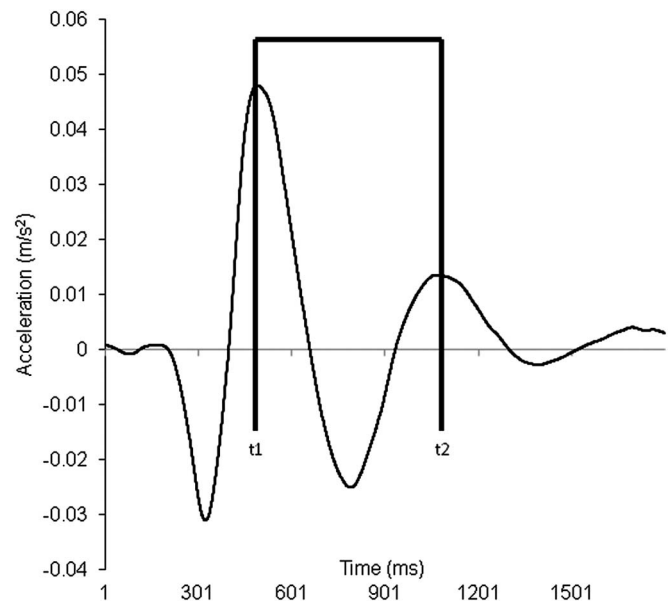


FIGURE 3. Tangential acceleration of the shank segment during hamstring stiffness assessments. Time instances of the first 2 peak accelerations were used to calculate the damped frequency of oscillation and hamstring stiffness.

the average of these trials was used for statistical analysis. All stiffness values were normalized to body mass.

Electromechanical delay, RFP, and T50% were calculated from a maximal voluntary isometric hamstring contraction.²⁷ Subjects were positioned prone with their right knee in 30 degrees of flexion similar to the hamstring stiffness protocol. Their foot was secured to a sled that prevented knee flexion from occurring, and a load cell (Honeywell Sensotec, Columbus, Ohio) was attached to the sled at the posterior aspect of the calcaneus. Subjects were instructed to perform a hamstring contraction as quickly and forcefully as possible in response to a light stimulus activated randomly to avoid anticipation by the subject. Each contraction lasted approximately 3 seconds, and 1 minute of rest was provided between the trials. Five trials were recorded, and the average was used for data analysis.

Vertical ground reaction force, EMG, accelerometer, and load cell data were sampled at 1000 Hz using The Motion Monitor motion capture software (Innovative Sports Training, Chicago, Illinois). Data filtering, processing, and onset identification parameters have been previously published.²⁷ The blood sample from 1 subject in the OC group was misplaced, thus hormonal concentrations were not available for this subject, and statistical analyses were conducted on the remaining 14 subjects in the OC group. Differences in dependent variables were evaluated via separate 2 (group) × 2 (phase) repeated measures analysis of variance. Significant main and/or interaction effects were evaluated post hoc via Tukey Honestly Significant Difference test. Statistical significance was established a priori as $\alpha \leq 0.05$.

RESULTS

Fifteen subjects were enrolled in each group, and no differences in subject descriptive statistics were noted (Table 1). Statistical information for reproductive hormones can be found in Table 2 and neuromechanical variables in Table 3.

As expected, we observed a significant group by phase interaction for the blood concentration of estradiol- β -17 ($F_{1,27} = 19.68$; $P < 0.001$), free testosterone ($F_{1,27} = 10.32$; $P = 0.003$), and progesterone ($F_{1,27} = 9.55$; $P = 0.005$). The Tukey post hoc revealed that each hormone was greater at ovulation in the non-OC group compared with the OC group. No significant group by phase interaction was present for resting knee angle ($F_{1,28} = 1.49$; $P = 0.23$) nor angular displacement ($F_{1,28} = 2.09$; $P = 0.16$) as a result of the perturbation during the active hamstring stiffness assessment. We observed no significant interactions for hamstring stiffness ($F_{1,28} = 0.34$; $P = 0.56$), lower extremity stiffness

($F_{1,28} = 2.58$; $P = 0.12$), EMD ($F_{1,28} = 0.18$; $P = 0.67$), T50% ($F_{1,28} = 0.97$; $P = 0.33$), or RFP ($F_{1,28} = 0.33$; $P = 0.57$). No group or phase main effects were observed for any neuromechanical variables ($P > 0.05$).

DISCUSSION

The findings of this investigation were contrary to our original hypotheses and suggest that muscle properties are not influenced by hormonal fluctuation across the MC or by OC use. No differences were observed in neuromechanical variables between samples of women who did and did not regularly use OC, and these variables did not change across the MC. These results are in agreement with Elliott et al,³² who demonstrated that muscle strength did not differ between OC users and nonusers. Similarly, Kubo et al³³ demonstrated that muscle stiffness did not differ between menses and ovulation, suggesting that these select hormones at 2 time points do not have an appreciable effect on muscle properties.

A number of factors may explain the lack of the significant effect of OC use on muscle properties. Shultz et al³⁴ reported a delayed effect of reproductive hormones on knee laxity across the MC. If these hormones have similar effects on skeletal muscle, our testing intervals may not have captured the appropriate time frame during the MC when these effects are exhibited; however, it is not known whether such a delay exists in muscle. Second, other sex steroid hormones (estradiol and progesterone) can have actions on androgen receptors that normally uptake testosterone and implement the physiological effects seen in tissues. We observed an increase in free testosterone at ovulation, which was expected,³⁵ without appreciable effects on muscle neuromechanics. If sex steroid hormones are limiting testosterone's ability to interact with androgen receptors (through competitive binding and blocking), then they would mitigate the effect on the elevated testosterone observed. Additionally, Eiling et al⁷ demonstrated a decreased lower extremity stiffness at ovulation using a single-leg hopping task. The lesser relative musculotendinous loading associated with the double-leg hopping used in this investigation, in comparison with the single-leg hopping used by Eiling et al,⁷ may have minimized the differences in lower extremity stiffness between the groups, thus masking any influence of OC use. Finally, the effect of these hormones on muscle may be too minute for the current testing protocol to quantify.

Additionally, the literature is inconsistent regarding the influence of hormonal fluctuations throughout the MC on muscle properties. Differences in muscle activation patterns,⁸ strength,^{10,36} endurance/fatiguability,^{10,36} stiffness,⁷ and extensibility⁹ have been reported across the MC as functions of fluctuating hormonal concentrations. However, other authors have reported a lack of changes in strength,^{11,13,32,37,38} endurance/fatiguability,^{37,38} and stiffness.^{9,33} The same is true of OC use in that some investigations have demonstrated that OC use limits changes in these characteristics across the MC,^{10,36} whereas others have demonstrated no effect of OC use.³² Collectively, these investigations encompass a wide variety of discrepancies in the experimental design, including (1) muscles being tested (eg, upper vs lower extremities), (2) sample

TABLE 1. Descriptive Statistics for Each Group

	Non-OC (n = 15)	OC (n = 15)	P*	t _(1,28)
Height, cm	163.9 ± 5.6	165.4 ± 7.9	0.57	-0.58
Mass, kg	62.8 ± 10.6	61.5 ± 13.1	0.78	0.28
Age, y	20.4 ± 1.6	19.9 ± 1.1	0.30	1.06

Values represent means ± SDs of respective group.

*Independent samples *t* tests were performed on each variable.

TABLE 2. Concentrations of Reproductive Hormones From the Selected Time Points

	Menses		Ovulation		Menses, 95% Confidence Interval		Ovulation, 95% Confidence Interval		Effect Size
	Mean	SD	Mean	SD	Lower	Upper	Lower	Upper	
Estradiol- β -17, pg/mL									
Non-OC	68.03	23.22	99.28*	31.29	53.51	82.55	84.75	113.32	1.18
OC	61.19	31.29	52.76	25.19	46.16	76.21	37.97	67.55	0.31
Free testosterone, ng/mL									
Non-OC	1.53	0.64	2.09*	0.64	1.20	1.87	1.79	2.41	0.91
OC	1.42	0.62	1.22	0.49	1.08	1.77	0.90	1.53	0.37
Progesterone, ng/mL									
Non-OC	0.80	0.54	6.66*	6.80	0.46	1.14	4.03	9.29	1.26
OC	1.05	0.73	1.42	1.17	0.70	1.40	-1.30	4.14	0.39

Effect sizes were calculated as mean 1 – mean 2/(pooled SD). All hormonal values were within the manufacturer’s recommended reference ranges.
 *Significantly greater than non-OC at menses and all OC values.

characteristics (eg, age and activity status), (3) testing points within the MC (eg, menses, ovulation, and mid luteal), (4) the method of identifying points in the MC (eg, ovulation kit, estimation based on length), and (5) outcome instruments used (eg, KT-1000, hopping). Yet, no consistent pattern emerges that predicts the likelihood of identifying significant influences

of either hormonal fluctuations or OC use. Our results in combination with those of Elliott et al³² and Kubo et al³³ suggest that reproductive hormone concentrations and OC use do not influence muscle properties in manners thought to alter ACL loading and injury risk, thus OC use does not seem to have a prophylactic effect on dynamic joint stability.

TABLE 3. Hamstring Muscle Neuromechanical Properties From the Selected Time Points

	Menses		Ovulation		Menses, 95% Confidence Interval		Ovulation, 95% Confidence Interval		Effect Size
	Mean	SD	Mean	SD	Lower	Upper	Lower	Upper	
Active stiffness, N·cm ⁻¹ ·kg ⁻¹									
Non-OC	0.19	0.02	0.19	0.03	0.17	0.21	0.16	0.20	N/A
OC	0.18	0.04	0.18	0.03	0.16	0.20	0.16	0.20	N/A
Perturbation amplitude, degrees*									
Non-OC	3.97	1.34	3.63	2.11	3.23	4.72	2.45	4.80	0.20
OC	3.84	2.18	4.67	2.26	2.64	5.06	3.42	5.94	0.34
Leg stiffness (N·cm ⁻¹ ·kg ⁻¹)									
Non-OC	334.36	71.77	366.47	97.98	296.86	371.86	322.14	410.80	0.39
OC	328.06	70.02	314.21	66.71	290.56	365.56	269.88	358.54	0.21
EMD, milliseconds									
Non-OC	117.31	27.43	117.27	20.83	101.72	132.91	99.68	134.87	N/A
OC	130.84	24.58	125.84	35.96	115.25	146.44	108.25	143.43	0.17
T50%, milliseconds									
Non-OC	129.57	33.93	128.67	31.49	105.99	153.16	107.41	149.93	0.03
OC	142.10	53.17	125.81	47.34	118.51	165.69	104.54	147.07	0.35
RFP									
Non-OC	490.98	185.23	512.32	262.30	381.00	600.95	374.52	650.12	0.10
OC	451.18	228.39	523.88	258.76	341.21	561.15	386.08	661.68	0.31

Effect sizes were calculated by mean 1 – mean 2/(pooled SD).
 *P = 0.16, F_{1,28} = 2.09; perturbation amplitude was not different between the groups nor phases.

Researchers agree that the preovulatory phase of the MC has a greater than expected number of ACL injuries.² However, fewer studies have investigated the influence of OC use on injury risk. Consensus does not exist, and some investigations suggest a decreased risk of injury with OC use¹⁵ and others reporting no difference.^{14,39} Estrogen fluctuation throughout the MC may influence muscle, but these changes seem too small to be clinically relevant. However, OC use has been demonstrated to improve the structural strength of the ACL in the animal model,⁴⁰ and thus the prophylactic effect may be limited to ligamentous tissues.

Limitations

Neuromechanical variables and blood hormone concentrations were assessed at 2 points in the MC. More testing sessions may have revealed larger group differences in hormonal concentrations and muscle stiffness, thus maximizing the potential to observe an influence of OC use. Additionally, as seen in ligament, it is possible that only some women are responsive to hormonal fluctuations (ie, responders vs nonresponders).^{34,41}

Another limitation is that OC use was limited to monophasic doses. A variety of common OC alternatives exist, which vary in cycle length and delivery methods that were not considered in this study. All OC types include a placebo interval during which no hormones are delivered, allowing menstruation to occur. Oral contraceptive regimens vary in cycle length, yet our study was limited to subjects using OC with 25-day to 32-day cycles. Extended periods of elevated estrogen concentrations associated with longer cycle OCs may have differing effects on muscle properties.^{7,42}

Only females with self-reported normal MCs were included in this study. Oral contraceptive is commonly prescribed for women with irregular MCs to stabilize hormone levels and cycle length. Larger hormonal surges in women with irregular MCs may have a larger effect on muscle properties compared with normally menstruating women. Finally, our study was powered to detect clinically meaningful changes in lower extremity and hamstring stiffness. Sample size may not be adequate to detect changes in other variables included in this study. However, these variables were associated with low effect sizes (Table 3), which indicate that large increases in sample size would be needed to reach statistical significance. Also, EMD was associated with reliability below clinically acceptable levels, and these results should be interpreted with caution.

In conclusion, we found no effect of MC phase or OC use on muscle properties across the time points studied. These results indicate that the use of OCs does not affect muscle properties in manners thought to reduce ACL injury risk.

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