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Human Kinetics
ORIGINAL INVESTIGATION

Indirect Markers of Muscle Damage Throughout the Menstrual Cycle

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Context: The indirect markers of muscle damage have been previously studied in females. However, inconclusive results have been found, possibly explained by the heterogeneity regarding monitoring and verification of menstrual-cycle phase. ***Purpose:*** To determine whether the fluctuations in sex hormones during the menstrual cycle influence muscle damage. ***Methods:*** A total of 19 well-trained eumenorrheic women (age 28.6 [5.9] y; height 163.4 [6.1] cm; weight 59.6 [5.8] kg body mass) performed an eccentric-based resistance protocol consisting of 10 × 10 back squats at 60% of their 1-repetition maximum on the early follicular phase (EFP), late follicular phase, and midluteal phase of the menstrual cycle. Range of motion, muscle soreness, countermovement jump, and limb circumferences were evaluated prior to 24 and 48 hours postexercise. Perceived exertion was evaluated after each set. ***Results:*** Differences in sex hormones indicated that tests were adequately performed in the different menstrual-cycle phases. Prior to exercise, muscle soreness was higher in the EFP (4.7 [7.7]) than in the late follicular phase (1.1 [3.2]; $P = .045$). No other variables showed significant differences between phases. Time-point differences (baseline, 24, and 48 h) were observed in knee range of motion ($P = .02$), muscle soreness, countermovement jump, and between sets for perceived exertion ($P < .001$). ***Conclusion:*** Although the protocol elicited muscle damage, hormonal fluctuations over the menstrual cycle did not seem to affect indirect markers of muscle damage, except for perceived muscle soreness. Muscle soreness was perceived to be more severe before exercise performed in EFP, when estrogen concentrations are relatively low. This may impair women's predisposition to perform strenuous exercise during EFP.

Keywords: female athlete, eumenorrheic, eccentric exercise, muscle soreness, perceived exertion

A bout of strenuous or unaccustomed exercise could trigger damage to muscle tissues, specifically sarcomeres and myofibers. Muscle-damage response is mainly characterized by loss of muscle strength, reduction in range of movement (ROM), increase in muscle soreness and limb girth, and release of myocellular enzymes and proteins into the bloodstream or a combination of these.[1] In addition, a decrease in countermovement jump (CMJ) height could also be considered as a marker of muscle damage as this test shows high fatigue sensitivity.[2] Altogether, these markers may be altered following a strenuous bout of exercise.

An increasing number of women are engaging in high-intensity training, which has led to a recent increase in women-based research studies,[3] in spite of the difficult nature of controlling the female hormonal environment.[4] Sex hormone concentrations dramatically change throughout the 2 major phases of the menstrual cycle as follows: the follicular phase, focused on maturing a reproductive cell, and the luteal phase, focused on its regression. Thus, muscle damage response to exercise could vary accordingly. During the early follicular phase (EFP), both estrogen and progesterone concentrations are at their lowest levels. Estrogen increases halfway through the midfollicular phase and reaches its peak during the late follicular phase (LFP), while progesterone remains low. After ovulation, progesterone starts rising while estrogen levels drop before being gradually recovering through the luteal phase. During the midluteal phase (MLP), both estrogen and progesterone are elevated and subsequently fall during the late luteal phase.[5]

Estrogens may influence muscle membrane stability and limit muscle damage, as suggested by studies with animal models.[6] Likewise, some research with postmenopausal women has also shown benefits in postexercise muscle disruption and inflammation in estrogen replacement therapies users.[7,8] However, muscle damage response in premenopausal women is not clear due to the lack of studies properly investigating sex hormone fluctuations by considering more than 1 phase of the menstrual cycle. In addition, further factors that may contribute to these inconclusive findings include the following: (1) the lack of accuracy in verifying menstrual cycle phases,[9] (2) the different modalities of exercise performed (eccentric exercise, downhill running, electrostimulation . . .), and (3) the different training status of the recruited participants. Therefore, the aim of this study was to determine whether sex hormone fluctuations throughout the menstrual cycle influence indirect markers of muscle damage in well-trained women. We hypothesize that muscle damage is lower when the estrogen concentration is higher, as it is observed in LFP.

Methods

Participants

A total of 19 healthy eumenorrheic women (age 28.6 [5.9] y; height 163.4 [6.1] cm; weight 59.9 [5.6] kg body mass, 14.8 [5.1] kg fat mass, 42.6 [3.1] kg fat free mass, and 2.6 [0.3] kg bone mineral content) were recruited to participate in this study. All participants self-reported their experience in resistance training (at least 1-h session 2 times per week during a minimum of a year). The exclusion criteria included the following: (1) irregular menstrual

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cycles, considering regularly occurring menstrual cycles those ranging from 24 to 35 days in length[10]; (2) the use of contraceptives in the 6 months preceding the study; (3) any existing disease and/or metabolic or hormonal disorder; (4) any musculoskeletal injury in the last 6 months; (5) any surgery interventions (ie, ovariectomy) or other medical conditions that would be exacerbated by an eccentric resistance exercise protocol; (6) the regular use of medication or dietary supplements that could affect the results; (7) pregnancies in the year preceding; and (8) smoking. Inclusion and exclusion criteria were determined through an individual questionnaire, and all participants signed written informed consent prior to inclusion in the study. All procedures complied with the Declaration of Helsinki and were approved by the Universidad Politécnica de Madrid ethics committee board.

Study Design

Participants first visited the laboratory within days 2 to 5 of their EFP, considering day 1 as the onset of menstrual bleeding. In this screening session, blood samples were drawn to determine and discount any existing disorder. Subsequently, the participants' body composition was assessed by dual-energy X-ray absorptiometry with a GE Lunar Prodigy apparatus using GE Encore 2002 software (version 6.10.029; GE Healthcare, Madison, WI). This session concluded with the strength assessment of the lower limb through the 1 repetition maximum (1RM) test for the parallel back squat exercise. The 1RM test consisted of 4 sets of 1 repetition with increasing loads from 75% to 90% of their estimated 1RM. The full ROM of each lift was recorded with an iPhone 6s (iOS version 12.1.3; Apple Inc, Cupertino, CA) and processed with PowerLift (iOS version 9.1.7; Carlos Balsalobre, Madrid, Spain).[11] Then, the participants visited the laboratory on 3 further occasions according to the EFP (3 [1] d), LFP (12 [3] d), and MLP (22 [3] d) of the menstrual cycle, to perform an eccentric-based resistance protocol as described below, to cause muscle damage (Figure 1). Data were collected over 2 menstrual cycles in a counterbalanced and randomized order to avoid learning effects. In addition, 2 menstrual cycles were also necessary to provide a minimum recovery of 1 week between the LFP and EFP or MLP.

Serum blood samples were obtained in each session to determine hormone concentrations. All measurements (muscle soreness, thigh and calf circumferences, hip and knee ROMs, and CMJ performance) were assessed prior to exercise (baseline), postexercise (24 h), and postexercise (48 h) by the same researcher. In addition, CMJ was assessed immediately postexercise (0 h) (Figure 1).

Determination of Menstrual-Cycle Phase

Participants reported the length of their last menstrual cycles before starting this study to determine the average cycle length

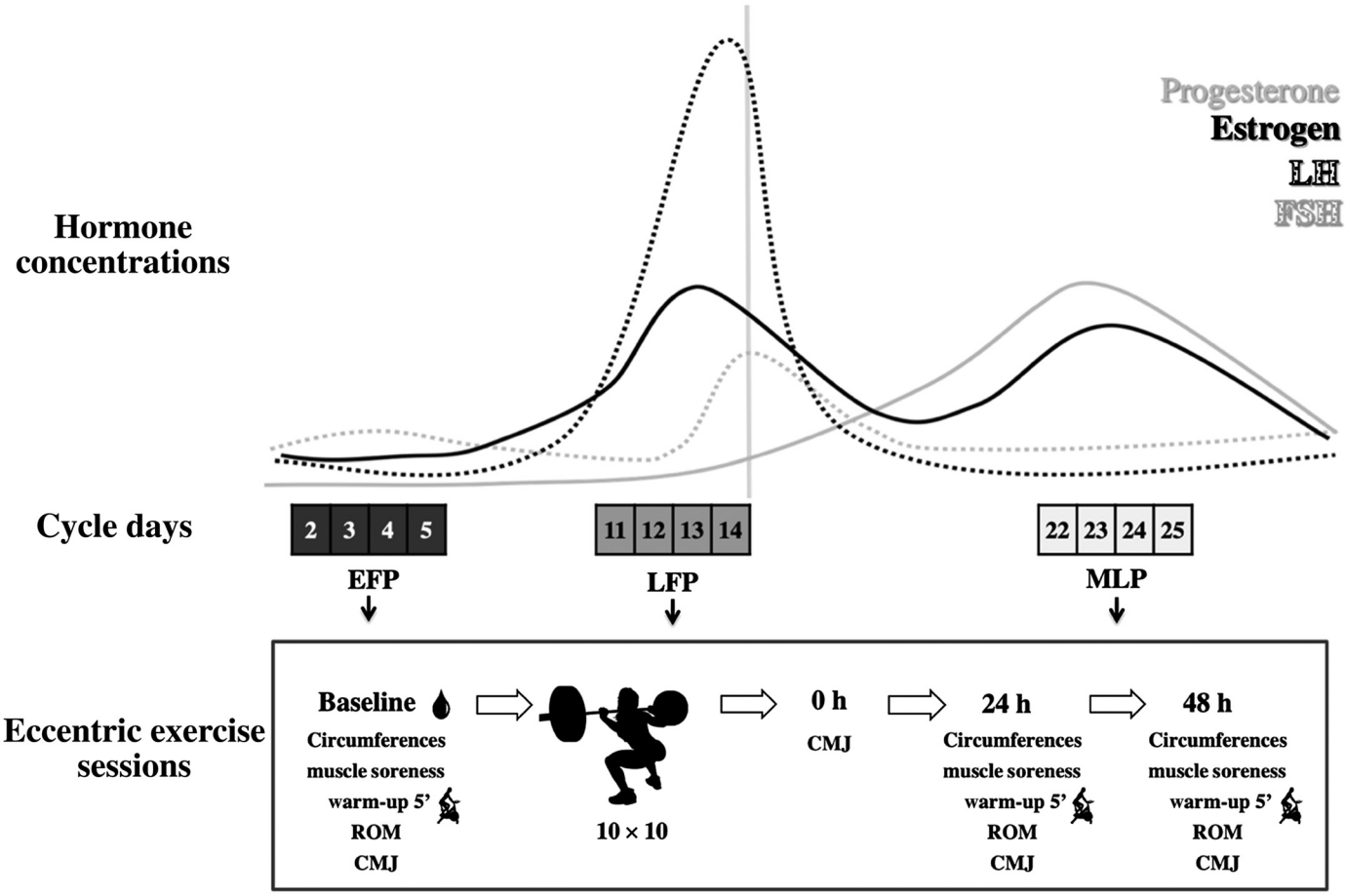

Figure 1 — Eccentric-based resistance protocol performed in the EFP, LFP, and MLP of the menstrual cycle. CMJ indicates countermovement jump; EFP, early follicular phase; FSH, follicle-stimulating hormone; LFP, late follicular phase; LH, luteinic hormone; MLP, midluteal phase; ROM, range of motion. 0 h: immediately postexercise; 24 and 48 h: 24 and 48 hours postexercise, respectively.

(considering the onset of menses as the start of the cycle). A gynecologist confirmed that the cycles were eumenorrheic and calculated the range of different phases in volunteers. From this retrospective analysis, the following cycle was prospectively estimated.

Ovulation was confirmed by using a home ovulation kit (Ellatest, Alicante, Spain) to adequately arrange LFP and MLP sessions. It is naturally occurring within the 14 to 26 hours after the LH surge is detected (14 [3] d) in the urine-based test.[5] Following the manufacturer's instructions, the second urine sample was collected at the same time each day, from 3 to 5 days before expected ovulation. Then, to confirm that the participants were conducting the trials in the correct phase, blood samples were collected at the beginning of each session to determine steroid hormone levels. In this regard, ovulation was confidently assured as the minimum conservative limit of 16 nmol/L (4.61 ng/mL) of postovulatory progesterone was accomplished.[5,9]

Procedures

Prior to the eccentric-based resistance protocol, perceived muscle soreness was measured using a visual analog scale.[12] Participants were requested to rate the level of soreness experienced in their thighs and glutes during a parallel unweighted squat from 0 mm (no pain at all) to 100 mm (unbearable pain). After that, a standard centimeter-marked tape was utilized to measure changes in muscle girth as an indirect marker of edema. Midthigh and midcalf points were marked with permanent marker to ensure reliability across measurements, and limb girths were assessed on the right side of the body according to the International Society for the Advancement of Kinanthropometry guidelines.

Later, after a 5-minute cycle ergometer warm-up, hip and knee passive ROMs were measured using a manual goniometer accurate to 1° (Jamar 360° steel goniometer; Greendale, WI). For hip ROM, a traditional hamstring passive stretching procedure was performed with the participant laid supine on the floor, whereas a researcher flexed the right hip (both knees extended) until the point of discomfort. Then, to measure knee ROM, participants performed a modified kneeling lunge with their left leg with the trunk in an upright position, placing their left knee in line with their left ankle and aligning their lower left leg perpendicular to the floor so that the right hip was stretched to the point of discomfort.[13] This hip angle was also registered and reproduced on subsequent occasions. After positioning, the researcher passively flexed the right knee until reaching the point of discomfort. An increase in both angles (increased ROM) indicated a decrease in mobility. Immediately after ROM assessment, CMJ was evaluated with the My Jump iOS App (version 5.0.6).[14] The jump was recorded with an iPhone 6s (Apple Inc, Cupertino, CA), and the flight time was calculated by identifying take-off, and landing frames providing jump height as outcome.

After some mobility and dynamic stretching exercises, a more specific squat-based warm-up was performed with moderate loads about 50% to 60% of the maximum value obtained during the screening session. After that, the 1RM was estimated again in each eccentric session by performing a quick test with the Powerlift App based on the full test previously performed in the screening session. Both in CMJ and 1RM recordings, the frequency was 240 frames per second. Since these procedures required manual selection by the researcher, 2 independent observers analyzed the same videos. Once the 1RM test was completed, the 1RM load was provided by the App from the force-velocity profile.

Eccentric-Based Resistance Exercise

The eccentric-based resistance exercise consisted of 10 sets of 10 repetitions, at 60% of their 1RM, of plate-loaded barbell parallel back squats, with a 2-minute rest between sets. Squats tempo (4-s eccentric movement, 1-s pause at the bottom, 1-s concentric movement, and 1-s pause at the top of the lift) was controlled using a timer, with the researcher signaling the changes in the lifting phase and providing verbal encouragement. The protocol was focused on the eccentric phase of the lift to achieve greater muscle damage.[13] Perceived exertion from every set was also obtained by administering a 10-point scale from 0 (extremely easy) to 10 (extremely hard).[15]

Blood Sampling

All blood samples were obtained by venipuncture into a vacutainer containing clot activator. Following inversion and clotting, the whole blood was centrifuged (Biosan LMC-3000, version V.5AD; Biosan, Riga, Latvia) for 10 minutes at 3000 rotations per minute and transferred into Eppendorf tubes before being stored frozen at −80°C until further analysis. Estradiol 17-β, progesterone, follicle-stimulating hormone (FSH), and luteinizing hormone (LH) were measured with a COBAS E411 (Roche Diagnostics GmbH, Mannheim, Germany), using ECLIA (Electrochemiluminescence immunoassay) technology. Proprietary reactives were calibrated following internal politics of laboratory calibration, and controls were assayed after calibration. Interassay and intra-assay coefficients of variation reported by the laboratory were 5.3% and 1.8% at 1.2 mIU/mL for FSH, 5.2% and 1.8% at 0.54 mIU/mL for LH, 11.9% and 8.5% at 93.3 pg/mL and 6.8% and 4.7% at 166 pg/mL for 17β-estradiol, and 23.1% and 11.8% at 0.7 ng/mL and 5.2% and 2.5% at 9.48 ng/mL for progesterone.

Statistical Analysis

Data are presented as mean (SD). The statistical analysis was conducted using the software package SPSS for Windows, (version 25.0; IBM Corp, Armonk, NY). A Shapiro–Wilk test for normality was used. One-way analysis of variance was used to analyze hormones concentrations and 1RM in the 3 phases studied. Then, 2-way analysis of variance with repeated measures (phases × time) was used to explore the objective. Perceived exertion, as data were not normally distributed, was analyzed with a nonparametric Friedman test to assess differences along sets and phases. Where appropriate, the Bonferroni post hoc test was applied to examine pairwise comparisons of each significant factor. Finally, once data were log-transformed, the standardized Cohen d with its confidence limit, and the magnitude-based inference (MBI) were analyzed to explore the effect size (ES) and the clinical relevance of the findings.[16,17] The ES was expressed with its 95% confidence limits and was interpreted based on the following criteria: <0.2, trivial; 0.2 to 0.6, small effect; 0.6 to 1.2, moderate effect; 1.2 to 2.0, large effect; and >2.0, very large. MBIs were carried out to determine the beneficial (negative), trivial, or harmful (positive) effects of the menstrual cycle phases. When a clear interpretation was possible, a qualitative inference was given as follows: 0.5% to 5%, very unlikely; 5% to 25%, unlikely; 25% to 75%, possibly; 75% to 95%, likely; 95% to 99.5%, very likely; and >99.5%, most likely. The significance level was set at $P < .05$.

Results

Results from hormone analysis are presented in Figure 2. A phase effect was observed for estradiol ($F_{1,26} = 14.53$), progesterone ($F_{1,19} = 117.27$), LH ($F_{2,19} = 8.54$), and FSH ($F_{2,19} = 23.33$; $P < .001$ for all variables) indicating higher LH and estrogen concentrations in LFP and higher estrogen and progesterone levels in MLP. Results from 1RM were 75.9 (16.8), 75.5 (17.4), and 74.7 (16.3) kg for EFP, LFP, and MLP, respectively, without significant differences between phases ($P = .57$).

Results from circumferences, ROM and CMJ are shown in Table 1. A trend for phase was observed for hip ROM ($F_{2,27} = 3.09$; $P = .07$) and CMJ ($F_{2,36} = 3.073$; $P = .06$). No effect of phase was observed for thigh and calf circumferences ($P = .65$ and $P = .88$, respectively) or knee ROM ($P = .20$). An effect of time was obtained for knee ROM ($F_{2,36} = 4.384$; $P = .02$) and CMJ ($F_{2,37} = 30.169$; $P < .001$). No effect of time was observed for thigh and calf circumferences ($P = .09$ and $P = .24$, respectively) and for hip ROM ($P = .11$). Results from ES and MBI for phase and time effects are shown in Table 2. No interaction was observed between phase and time for either thigh or calf circumferences ($P = .63$ and $P = .88$, respectively); hip ROM ($P = .78$); knee ROM ($P = .90$); and CMJ ($P = .46$). Likewise, ES and MBI for interactions were negligible (data not shown).

Muscle soreness results are shown in Figure 3. No effect of phase ($P = .24$) was observed while an effect of time ($F_{2,36} = 64.414$; $P < .001$) was shown for this variable. It was supported by clinical inference indicating that 24 hour (2.9 [1.3]) and 48 hour (2.5 [1.2]) postexercise soreness values were higher than at baseline (0.3 [0.5]) (Table 2). In addition, an interaction between phase and time ($F_{2,44} = 3.098$; $P = .046$) was observed (Figure 3). In accordance, clinical inference regarding interaction effects revealed that soreness was possibly higher in the EFP than in the LFP at baseline (1.07). In addition, a moderate ES showed that soreness seemed likely to be higher in the LFP than in the EFP 48-hour postexercise (0.55) despite no significant difference ($P = .12$) being observed between LFP and EFP 48-hour postexercise. However, this difference between the 2 statistical approaches is worth mentioning.

Perceived exertion values during the protocol are shown in Figure 4. The nonparametric test revealed no significant differences ($P = .689$) between EFP (6.7 [1.4]), LFP (6.5 [1.2]), and MLP (6.6 [0.9]). Finally, differences between sets were observed ($\chi^2 = 145.73$; $P < .001$).

Discussion

The major finding of our study is that the fluctuation of sex hormones over the menstrual cycle does not seem to affect indirect markers of muscle damage, except muscle soreness. To the best of our knowledge, no studies in the literature have analyzed muscle damage responses in the same participants after an eccentric-based protocol in the EFP, LFP, and MLP of the menstrual cycle. Muscle pain perception before exercise increased in the EFP when estrogen and progesterone were at their nadirs. This could be related to the menstrual disturbances reported by some women during menstruation, such as cramps or pain, which are mainly experienced in the lower abdomen but it may radiate to the lower back and/or upper legs eliciting physical distress.[18] Similar results have also been reported by other studies where participant soreness was evaluated at least twice.[19,20] In these studies, higher soreness values in the EFP were also shown following exercise. On the contrary, in our study, a moderate ES could suggest a different postexercise pattern, with higher values of soreness 24-hour postexercise in the MLP and 48-hour postexercise in the LFP. According to this result, the protective role of estrogen suggested by the literature[6,21] might not have attenuated exercise-

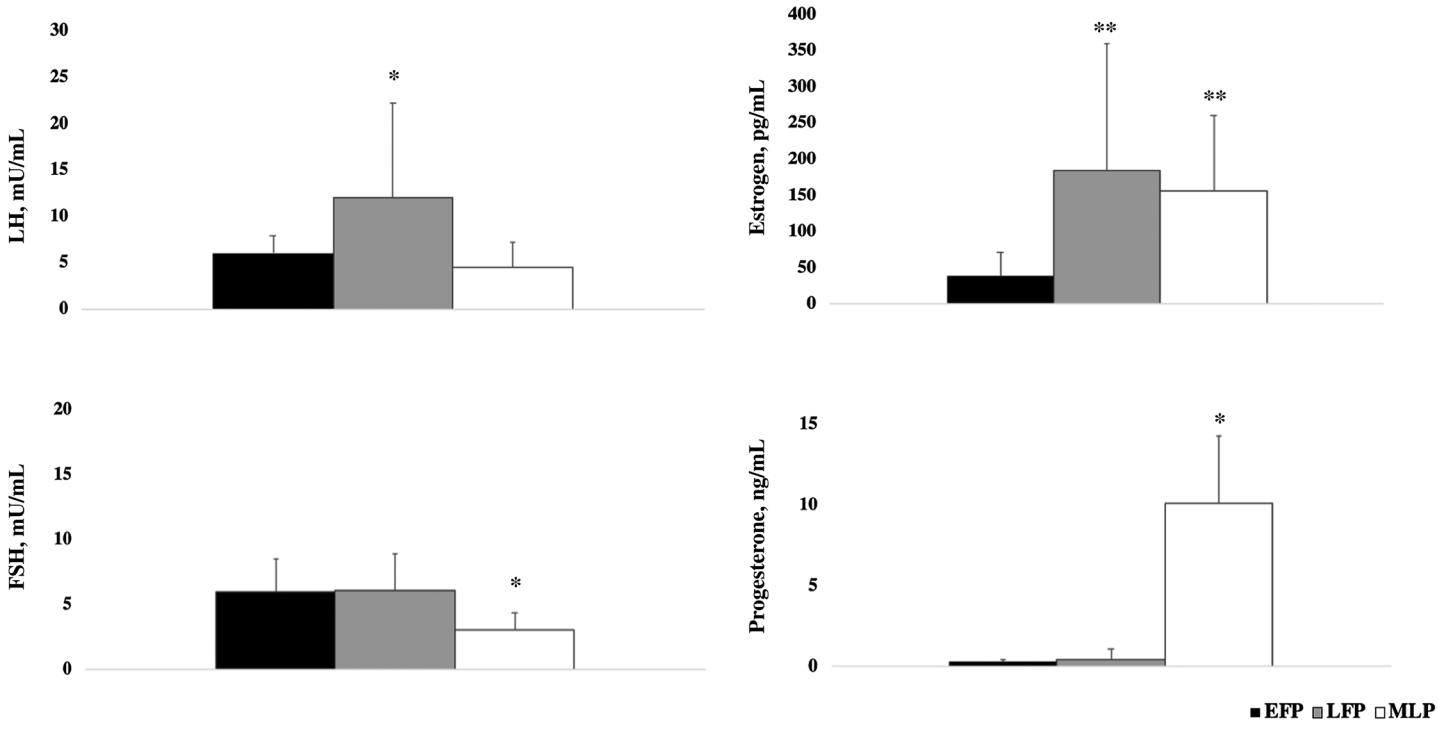

Figure 2 — Hormone concentrations in the different phases of the menstrual cycle. EFP indicates early follicular phase; LFP, late follicular phase; MLP, midluteal phase. *Different from the other phases ($P < .001$); **Different from the EFP ($P = .002$).

Table 1 Circumferences, Range of Motion, and Countermovement-Jump Height Throughout the Menstrual Cycle, Mean (SD)

	Early Follicular Phase	Late Follicular Phase	Midluteal Phase	Total
Thigh circumference				
Baseline	51.95 (3.82)	51.56 (4.19)	51.67 (3.74)	51.73 (3.92)
24 h	52.04 (3.82)	51.74 (4.34)	51.68 (3.81)	51.82 (3.99)
48 h	52.10 (3.77)	51.70 (4.18)	51.75 (3.90)	51.85 (3.95)
Total	52.03 (3.81)	51.66 (4.24)	51.70 (3.82)	
Calf circumference				
Baseline	35.02 (1.50)	35.01 (1.66)	34.97 (1.61)	35.00 (1.59)
24 h	34.98 (1.54)	34.98 (1.55)	34.93 (1.65)	34.96 (1.58)
48 h	34.98 (1.51)	34.94 (1.57)	34.96 (1.69)	34.96 (1.59)
Total	34.99 (1.52)	34.98 (1.59)	34.95 (1.65)	
Hip range of motion				
Baseline	82.00 (14.85)	85.00 (17.12)	80.94 (15.90)	82.65 (15.96)
24 h	80.39 (11.12)	83.94 (13.60)	82.06 (15.13)	82.13 (13.28)
48 h	80.17 (13.64)	81.78 (15.18)	79.72 (14.71)	80.56 (14.51)
Total	80.85 (13.20)	83.57† (15.30)	80.91 (15.25)	
Knee range of motion				
Baseline	55.47 (11.99)	57.53 (12.71)	59.21 (11.62)	57.40 (12.11)
24 h	55.37 (10.68)	59.11 (9.92)	61.37 (11.33)	58.61* (10.64)
48 h	53.56 (12.05)	56.11 (9.80)	56.74 (9.03)	55.47 (10.29)
Total	54.80 (11.57)	57.58 (10.81)	59.11 (10.66)	
Countermovement-jump height				
Baseline	25,70 (5.34)	25.73 (5.47)	26.40 (6.21)	25.94 (5.67)
0 h	21.47 (4.50)	22.10 (4.86)	22.69 (4.83)	22.10** (4.73)
24 h	24.25 (5.51)	24.55 (5.61)	25.65 (6.47)	24.82*** (5.87)
48 h	25.24 (6.08)	24.62 (5.27)	25.79 (6.15)	25.22 (5.84)
Total	24.17 (5.36)	24.25 (5.30)	25.13 (5.91)	

*Different from 48 h ($P = .030$). **Different from the other time points ($P < .001$). ***Different from baseline ($P = .05$). †Trend ($P = .07$) to be different from early follicular phase and midluteal phase.

induced muscle soreness. In contrast, some studies did not report changes in muscle soreness at any postexercise time point in comparison to baseline.[22,23] All of these disparities in findings could not be attributed to different rating scales, as studies in general used a 100-mm visual analog scale [19,20] or 10-point scale, as reported in this study.[19,22,23] However, they may be attributed to differences in exercise modalities or the training status of participants. In addition, the previous literature barely includes both blood hormones confirmation and LH urine surge detection[9] in the methodology to verify menstrual cycle phases, hence impairing comparisons of participant's hormonal status between studies. As a result, further research is needed to confirm whether the EFP is the most pain sensitive phase and the possible relationship between sex hormones and pain mechanisms.

Regarding the rest of the parameters analyzed, only significant differences between time points were observed, indicating that our squat-based protocol elicited muscle damage. The previous literature is in agreement with our lack of significant differences between phases in CMJ.[24] In addition, another study assessed muscle function and fatigability in the same phases as in our study, and no differences between menstrual cycles phases were observed.[25] Despite no exercise protocol being performed in these studies, it could be suggested that hormone fluctuations during the menstrual cycle may not influence CMJ in terms of both performance and as a muscle damage or fatigue marker on the basis of these findings. Moreover, as in our study, no significant differences were found in 1RM, and sex hormones may not either influence maximum strength. However, other factors could be influencing CMJ and 1RM, such as technique or the fact that both exercises are highly related to nervous system response. In our study, CMJ was reduced 24-hour postexercise in comparison with baseline, which was inconsistent with other studies reporting reduced CMJ height at 48-hour postexercise.[26,27] However, the findings are not entirely comparable as they only evaluated the MLP and used a different exercise protocol to induce muscle damage.

Not many studies in the literature have evaluated decreases in ROM after damaging exercises throughout the menstrual cycle. Our findings showed a higher ROM, and as a result, worse mobility 24-hour postexercise in comparison with 48-hour postexercise for knee ROM, suggesting that the impairment observed 24 hour postexercise recovered 48-hour postexercise, independent of the menstrual cycle phase. This could be associated to muscle soreness, as women used to report higher soreness 24 hour rather than 48-hour postexercise, although no significant differences were

Table 2 Percentange Changes Both Between Time Points and Phases for Circumferences, Range of Motion, Countermovement-Jump Height, and Muscle Soreness, Mean ± CL

Time effects			Phase effects		
	% Change	ES		% Change	ES
Thigh circumference					
Baseline—24 h	0.17 ± 0.19	0.03	EFP—LFP	−0.74 ± 1.19	0.10
Baseline—48 h	0.25 ± 0.30	0.02	EFP—MLP	−0.59 ± 1.49	0.08
24–48 h	0.06 ± 0.27	0.01	LFP—MLP	0.16 ± 1.27	0.02
Calf circumference					
Baseline—24 h	0.11 ± 0.17	0.02	EFP—LFP	0.09 ± 1.81	0.01
Baseline—48 h	−0.12 ± 0.14	0.03	EFP – MLP	0.02 ± 1.81	0.03
24–48 h	−0.01 ± 0.17	0.00	LFP—MLP	−0.07 ± 0.29	0.02
Hip range of motion					
Baseline—24 h	0.12 ± 3.18	0.00	EFP—LFP	3.20 ± 3.69	0.15
Baseline—48 h	−8.66 ± 4.06	0.09	EFP—MLP	−6.84 ± 3.56	0.02
24–48 h	−8.81 ± 2.41	0.15	LFP—MLP	−9.59 ± 2.80	0.17
Knee range of motion					
Baseline—24 h	2.71 ± 3.73	0.15	EFP—LFP	7.70 ± 9.31	0.27†
Baseline—48 h	−2.64 ± 4.68	0.25†	EFP—MLP	11.14 ± 11.71	0.50††
24–48 h	−5.10 ± 3.95	0.40††	LFP—MLP	3.32 ± 6.98	0.23†
Countermovement jump					
Baseline—0 h	−14.51 ± 3.54	0.70†††	EFP—LFP	0.72 ± 3.84	0.02
Baseline—24 h	−4.53 ± 1.87	0.21†	EFP—MLP	4.01 ± 3.21	0.16
Baseline—48 h	−2.79 ± 2.74	0.13	LFP—MLP	3.54 ± 3.06	0.14
0–24 h	12.32 ± 4.35	0.49†††			
0–48 h	14.28 ± 4.74	0.57†††			
24–48 h	1.86 ± 2.53	0.07			
Muscle soreness					
Baseline—24 h	26.84 ± 5.81	3.06†††	EFP—LFP	5.26 ± 6.09	0.34†
Baseline—48 h	22.63 ± 5.98	2.67†††	EFP—MLP	4.21 ± 5.63	0.33†
24–48 h	−4.21 ± 4.34	0.35††	LFP—MLP	−1.05 ± 5.30	0.01

Abbreviations: CL, confidence limit; EFP, early follicular phase; ES, effect size; LFP, late follicular phase; MLP, midluteal phase. Note: Also shown are ES and magnitude-based inference for the substantial changes in mean.
†Possibly, ††likely, †††most likely (positive or negative).

observed between these phases to confirm this statement. Other studies with women where knee ROM was evaluated after damaging cycling[28] or bench stepping protocols[29] did not test 24-hour postexercise measurement, but instead showed impairments in ROM 48-hour postexercise. However, none of these studies evaluated more than 1 menstrual cycle phase. In addition, further analysis of ROM should also include blood samples along the time points to confirm that ROM changes are not influenced by the levels of certain female hormones like estrogen or even relaxin.

As for ROM, there is little literature providing an assessment of perceived exertion during resistance protocols in women considering their menstrual cycle. In our study, no differences were observed between menstrual-cycle phases in perceived exertion through the exercise sets. In comparison, perceived exertion during aerobic exercise has been previously investigated, revealing higher values through the exercise in the EFP in sedentary women, while the lowest values were obtained in the MLP.[30] They suggest that the increase in perceived exertion could be due to the rising pain perception observed during the exercise. This was possibly triggered by the increasing pain sensitivity in the EFP as a consequence of the endogenous hormone drop. In fact, young female athletes also reported higher training monotony and training strain in the EFP than in the LFP also during an endurance protocol.[4] In addition, the time of recovery required was shorter in the LFP in comparison to the EFP and MLP. Altogether, this may indicate that lower concentrations of sex hormones could impair the predisposition of achieving a physical task but not necessarily the performance. Nonetheless, methodological aspects as differences in sample size or in participants' training status or hormonal profiles should be cautiously considered when exploring perceived exertion during exercise.[4]

Finally, our results did not show any significant differences between time points or phases in circumference measurements, which is in accordance with the previous literature.[27,29] Not only does this suggest that our protocol could not be strenuous enough to trigger significant inflammation or edema, but it also indicates that the participants' training status may be sufficiently adapted to not suffer from edema, even though the protocol was intense enough to elicit muscle damage.

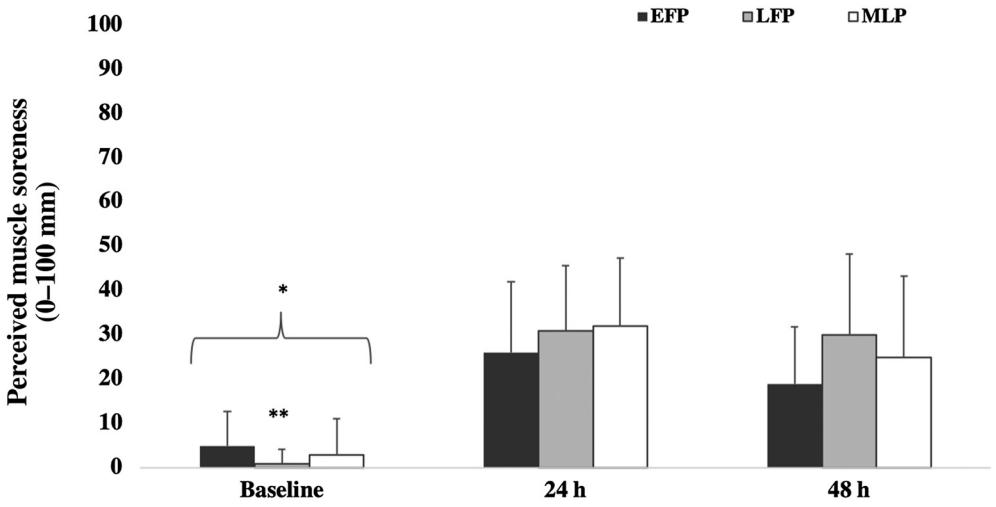

Figure 3 — Perceived muscle soreness in the different phases of the menstrual cycle (0 indicating no pain at all and 10 unbearable pain). *Different from the other time points ($P < .001$). **Different from the EFP ($P = .045$). EFP indicates early follicular phase; LFP, late follicular phase; MLP, midluteal phase.

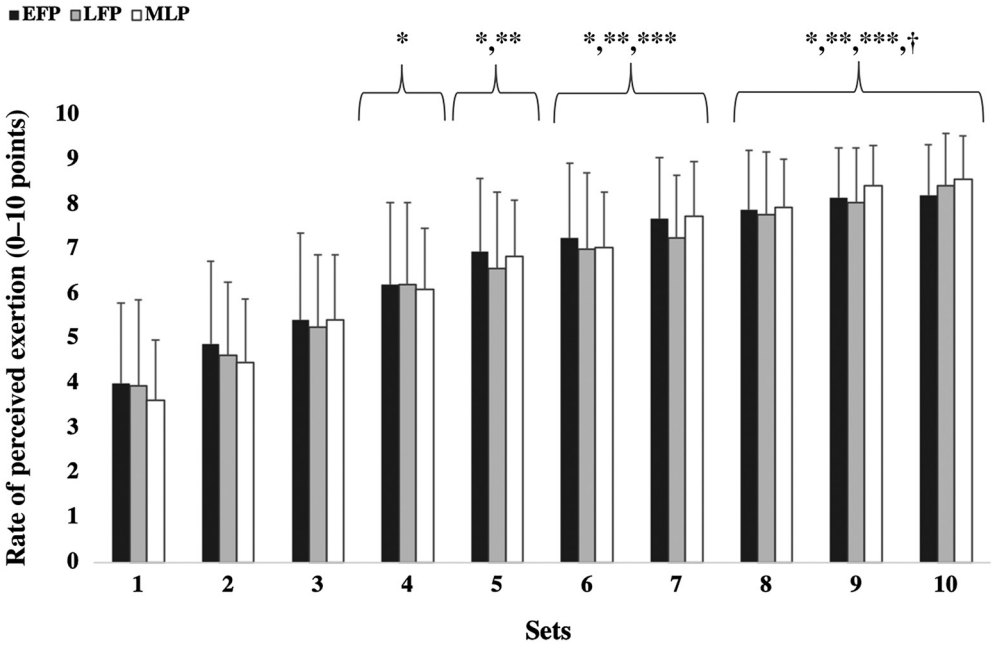

Figure 4 — Rating of perceived exertion during the eccentric-based resistance protocol. *Different from set 1 ($P < .001$); **different from set 2 ($P = .020$ for set 5 in the EFP; $P = .006$ for set 5 in MLP; $P = .003$ for the other comparisons); ***different from set 3 ($P < .001$); †different from set 4 ($P = .049$ for set 8 in the EFP; $P = .006$ for set 9 and 10 in the EFP; $P = .016$ for set 9 in the LFP; $P < .001$ for the other comparisons). EFP indicates early follicular phase; LFP, late follicular phase; MLP, midluteal phase.

Our study provides an approach toward exercise-induced muscle damage response throughout the menstrual cycle, evaluating some indirect markers with attainable tools and devices. In addition, to the best of our knowledge, it is the first study to evaluate the enrolled participants in all of the previously mentioned menstrual cycle phases, performing an exercise protocol that could belong to any female's training program, under a complete methodology for menstrual cycle phase verification,[9] consisting of the combination of 3 methods (blood analysis, urine-based ovulation kits, and calendar counting). However, it would be more accurate to measure sex hormone concentrations on a daily basis during the entire cycle. Furthermore, another possible limitation of the study is that despite the phase order being counterbalanced, the repeated bout effect has not been studied. Even though this effect could be less acute in our study due to the training status of our participants, it is a key aspect to consider in future studies. In addition, the inclusion of blood markers of muscle damage could provide helpful information about tissue damage[3] relevant to address this response.

Finally, another limitation could be that our squat exercise protocol may not have been strenuous enough to induce differences in markers between menstrual cycle phases in comparison to a less lifelike but more damaging exercise.

Practical Application

This study could provide useful information for training prescription to determine training loads and recovery times for female athletes, especially in phases where estrogen concentrations are lower and pain perception seems to be higher. As previously mentioned, an increasing number of women are engaging in sports and training; therefore, coaches should be provided with as many tools as possible to meet female sport requirements. According to this alleged increase in muscle soreness perception before exercise, dealing with an intense workout may seem initially less attainable during this phase but should not necessarily be accompanied by impairments in performance or higher acute muscle damage. Perceived side effects may be individually considered when possible to facilitate training achievement, especially when pain perception may be higher. An additional feature from this research could be the contribution to future investigations to show the markers with the greatest potential that are influenced by female sex hormone fluctuations and to focus on a deeper study of these responses.

Conclusion

The current results suggest that an eccentric squat-based exercise in well-trained females elicits muscle damage, which could be observed from soreness, ROM and CMJ postexercise changes in comparison to baseline. However, the sole marker that seems to be affected by hormone fluctuations during the menstrual cycle is muscle soreness. The lower estrogen concentration during the EFP resulted in a higher perception of muscle soreness before the start of the exercise bout. However, in postexercise time points, this pattern seems to change, so estrogen might not attenuate soreness related to exercise-induced muscle damage. Therefore, further research is needed to clarify whether menstrual disturbances affect this pain perception.

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