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Original Article

Changes in hamstring flexibility and muscle strength during the menstrual cycle in healthy young females

Manabu Miyazaki, RPT, MS[1, 2)*], Seiji Maeda, PhD[2, 3)]

[1)] *Department of Physical Therapy, Faculty of Medical Science for Health, Teikyo Heisei University: 2-51-4 Higashi-Ikebukuro, Toshima-ku, Tokyo 170-8445, Japan*
[2)] *Graduate School of Comprehensive Human Sciences, University of Tsukuba, Japan*
[3)] *Faculty of Sport Sciences, Waseda University, Japan*

Abstract. [Purpose] The purpose of this study was to elucidate changes in flexibility and muscle strength during the menstrual cycle in detail and to investigate the relationship between flexibility and muscle strength. [Participants and Methods] Sixteen healthy young female and eight male participants were measured during the follicular, ovulation and luteal phases. Range of motion, passive torque at the onset of pain, passive stiffness and muscle strength were measured using an isokinetic dynamometer. Additionally, electromyography was measured during muscle strength measurement. [Results] In the female group, range of motion and passive torque at the onset of pain were significantly increased during the ovulatory and luteal phases compared with the follicular phase. Passive stiffness decreased significantly during the ovulatory phase compared with the follicular phase. Isometric muscle force and electromyographic activity were significantly increased during the luteal phase compared with the ovulation phase. There was no correlation between stiffness and muscle strength. However, there was a positive correlation between electromyographic activity and muscle strength. [Conclusion] Our findings suggest that changes in flexibility during the ovulatory and luteal phases are influenced by fluctuations in sex hormones. However, the changes in muscle strength showed little relation to flexibility, suggesting the involvement of neural mechanisms.
Key words: Menstrual cycle, Flexibility, Muscle strength

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INTRODUCTION

In recent years, with the progress of female participation in society, female athletes have played an increasingly active role in sports. Consequently, the impacts of changes in sex hormones caused by the female menstrual cycle on flexibility, strength and performance have received substantial attention and have been widely discussed[1)]. The menstrual cycle is typically classified into the follicular phase (low estrogen, low progesterone), the ovulation phase (high estrogen) and the luteal phase (high estrogen, high progesterone) according to the fluctuation of ovarian hormones[2)]. These sex hormones have potential effects on exercise capacity and performance through various mechanisms, including substrate metabolism, cardiorespiratory function, thermoregulation and psychological factors[3)]. These changes may alter the incidence of sports injuries during the menstrual cycle.

Hamstring injury is one of the most common sports injuries. Previous studies have indicated that increased muscle stiffness[4)] and decreased muscle strength[5)] are associated with the risk of hamstring muscle injury. In particular, female have a lower risk of hamstring injuries than male, which has been reported to be related to estrogen-induced muscle stiffness. Estrogen has been reported to contribute to a decrease in muscle stiffness[6)] and to increase muscle strength[7)], and it is likely

*Corresponding author. Manabu Miyazaki (E-mail: m.miyazaki@thu.ac.jp)

that these indices fluctuate during the menstrual cycle. Range of motion (ROM) is used as an indicator of flexibility and includes not only stiffness, but also passive torque (PT) changes that reflect the pain threshold of extension stimulation[8]. Progesterone has been reported to have a pain-suppressing effect[9] and fluctuations in PT may contribute to increased ROM. However, to the best of our knowledge, no studies have investigated changes in flexibility of the hamstrings over time during the menstrual cycle, and there is currently not sufficient evidence for clinical applications. On the other hand, among previous studies examining the effects of the menstrual cycle on maximal muscle strength (isokinetic contraction) of the hamstrings, one study reported that the menstrual cycle did not affect the maximal muscle strength[10], whereas another study reported that muscle strength decreased during the follicular phase[11]. Thus, this issue remains to be clarified. Potential reasons for the discrepancy between previous studies include differences in the day of ovulation, the biphasic nature of the body temperature and a failure to take associated menstrual symptoms into account, including premenstrual dysphoric disorder (PMDD).

The objectives of the current study were: 1) to determine the details of changes in hamstring flexibility during the menstrual cycle; 2) to determine the effect of the menstrual cycle on hamstring muscle strength; and 3) to examine the relationships between changes in flexibility and changes in muscle strength. Clarifying these changes could inform the development of training programs and injury prevention programs that take the menstrual cycle into account, with potential practical applications in sports. We hypothesized that: 1) stiffness decreases during the ovulatory and luteal phases, when estrogen is high, and PT during pain onset increases during the luteal phase, when progesterone is high; 2) muscle strength decreases during the ovulatory phase, when stiffness is expected to decrease; and 3) there is a correlation between stiffness, a measure of flexibility, and muscle strength.

PARTICIPANTS AND METHODS

We conducted an experiment with a repeated-measures design. Participants underwent measurements on 3 days, in the follicular, ovulation and luteal phases. The criterion measures consisted of ROM of passive knee extension, PT at the onset of pain, passive stiffness, isometric muscle force and electromyography (EMG) activity. All participants attended a familiarization session before the first testing day.

G*Power (version 3.1.9.6 for macOS; Heine-Universität, Düsseldorf, Germany) was used to estimate the sample size required for this study. In a similar previous study[12], the effect size (Cohen's d) that showed a significant difference in ROM between the follicular phase and the ovulation phase was 0.89. Therefore, the required number of participants in the current study was estimated at 12, with an alpha level of 0.05, power of 0.80 and d of 0.89. To allow for possible dropouts, 22 healthy females were recruited to participate in this study. Additionally, eight healthy males who do not perform regular exercise were recruited to participate in the study as a control group. The inclusion criteria selected females with a normal and consistent menstrual cycle (25–35 days between menses[13]). The exclusion criteria for females were: lower extremity joint contractures, history of surgical operation on the back or lower extremities, neurological disorders, PMDD, current regiment of hormones or muscle-affecting drugs, history of oral birth contraceptive pill use, engagement in competitive sports and regular resistance, aerobics or flexibility training. Participants were asked to refrain from vigorous physical activity during the experimental period. This study was approved by the Ethical Committee for Research on Human Subjects at Teikyo Heisei University (approval number: 28-128). All participants were informed about the purpose of the study and the experimental procedures prior to participation in this study. All participants gave written informed consent to participate in the study.

Female participants measured their body temperature using a basal thermometer (WOMAN°C531; Terumo Corp., Tokyo, Japan) from two cycles before the experiment. Participants were instructed to measure body temperature under the tongue in a supine position immediately after waking up each morning. Additionally, before measurement, participants were asked to complete a questionnaire using the PMDD evaluation scale[14]. Participants with confirmed biphasic basal body temperature and no PMDD were recruited and their flexibility was measured. The measurement was performed in the following order: follicular phase, ovulatory phase, then luteal phase. The follicular phase was measured within 3 days after the end of menstruation, and the luteal phase was measured 6–8 days before the next scheduled start of menstruation. The ovulation phase was measured 2–3 days after the ovulation test was positive because the probability of ovulation within 2 days of a positive test is reported to be 91.1%[15]. Participants were given a urine-based ovulation predictor kit (dotest LHa; ROHTO Pharmaceutical Co., Ltd., Osaka, Japan) and tested themselves daily, starting 17 days before the next scheduled menstrual period. Participants were instructed to contact the investigator when the test strip showed positive ovulation. In contrast, measurements for males were conducted three times in total with one week between each measurement.

The experiment was performed in a university laboratory with the room temperature maintained at 26°C throughout the study. On the basis of the findings of previous studies[16], we used a sitting position that has previously been shown to efficiently stretch the hamstrings (Fig. 1). Participants were seated in an isokinetic dynamometer (Primus RS; BTE Technologies, Hanover, MD, USA) with the seat raised maximally. A wedge-shaped cushion was inserted behind the trunk, and the angle between the backrest and the seat surface was set to approximately 60°. Each participant's chest, pelvis and distal right thigh were stabilized with Velcro straps. The knee joint was aligned with the axis of rotation of the isokinetic dynamometer. The lever arm attachment was placed just proximal to the medial malleolus and stabilized against the limb with straps. When participants sat in the chair, their knee was extended passively at 5°/sec to the point of maximum knee extension just before the onset of pain. We used EMG to confirm that the hamstrings did not contract during this passive knee extension. With the

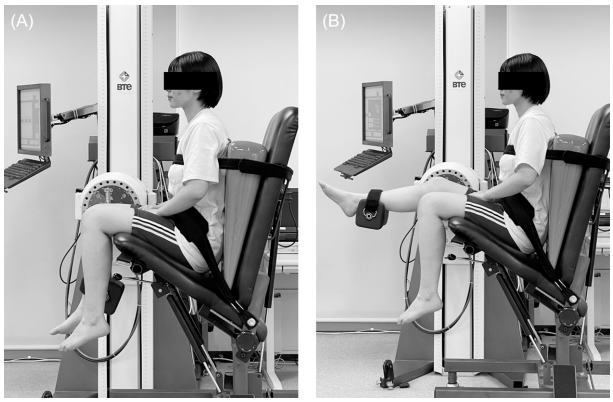

Fig. 1. Positioning for flexibility measurement. (A) Starting position. (B) The knee is extended passively until the participant experiences the onset of pain.

isokinetic dynamometer programmed in continuous passive motion mode, the torque and angle signals were continuously measured and recorded. Gravity correction was not performed when the torque-angle curve was measured, in accord with previous protocols[16]. ROM (°) was defined as the maximum knee extension angle from the initial position (0°), and PT at the onset of pain (Nm) was defined as the torque at the onset of pain[16]. Passive stiffness (Nm/°) was defined as the slope of the regression line calculated from the torque–angle relationship using the least squares method[16]. Stiffness was calculated using the same knee extension angle range. The knee joint angle for which stiffness was calculated was 50% to 100% of the minimum knee joint extension angle among the three torque–angle curves of the follicular, ovulatory and luteal phases.

Isometric muscle force (Nm) was measured in the prone position with knee flexion of 90°. Peak torque was recorded. To avoid the effects of participant fatigue, only one test was performed. Maximal muscle strength was defined as the exertion of force at maximal effort for approximately 3 sec using an isokinetic dynamometer. The participants lay prone on a platform and the chest and pelvis were secured with non-elastic straps. The center of the right knee joint was aligned to coincide with the axis of rotation of the isokinetic dynamometer, and the right ankle was fixed to the lever arm attachment of the dynamometer. Verbal encouragement was given to the participants in all measurements.

EMG activity during the maximal isometric and concentric contractions was recorded from the lateral and medial hamstrings using a MyoSystem 1200 (Noraxon, Scottsdale, AZ, USA) device with a sampling frequency of 1 kHz. The skin under the electrodes was shaved, abraded and cleaned with alcohol before placement of the electrodes. The electrodes were Ag/AgCl sensors (Blue Sensor M-00-s, Ambu, Ballerup, Denmark), and the distance between the electrodes was set to 35 mm. The electrodes were attached to the lateral hamstrings at the center of the line connecting the ischial tuberosity and the lateral condyle of the tibia, and to the medial hamstrings at the center of the line connecting the ischial tuberosity and the medial condyle of the tibia. EMG signals during maximal isometric muscle force were stored on a PC, and root mean square (RMS) amplitude values for 100 ms intervals were calculated using software (MyoResearch, Noraxon). Data were measured in 3.0 sec starting 0.5 sec after the start of muscle force measurement by verbal instructions.

We confirmed the test–retest reliability values for all dependent variables by calculating intra-class correlation coefficients (ICC) and coefficients of variation (CV). Prior to data collection in the present study, we conducted a pilot study to examine the test–retest reliability for all dependent variables. The participants were eight males. The two tests were performed on two separate days. We calculated ICC and CV, and the results of the assessments showed that reliability was acceptable for all measures (ROM: 0.97 [ICC], 2.26% [CV]; PT at the onset of pain: 0.90, 5.84%; passive stiffness: 0.95, 6.83%; isometric muscle force: 0.98, 2.43%; RMS [lateral hamstrings]: 0.92, 7.72%; and RMS [medial hamstrings]: 0.97, 7.38%).

We assessed the normality of the data using the Shapiro-Wilk test. This test showed that the data of all participants were normally distributed. Thus, we applied parametric tests to all absolute values. We performed a one-way repeated-measures analysis of variance to identify significant differences between the follicular, ovulation and luteal phases. The Bonferroni method was used as a post-hoc test. In addition, we investigated the relationship between the rate of change in muscle strength and the rate of change in stiffness and muscle activity based on the ovulation phase using Pearson's product moment correlation coefficient. Statistical analyses were performed using statistical software (SPSS Statistics 27, IBM Corp., Armonk, NY, USA). The statistical significance level was set at $p<0.05$. All results are expressed as mean ± standard deviation (SD).

RESULTS

During the data measurement of this experiment, four participants did not test positive for ovulation. Additionally, two participants did not show biphasic basal body temperature in the measured cycle. Data from these participants were excluded and analysis was performed on data from 16 female participants (mean ± SD: age 21.0 ± 1.0 years, height 159.5 ± 4.7 cm,

body mass 52.5 ± 5.0 kg, body mass index 20.6 ± 1.9 kg/m^2). In addition, the results of the questionnaire using the PMDD evaluation scale revealed that no participants exhibited PMDD. The mean duration of menstrual cycle was 31.1 ± 2.4 days overall. Measurements in the follicular, ovulatory and luteal phases in females were performed on 7.5 ± 1.6 days, 19.8 ± 2.5 days and 26.1 ± 2.2 days from the first day of menstruation, respectively. All participants conducted the measurements according to the protocols of this study. Furthermore, eight male participants (mean ± SD: age 21.1 ± 0.8 years, height 171.0 ± 6.9 cm, body mass 64.8 ± 10.8 kg, body mass index 21.1 ± 0.8 kg/m^2) completed all measurements.

The results of ROM, PT at the onset of pain, passive stiffness, isometric muscle force and RMS are shown in Table 1. In the female group, ROM and PT at the onset of pain increased significantly during the ovulatory phase compared with the follicular phase (η^2=0.79, p<0.05; η^2=0.38, p<0.05, respectively). Additionally, ROM and PT at the onset of pain increased significantly during the luteal phase (η^2=0.79, p<0.05; η^2=0.50, p<0.05, respectively) compared with the follicular phase. Passive stiffness decreased significantly during the ovulatory phase compared with the follicular phase (η^2=0.32, p<0.05). However, we observed no significant differences between the follicular and luteal phases (η^2=0.16, p=0.30). Isometric muscle force did not show any significant difference between the follicular and ovulatory phases (η^2=0.19, p=0.24). However, there was a significant increase in the luteal phase compared with the ovulatory phase (η^2=0.51, p<0.05). RMS of the lateral hamstrings exhibited a significant increase during the luteal phase compared with the ovulatory phase (η^2=0.59, p<0.05). However, RMS of the medial hamstrings was not significantly different in all phases (η^2=0.06, p=0.40). Additionally, there were no significant correlations in terms of relative change from the ovulation phase to the luteal phase between isometric muscle strength and passive stiffness, and isometric muscle strength and RMS in the medial hamstrings (r=−0.30, p=0.24; r=−0.08, p=0.75, respectively). However, we found a positive correlation between isometric strength and RMS in the lateral hamstrings (r=0.50, p<0.05). On the other hand, the male group, which was set as the control group, did not have significant differences in all the evaluation indices.

DISCUSSION

In the present study, we tested the hypothesis that an increase in ROM caused by increased estrogen during ovulation decreases passive stiffness, which in turn decreases isometric muscle strength. The results revealed that passive stiffness decreased significantly during the ovulatory phase compared with the follicular phase, whereas muscle strength did not differ significantly, and there was no correlation between changes in muscle strength and passive stiffness. These results provide partial support for our hypothesis, suggesting that the ovulation phase can have a positive effect on the decrease in passive stiffness without muscle weakness. This study was a repeated measures design, and the possibility of measurement bias had to be considered. The control group of males did not show significant differences in each of all the assessment indices. This result indicates that the variability of the evaluation indices due to the effect of repeated measurements is small.

During the ovulation period, when the blood concentration of estrogen is increased by 200–300 pg/mL, a luteinizing hormone surge occurs for 2–3 days[17] and ovulation predictor kits indicate a positive result. Furthermore, because there is

Table 1. Changes in range of motion, passive torque at the onset of pain, passive stiffness, isometric muscle force and mean value of root mean square of electromyography during the menstrual cycle

Outcome measure		Follicular phase	Ovulation phase	Luteal phase
Female group (n=16)				
ROM (°)		83.0 (8.8)	88.3 (8.6)*	89.6 (9.6)*
PT at the onset of pain (Nm)		23.5 (4.7)	25.0 (4.1)*	26.3 (5.0)*
Passive stiffness (Nm/°)		0.31 (0.06)	0.29 (0.04)*	0.30 (0.05)
Isometric muscle strength (Nm)		38.9 (9.9)	35.3 (8.2)	39.0 (5.8)†
Mean value of RMS-EMG (mV)	Lateral hamstrings	0.35 (0.09)	0.32 (0.10)	0.39 (0.12)†
	Medial hamstrings	0.42 (0.11)	0.40 (0.14)	0.43 (0.15)
Male group (n=8)				
ROM (°)		78.0 (10.7)	76.2 (14.4)	78.1 (15.4)
PT at the onset of pain (Nm)		30.9 (9.3)	30.9 (12.5)	30.8 (12.7)
Passive stiffness (Nm/°)		0.41 (0.11)	0.42 (0.11)	0.41 (0.09)
Isometric muscle strength (Nm)		63.4 (8.2)	61.6 (9.0)	61.7 (9.4)
Mean value of RMS-EMG (mV)	Lateral hamstrings	0.54 (0.21)	0.55 (0.29)	0.54 (0.26)
	Medial hamstrings	0.51 (0.24)	0.49 (0.22)	0.50 (0.18)

Values are presented as mean (standard deviation). The asterisk indicates a significant (p<0.05) difference from the follicular phase. The dagger indicates a significant (p<0.05) difference from the ovulation phase. ROM: range of motion; PT: passive torque; RMS: root mean square; EMG: electromyography.

a time delay of approximately 3 days from the increase in blood estrogen concentration to the change in joint laxity[18], we assumed that the ovulation period in the high estrogen state could be identified in this experiment.

A decrease in passive stiffness was observed during the ovulation period, which involved high estrogen concentration. In contrast to the current findings, Bell et al.[12] reported that active stiffness of the hamstrings did not change during the follicular and ovulatory phases. A possible reason for this discrepancy is that the sample size in their study was small (n=8) and the effect size was low, potentially leading to a lack of significant differences[12]. Estrogen is known to have receptors in skeletal muscle[19]. Moreover recent study reported a negative correlation between estrogen and active stiffness of hamstrings[6]. In the context of these reports, the results of the current study suggest that estrogen during the ovulatory period may decrease passive stiffness. In addition, in the current study, stiffness did not change during the luteal phase, in contrast to our hypothesis. This is because the estrogen concentration in the luteal phase is higher than in the follicular phase and lower than in the ovulatory phase[20], and therefore was not reflected in the passive stiffness.

In contrast to our expectations, PT at the onset of pain increased not only during the luteal phase but also during ovulation. The PT in the final ROM region is the pain threshold for stretch stimuli, reflecting stretch tolerance, and is an indicator of the maximum permissible change in stretch sensation for each participant[21]. Variations in stretch tolerance have been suggested to be caused by changes in the participant's pain sensation and the involvement of psychological factors[22], however the details of these factors have not been clarified. Previous studies in humans have reported that progesterone, which is secreted after ovulation, suppresses pain[23] and the central nervous system is involved in this process[24]. PT at the onset of pain in the current study is considered to reflect an unpleasant state of extension, although it does not cause pain. Because the secretion of progesterone increases immediately after ovulation and reaches its peak in the mid-luteal phase, the ovulatory and luteal phases measured in this experiment were considered to have higher progesterone levels than the follicular phase. Therefore, the discomfort associated with pain stimulation was reduced during the ovulatory and luteal phases compared with the follicular phase, suggesting that the awareness of pain was reduced.

The factors that increased ROM during the ovulation and luteal phases compared with the follicular phase may be related to different causes. The increase in ROM during the luteal phase resulted from an increase in ROM caused by changes in stretch tolerance. Furthermore, the increase in ROM during ovulation was caused by a decrease in passive stiffness, in addition to the change in stretch tolerance. Thus, increased ROM during the luteal phase does not reflect a change in passive stiffness, but a change in apparent flexibility caused by the habituation of pain.

Despite the hypothesis that muscle force decreases with decreasing passive stiffness, there was no significant difference in isometric muscle force during the ovulatory phase compared with the follicular phase. Previous studies have reported that changes in muscle strength involve a decrease in muscle activity[25], a change in the relationship between muscle length and tension[26], and a decrease in the efficiency of force conduction caused by reduced passive stiffness[27]. Additionally, previous studies have indicated that estrogen may contribute to contractile properties such as the number of cross-bridges in muscle cells and the force per cross-bridge[7, 28]. These previous findings suggest that the conflicting phenomenon of estrogen inhibiting muscle weakness may have occurred in the current study. However, the luteal phase was associated with a significant increase in muscle strength compared with the ovulatory phase. This increase in muscle strength was caused by the correlation between muscle strength and the muscle activity of the lateral hamstrings. Estrogen is thought to have a neuroexcitatory effect, which has been reported to reduce inhibition and increase spontaneous activation[29]. Therefore, the current findings suggest that estrogen may have increased muscle activity during the ovulation and luteal phase, resulting in increased muscle strength. However, in this study, myoelectric activity during the ovulatory phase did not show an increase compared with the follicular phase. This result may have been caused by bias in the study design, which was conducted in a non-randomized chronological order starting from the beginning of menstruation. Females who do not train regularly are more likely to learn and benefit from later tests if they are not accustomed to fitness testing[30]. In the current experiment, participants underwent an orientation period before the experiment, and the test was practiced. However, the possibility of such a bias cannot be ruled out.

Our study involved several limitations that should be considered. First, because we did not measure estrogen and progesterone, the relationship between the ways in which sex hormones affected flexibility and muscle strength remains uncertain. Janse de Jonge et al.[1] recommended using a combination of three methods to determine the phases of the menstrual cycle: calendar-based counting, using an ovulation day prediction test, and measuring serum estrogen and progesterone levels. Additionally, all participants were healthy young females who did not perform regular training. Kuwahara et al.[31] reported that when females continue training, the amount of female hormones secreted decreases even if they have a normal menstrual cycle, and the fluctuation range also decreases. The results of the current study may not enable comparisons of changes in athletes' flexibility and muscle strength during the menstrual cycle. Further studies will be required to measure sex hormones during the menstrual cycle of athletes and to examine the effects of flexibility, muscle strength and performance from various perspectives in addition to exercise physiology, including higher brain function, such as brain waves and cerebral blood flow.

In conclusion, we compared the flexibility of hamstrings and isometric muscle force during the follicular, ovulation and luteal phases of healthy young females. The results revealed that ROM increased in the ovulation and luteal phases compared with the follicular phase. Furthermore, although passive stiffness decreased during the ovulatory phase compared with the follicular phase, muscle strength did not change. In addition, isometric muscle force increased in the follicular phase compared with the ovulatory phase. This finding may have been caused by an increase in myoelectric activity. The current

results suggest that even though passive stiffness decreases during ovulation, muscle strength does not, and the level of exercise capacity in the follicular phase can be maintained in the other phases. Decreased muscle stiffness has been shown to reduce the risk of muscle injury[4], but also to decrease muscle strength[27] and exercise performance using stretch-shortening cycle such as sprinting[32]. However, in the current study, reduced passive stiffness during ovulation did not lead to muscle weakness, suggesting that decreased passive stiffness during the menstrual cycle is unlikely to reduce exercise performance. Additionally, motor performance may be altered by fluctuations in sex hormones.

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Conflicts of interest

All the authors declare that there are no potential conflicts of interest regarding this article.

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