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Greater Muscle Stiffness during Contraction at Menstruation as Measured by Shear-Wave Elastography

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It is important to measure mechanical properties of muscle, since muscle stiffness is an important component of stabilizing or controlling joint stability. The levels of sex hormones especially estrogen vary over the phase of the menstrual cycle and impact the mechanical properties of soft tissue such as muscle, tendon, and ligaments due to the presence of 17-β estradiol receptor in human connective tissues. Recently, shear-wave elastography (SWE), based on ultrasound imaging, has been used as an accurate technique for visualizing and assessing tissue stiffness. The purpose of this study was to compare the muscle stiffness at rest and during contraction condition between the early follicular phase (menstruation) and ovulation in young women, measured using SWE. Thirty-seven young women with regular menstrual cycles completed this study throughout one full menstrual cycle. Stiffness of lower limb muscles such as the rectus femoris, biceps femoris, tibialis anterior, and medial gastrocnemius was measured at resting and during contraction conditions using SWE during menstruation and ovulation. All muscles showed significantly greater stiffness during the menstruation than ovulation when muscles were actively contracted ($P < 0.05$), whereas no significant differences in muscle stiffness at rest were noted across phase of the menstrual cycle. These significant findings suggest that muscular factors are changed with estradiol fluctuations; muscles are less stiff during ovulation where the levels of estradiol peak when muscles in a contraction condition. As muscle stiffness is an important part of joint stability, these differences should be recognized to prevent the risk of musculoskeletal injuries.

Keywords: estrogen; menstrual cycle; muscle; shear-wave elastography; ultrasound
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Introduction

Skeletal muscles are primarily involved in the human body, supporting body structure and locomotor activity (Mannarino et al. 2019). In order to recognize the significance of muscle function, it is important to understand the mechanical properties such as tone, elasticity and stiffness of skeletal muscle (Green et al. 2012). It is also important to quantify mechanical properties, since muscle conditions such as pain, fatigue or cramps can be detected (Feng et al. 2018). Various disease states such as spinal cord injury, stroke, and muscular dystrophy have been shown to alter skeletal muscle mechanical properties (Green et al. 2012; Chen et al. 2017).

In human connective tissues, the 17-β estradiol receptor (Kjaer and Hansen 2008; Hansen et al. 2009; Silbernagel et al. 2015), a well-known estrogen receptor, alters properties of connective tissues such as the ligaments, muscles, and tendons according to the serum estrogen level (Petrofsky et al. 2013; Lee et al. 2014). In addition to the functional myosin and actin components, muscles contain densely packed collagen fibers. Sex-related differences in the role of estrogen in regulating muscle mass have been studied (Hansen et al. 2009; Lee et al. 2013). Higher estrogen levels result in decreased collaged formation and suppressed fibroblast proliferation, leading to muscle weakening (Yu et al. 1999; Lee et al. 2013). The resulting laxity of connective tissues is thought to increase the risk of musculoskeletal injuries during sporting activities by 2-8 times in women compared to that in men (Boden et al. 2010; Lee et al. 2013, Stijak et al. 2015), although a causal link has not been established. Nevertheless, muscle and tendon stiffness

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plays an important role in stabilizing and articulating joints; therefore, sex-related differences in joint stability owing to these mechanical properties may explain why women have a higher incidence of musculoskeletal injuries (Yim et al. 2018; Tas and Salkin 2019).

Muscle and tendon stiffness has been found to vary with menstrual cycle phase (Lee and Yim 2016; Yim et al. 2018). Fluctuations in 17-β estradiol levels and injury risk have also been linked, and these are thought to underlie women's greater susceptibility to injuries (Lee et al. 2013; Khowailed et al. 2015).

Yim et al. (2018) assessed the muscle tone, stiffness, and elasticity of the tibialis anterior (TA), peroneus longus (PL), and lateral gastrocnemius using myometry during different phase of the menstrual cycle in young women. Women had stiffer PL and TA muscles during the early follicular phase than during ovulation when the estrogen levels were the lowest (Yim et al. 2018).

Conventional grey scale and Doppler ultrasound techniques have been used for assessing various musculoskeletal tissues in the past, but recently, shear-wave elastography (SWE) has emerged with promising results in measuring mechanical properties and diagnosing pathological condition of muscles (Taljanovic et al. 2017; Kelly et al. 2018). SWE is a quantitative technique for analyzing tissue stiffness and also acts as a direct measurement tool (Carlsen et al. 2015). SWE can measure soft tissue stiffness and produce a visualized image. It is a non-invasive method of measuring the mechanical properties of deep tissues, and recently, SWE has been increasingly used to measure the stiffness of connective tissues such as muscles, tendons, and aponeuroses in biomechanics (Feng et al. 2018). In comparison with other imaging modalities, such as magnetic resonance imaging, SWE is cheaper, faster, more reliable, and more valid in measuring the mechanical properties of tissues (Andonian et al. 2016; Heales et al. 2018). SWE calculates the tissue stiffness using Young's modulus by examining the propagation velocity of the induced shear waves from a region of interest (ROI) in deep muscles, superficial muscles, and any other soft tissues (Chen et al. 2017; Feng et al. 2018; Caliskan et al. 2019; Siracusa et al. 2019).

One study examined the differences in muscle stiffness measured using SWE in each phase of the menstrual cycle, but researchers found no significant effect of the menstrual cycle phase in medial gastrocnemius muscle (MG) stiffness. However, the experiment was conducted with only eight women (Miyamoto et al. 2018). Given this limited and replicated evidence, it still remains unclear whether muscle stiffness changes occur during the menstrual cycle in young women.

The primary purpose of this study was to assess the differences in muscle stiffness at rest and during active voluntary contraction between the early follicular phase (menstruation) and ovulation in young healthy women, measured using SWE. We hypothesized that muscle stiffness will be higher both at rest and during active voluntary contraction during the early follicular phase than during ovulation.

Methods

Participants

A convenience sample of 46 physically active women volunteered to participate in this investigation. Data were collected between October and December of 2019 and participants were recruited through advertisements and posters on a bulletin board. All participants were healthy young women aged 18 to 30 years with normal and regular menstrual cycles over at least the past year. They were non-smokers and had a low to moderate self-reported physical activity level; participants were excluded if they regularly exercised more than 3 days per week. To reduce training bias and muscle fatigue, the participants were asked not to perform intense exercise for least 5 days before potential measurement days.

Participants were excluded according to the following criteria: body mass index (BMI) was < 18 or > 25 kg/m^2; a past medical history of cardiovascular disease, liver disease, or diabetes; use of oral contraceptives or other medicines that affect sex hormones; a history of pregnancy, abortion, or damage to the musculoskeletal system of the lower limbs; and a history of neurological abnormalities.

Hormonal assessment

All participants visited the laboratory twice for the study: first, during the early follicular phase (menstruation), which is between days 1 and 3 from the beginning of the menstrual cycle; second, during the ovulatory phase in which all participants used a Surearly digital ovulation test (Sugentech, Daejeon, Korea), which is > 99% accurate according to the manufacturer's manual.

Self-reported menstrual cycle: The menstrual cycle length was reported as the average value for the last 3 months. Based on the instructions in the ovulation kit, participants used the kit until a positive sign was detected. The research coordinator contacted participants a day before test commencement and instructed them to use the kit at the same time every day. For example, for a participant with a menstrual cycle length of 28 days, the test commencement date was 11 days after day 1. The specimen was taken either directly from the urine stream or from urine sample was collected in a clean tube. Drinking beverages and micturition (urination) were prohibited for 4 hours before taking the test, and participants were also asked to avoid using urine from the first micturition of the day. Once a positive sign was detected, the participants contacted the research coordinator and were scheduled for a visit within 3 days.

Salivary estradiol (E2) level: The levels of 17-β estradiol were analyzed from saliva specimens. All participants were given 15 ml conical tubes. On the day of data collection, participants collected at least 3 ml of saliva in the morning. They were instructed to collect the sample before or 30 minutes after brushing their teeth and to avoid con-

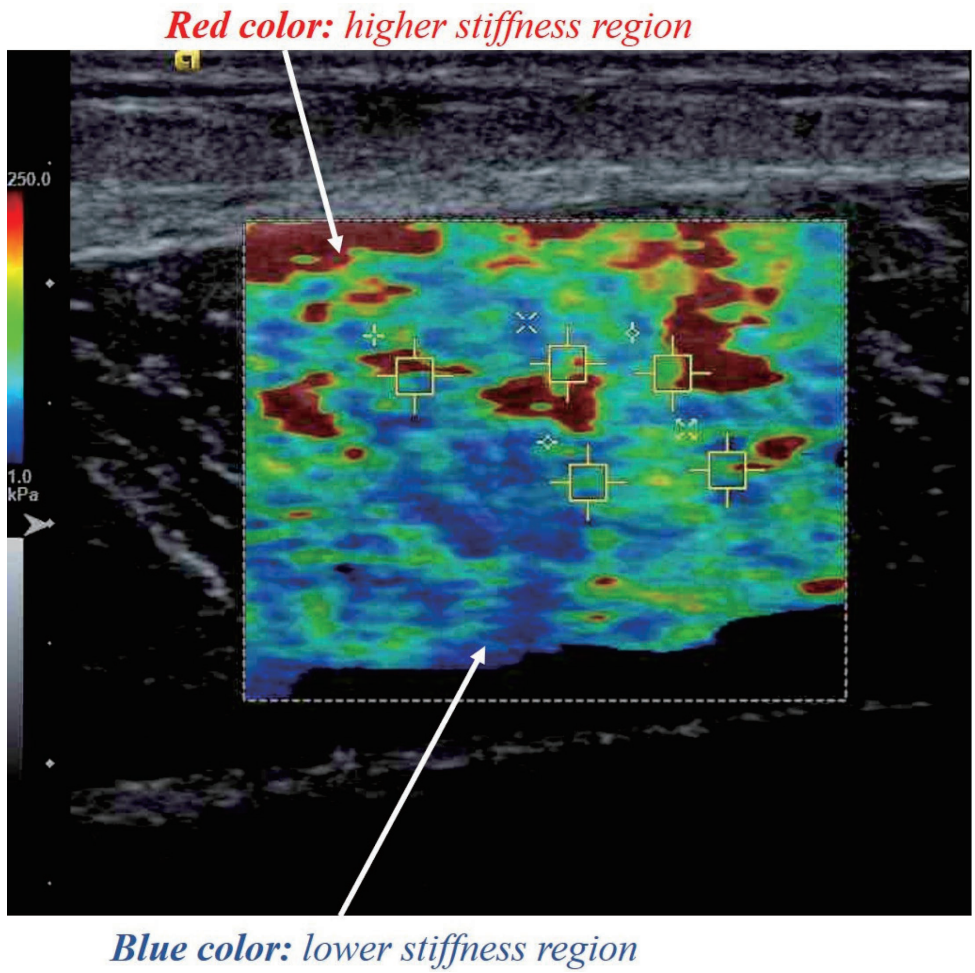

Fig. 1. The representative image for shear modulus measurement of medial gastrocnemius using shear-wave elastography. The scale for the color is provided to the left as estimated shear modulus (blue color indicates soft; red color indicates hardness)

suming food and drinks or smoking at least 30 minutes before collecting saliva, as this may compromise the sample by lowering the saliva pH. Collecting of the saliva first thing in the morning was recommended. Before saliva collection, the mouth was rinsed with cold water. Once a saliva sample was collected, it was stored at −30℃ until all samples of saliva were collected for the hormone analysis. After all samples of saliva were collected, 17-β estradiol levels were assessed using a 17-β estradiol saliva enzyme-linked immunosorbent assay (ELISA; Tecan. Switzerland).

Muscle stiffness

Shear-wave elastography: An ACUSON S3000 ultrasound device (Siemens Healthcare, Erlsangen, Germany) and 9-MHz linear probe were used for SWE measurements. SWE measurements were based on the acoustic radiation force impulse imaging technique, which can acquire color maps and measurements of stiffness of each tissue in the human body. This imaging technique allows real time quantitative analysis, and displacement is induced by push and detection pulses. Elastography images are represented in red and blue colors using the SWE imaging technique. The red color indicates relatively high tissue stiffness and blue indicates low stiffness. Fig. 1 shows an example SWE image obtained using the ACUSON S3000 ultrasound device. SWE allows the operator to set the ROI in a user-friendly manner, and this study used 5 ROIs.

Procedure

This study was approved by the Gachon University Institutional Review Board. All participants provided written informed consent at the beginning of the study. Once participants signed an informed consent, the researcher obtained their demographic data, including age, height, weight, menstrual cycle length, self-reported cycle, and presence of subjective dysmenorrhea. Then, one researcher marked measuring points on each participant's dominant lower extremity muscles before data collection. The investigator used anatomical landmarks and a measuring tape to establish accurate measuring points. Muscle stiffness was measured both with the muscle in active voluntary contraction and in a relaxation position. All participations were instructed on how to contract their muscles before measurement. When the muscles were properly contracted, partici-

pants were reinstructed on how to contract each muscle and the measurement was repeated three times.

Muscles

Rectus femoris (RF): In the supine position, stiffness of the resting rectus femoris (RF) was measured on the distal 1/3rd of the anterior superior iliac spine and patella. Contraction of the RF was performed with the participant seated on the edge of a table. Participants were asked to sit up with the arms across their chest and to perform 30-45° flexion of hip joint.

Biceps femoris; long head (BF): The distal 1/4th point between the posterior superior iliac spine and fibular head was marked in the prone position for measuring stiffness of the biceps femoris (BF). BF contraction was maintained at 30-45° of knee flexion.

Tibialis anterior (TA): Landmarks of the resting TA were marked on the proximal 1/3rd between the tibial tuberosity and medial malleolus in the supine position. TA stiffness at contraction was measured with maximum active dorsiflexion at the ankle.

Medial gastrocnemius (MG): The most prominently contracting point of the MG was marked. In prone position, the ankle was placed at the end of the table. Contraction of the MG was induced by active plantar flexion through the range of motion (ROM).

Reliability tests

The inter-operator reliability measurements for muscle stiffness were performed in randomly selected five participants. All measurements were performed two times at each phase of the menstrual cycle both at rest and during contraction.

Statistical analyses

G Power 3.1 software (Heinrich-Heine-University Dusseldorf, Düsseldorf, Germany) was used to calculate the sample size needed for the study. Based on a previous study, an effect size of 0.48 between the two menstrual cycle phases was applied (Yim et al. 2018), with an alpha error probability of 0.05 and a power of 0.90. A sample size of 40 participants was required to provide a statistical power of 90.65%. Taking into account potential dropouts, 46 participants were recruited.

SPSS 23.0 software for Windows 10 (IBM Corp., Armonk, NY, USA) was used to analyze the data, which were summarized as means and standard deviations. The assumption of normality of distribution of continuous variables was tested using the Kolmogorov-Smirnov test. A paired *t*-test was used to compare the mean estradiol level and muscle stiffness obtained by SWE between the early follicular phase and ovulation at each measurement site. To assess inter-operator reliability, the two-way random, absolute agreement, average measured infraclass correlation coefficient (ICC) was calculated using mean stiffness measured by each operator. The level of significance was set at $\alpha = 0.05$.

Results

Thirty-seven young healthy women completed the study. We recruited 46 participants; however, six did not meet the inclusion criteria and three withdrew because of scheduling conflicts. The general characteristics of the participants are described in Table 1.

Estradiol levels were significantly different between the early follicular phase and ovulation (9.0 ± 2.6 pg/ml and 11.7 ± 4.1 pg/ml, respectively, 95% confidence interval: 1.48 to 3.89, $P < 0.001$; Fig. 2).

Fig. 3 shows representative images for each muscle. When the muscle was in the relaxed position, no significant difference in mean stiffness of the RF, TA, BF, and MG as measured by SWE was noted between the two phases of menstrual cycle ($P > 0.05$). By contrast, all muscles showed significantly higher stiffness in the early follicular phase than during ovulation when the muscles were contracted (RF: $d = 0.51$; TA: $d = 0.84$; BF: $d = 0.90$; MG: $d = 0.70$, all $P < 0.05$; Table 2).

Inter-operator reliability of the SWE measurements of muscle stiffness

The inter-operator reliabilities of the stiffness measurements of all four muscles measured with SWE were good to excellent at rest and during active contraction. The inter-operator reliabilities of the ICC were 0.88 to 0.97 at rest and 0.79 to 0.91 during contraction in the early follicular phase, and 0.86 to 0.95 at rest and 0.70 to 0.87 during contraction in the ovulatory phase.

Discussion

Recently, many researchers have focused on determining sex-related differences in the musculoskeletal system that may be risk factors for musculoskeletal disorders during physical activity in women (Lee and Petrofsky 2018; Stanev and Moustakas 2019; Tas and Salkin 2019). In the present investigation, we aimed to assess the differences in muscle stiffness at rest and during active voluntary contraction between the early follicular phase and ovulation in young healthy women, as measured using SWE.

Very few existing studies have assessed muscle stiffness changes with SWE during the menstrual cycle in

Table 1. General characteristics of participants.

	Women (n = 37)
Age (years)	21.24 ± 1.28
Height (cm)	161.12 ± 4.78
Weight (kg)	53.78 ± 6.21
BMI (kg/m^2)	20.67 ± 1.85
Cycle (day)	29.9 ± 3.43
Subjective dysmenorrhea	4.7 ± 2.95

BMI, body mass index.

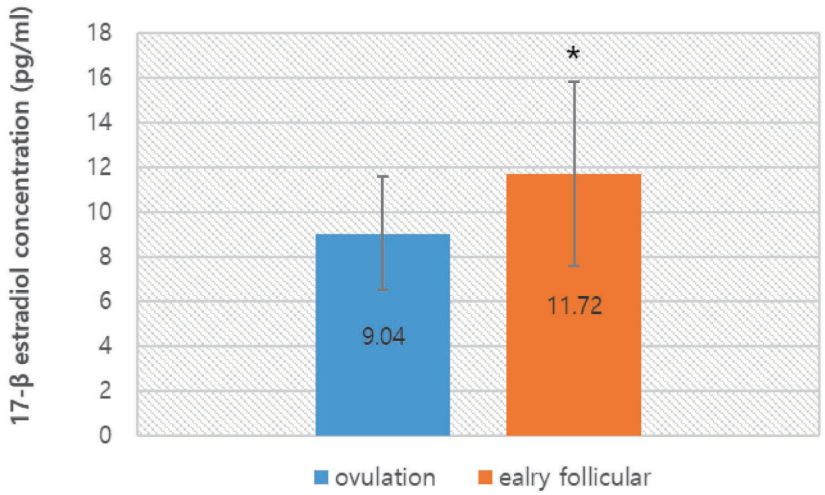

Fig. 2. 17-β estradiol concentration during ovulation and the early follicular phase (n = 37).
*significant higher in 17-β estradiol level at early follicular phase than ovulation.

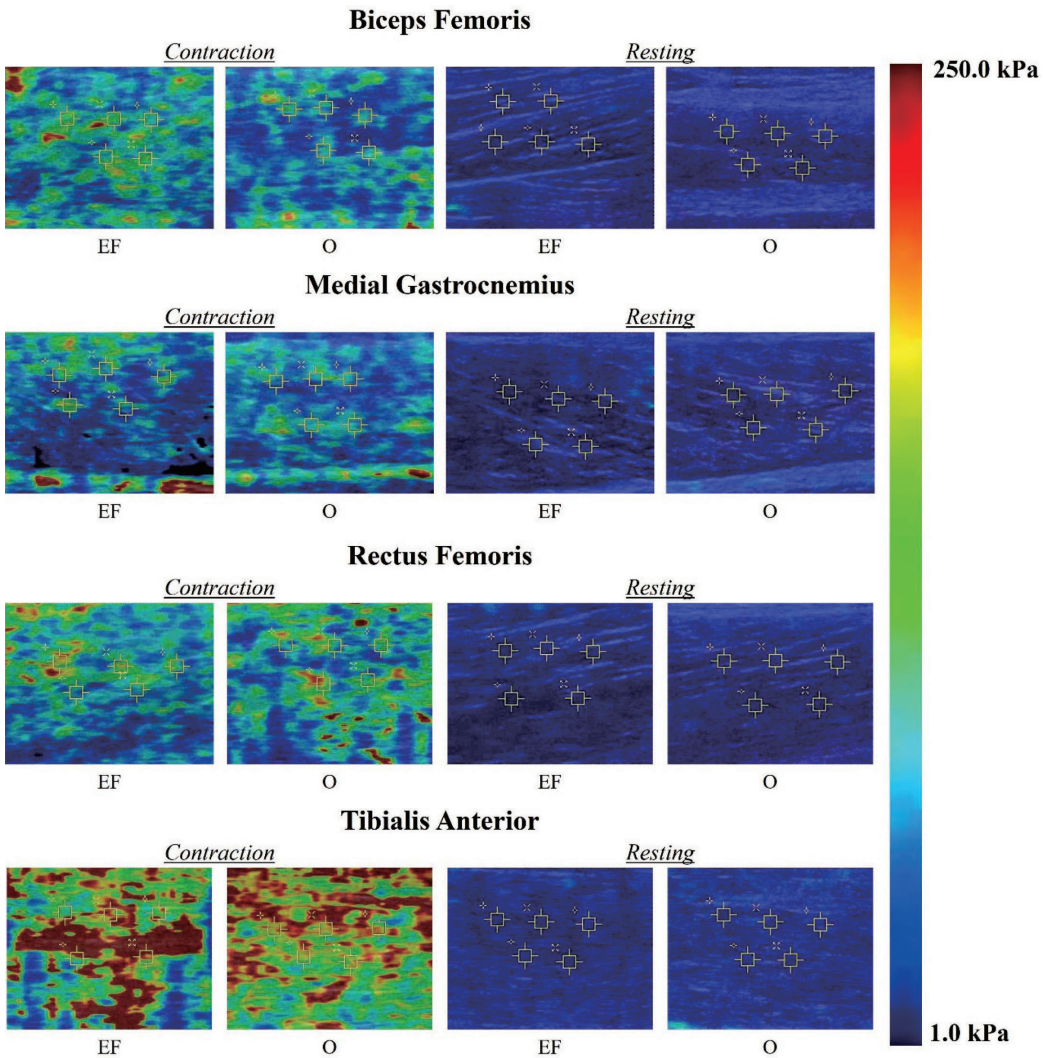

Fig. 3. The typical examples of shear-wave elastography measurements of rectus femoris, and tibialis anterior obtained at rest and during active voluntary contraction at the early follicular phase and ovulatory phase.
The colored area represents the shear modulus map with the scale next to the images.
EF, early follicular phase; O, ovulation.

Table 2. Differences in muscle stiffness measured using SWE between the early follicular phase and ovulation (n = 37).

Muscle		SWE (kPa) (Mean ± SD)			
		Early follicular phase	Ovulation	95% CI	Cohen's *d*
Rectus Femoris	R	12.8 ± 2.6	11.6 ± 2.6	−0.06 to 2.55	0.32
	C	82.2 ± 31.8	67.3 ± 14.8	5.04 to 24.7	0.51**
Tibialis Anterior	R	18.8 ± 5.2	18.1 ± 4.3	1.19 to 2.78	0.13
	C	149.4 ± 31.9	122.2 ± 36.7	16.39 to 38.07	0.84**
Biceps Femoris	R	11.7 ± 3.3	11.3 ± 2.2	−0.89 to 1.68	0.10
	C	75.9 ± 12.8	58.7 ± 17.3	10.85 to 23.6	0.90**
Medial Gastrocnemius	R	11.0 ± 2.7	10.8 ± 2.5	−0.66 to 1.15	0.09
	C	69.8 ± 17.8	59.2 ± 12.1	0.46 to 19.24	0.70*

R, resting; C, contraction; CI, confidence interval.

**significant difference between the early follicular phase and ovulation ($P < 0.01$).

*significant difference between the early follicular phase and ovulation ($P < 0.05$).

young women. To our knowledge, this is the first adequately powered study to assess the muscle stiffness during the menstrual cycle, using SWE, in order to determine if there is any effect of 17-β estradiol level on muscle stiffness. SWE measures muscle stiffness, and this can be graphically represented as a color-coded image. In addition, SWE measures the mechanical properties of the deep tissues while myometry can only assess the superficial tissues.

The study results indicated muscle stiffness of all four muscles of interest was similar both tested phases of the menstrual cycle with the muscles at rest; however, all muscles showed higher stiffness in the early follicular phase than during ovulation when tested during active voluntary contraction. The results of the present study do not agree with previous findings of stiffer PL and TA muscles in the early follicular phase in the resting position (Yim et al. 2018). This might be because the MyotonPRO, which was used for measurement in the previous study, has a 3-mm-diameter probe that assesses smaller ROI than the linear ultrasound probe used in this study. The main disadvantage when evaluating SWE images is that the evaluation results are highly dependent on the ROI setting. To mitigate this limitation, a software-based color mapping program that can extract red, green, and blue regions must be developed.

Another factor that should be considered when measuring stiffness with SWE is stiffness heterogeneity in different type of tissues. According to the previous studies, higher heterogeneity was observed between fat tissue and glandular tissue in breasts. Heterogeneity of fat tissue itself correlates with BMI and glandular elasticity heterogeneity. Even though a weak negative correlation was found between the heterogeneity of glandular or fat tissue and elasticity (Rzymski et al. 2011a, b, c), difference in tissue types should be considered when measuring stiffness using SWE. The BMI of participants in the present study ranged from 18.54 to 24.03 kg/m^2 which is considered ideal. However, further studies should measure fat and/or skin thickness and use a controlling variable for more accurate results.

The present study has some limitations. First, measurements were compared only between two phases of menstrual cycle even though the 17-β estradiol levels were evaluated using saliva samples to confirm the menstrual cycle phase. More frequent testing sessions may show more precise hormonal effects on muscle stiffness. Second, active voluntary contraction was not measured quantitatively using a dynamometer or joint angles. Because of this, contraction intensity was not strictly controlled, and these factors can independently influence muscle stiffness. Differences in activation levels and recruitment patterns of muscles may have led to ambiguous results; however, our intention was to observe the differences between the rested and actively contracted muscle states under real-world conditions.

In summary, the present study showed that the stiffness of actively contracting lower limb muscles was significantly different between the two phases of the menstrual cycle. As muscle stiffness is an important component of stabilizing or controlling joint stability, health professionals and athletic trainers should be aware of these changes to prevent injuries. Finally, this study lays the groundwork for further clinical research into various changes in and conditions of women with or without musculoskeletal injuries and also establishes general methodological guidelines for further studies.

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Conflict of Interest

The authors declare no conflict of interest.

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