INTRODUCTION

Women are increasingly participating in sports activities of all kinds, both amateur and professional (1). For instance, women made up 26% of all athletes who participated in the 1988 Seoul Olympics and 45% in the 2016 Rio Olympics. This number is expected to increase to 49% in the 2021 Tokyo Olympics (2).

With increasing female participation in sports, gender differences in exercise performances and development have become of interest. Anatomical, physiological, endocrinological, and psychological factors could explain the differences between male and female athletes' physical performances, and menstrual cycle is especially prominent (3).

Keywords: Female athlete, menstrual cycle, sports performance
The menstrual cycle is divided into three phases: the early follicular phase with low estrogen and progesterone, the ovulatory phase with high estrogen and low progesterone, and the midluteal phase with high estrogen and high progesterone (4). Serum luteinizing hormone (LH), follicle-stimulating hormone (FSH), estradiol (E2), and progesterone (Prog) concentrations fluctuate over the menstrual cycle, and androstenedione and testosterone levels peak immediately before and during ovulation (5). These hormones primarily regulate the reproductive cycle but also impact various physiological functions, which may translate into altered physical performance (6).

Estrogen has anabolic influence on skeletal muscles and is argued to improve endurance by altering carbohydrate, fat, and protein metabolisms. Progesterone, on the other hand, has anti-estrogenic effects. For type-1 muscle fibers, estrogen increases glucose uptake and availability during short-term exercise, while the opposite is observed with progesterone (6).

Studies concerning the effect of menstrual cycle on exercise performance have yielded various and inconsistent results. Clarifying the changes in performance according to the phases of the menstrual cycle, and organizing athlete training programs according to the effects, including them in acute/chronic workload calculations may be effective in protecting female athletes from injuries. For this purpose, it was aimed to examine the effects of menstrual cycle phases on physical fitness parameters. The hypothesis of the research is that the effect of phase changes on physical fitness components, flexibility, and time from peak effort to relaxation would be in the direction of pleasure.

MATERIALS and METHODS

After the study was approved by the local ethics committee (date 29/11/2019 number 323), voluntary and healthy women who conformed to the inclusion criteria were recruited. The study included healthy women with regular normal-length (21-35 days) menstrual cycles in the last six months. The exclusion criteria were as follows: oligo-, poly- or amenorrhea or metromenorrhagia, use of Depo-Provera, Norplant or intrauterine medication in the last 12 months, oral contraceptive use or hormone therapy in the last three months, endometriosis, polycystic ovary syndrome, uterine fibroids, body mass index <18 kg/m² or >35 kg/m², smoking, alcohol or illegal drug use, a calorie-restricted diet, pregnancy, musculoskeletal disease in the last six months, heart disease, inflammatory or autoimmune diseases, diabetes, any endocrine disorder (e.g. hypo-/hyperthyroidism, etc.), anemia, or using medication or nutritional supplements.

Physical activity was evaluated using the "International Physical Activity Questionnaire-Short Form" (IPAQ-SF) (7). Participants' menstrual data (cycle length, date of the last period, etc.) were recorded. Cross-randomization was performed in multiple performance tests.

Determining the phase of menstrual cycle: The early follicular phase was estimated to be cycle days 2-5, and the midluteal phase was defined as days 4-8 after ovulation. Ovulation was determined using home LH ovulation cassette tests (Laboquick, Izmir, Turkey) that can detect LH hormone in urine just in minutes with a sensitivity of 30mIU/ml (8). Participants were provided with five disposable cassettes. Starting from two days before estimated ovulation, LH levels were tested using urine samples retrieved after 10 AM for five days. The 24-hour period after a positive test was accepted as the day of ovulation (9).

Determining body composition: Height, body weight (SECA 700, Germany), waist circumference, and hip circumference were measured (10). Four skinfold measurements (biceps, triceps, subscapular, and suprailiac) were made using a caliper (Baseline® Skinfold Caliper, 12-1110, United States). Body fat ratio was calculated by averaging the results as described by Yuhasz (11) and Siri (12).

Before the testing session started, the participants were allowed a 5-min warm up at light intensity (less than 50 W). Tests were performed in the order of Y-balance test, 20-m shuttle run test, vertical jump, hexagon agility test, isokinetic muscle test, sit-and-reach test and Wingate anaerobic capacity test. Between each test, the participants rested for 15 minutes, and for 30 minutes before the isokinetic- and Wingate tests. Participants had access to water and carbohydrate compounds during their rest periods. They were verbally motivated during the tests. All measurement procedures were applied in the same way in both phases.

Sit-and-reach test: It was used to assess flexibility by doing as described in the literature (13). The test was repeated twice and the average value was recorded.

Y balance test: It was used to assess dynamic balance by doing as described in the literature (14). The test was repeated three times in each direction, and the highest score was recorded. Total score was calculated using the following formula:

\[
\text{Total score} = \frac{(\text{anterior} + \text{posteromedial} + \text{posterolateral})}{(3 \times \text{lower limb length}) \times 100}
\]

Hexagon agility test: It was used to assess agility by doing as described in the literature (15). After 5-min rest, the test was repeated and the average of the two results was recorded.
**Vertical jump test:** The test was repeated three times and vertical jump distances were recorded and averaged. Anaerobic power was calculated using the formula described by Lewis (16).

**20-m shuttle run test** To determine endurance, participants were asked to run a 20-m distance back and forth until exhaustion (17). The test was terminated when the participants were ‘beeped’ twice in a row, when they could not catch the run, or stated that they were exhausted.

**Wingate anaerobic capacity test:** The participants were asked to perform for 30 seconds against external resistance of 7.5% of their body weight on a cycle ergometer (Wingate Test System 894E) (18).

**Isokinetic muscle test:** Before starting the test, the participants were allowed two tries at 60°/s and 180°/s for adaptation. For the determination of muscle strength, the isokinetic test protocol (Isoforce, Germany) (19) was applied to both knees five times at an angular velocity of 60°/s and 20 times at an angular velocity of 180°/s.

**Statistical analysis**
Data were analyzed using the SPSS v23 software package. Data were evaluated using frequency and descriptive statistical analysis. Upon determining normal distribution of data with the Shapiro Wilk test, the Wilcoxon test was preferred. The differences between the measurements were investigated using the Wilcoxon Signed Rank test. Statistical significance was considered at 0.05. The results were presented as median ± standard error.

**RESULTS**
A total of 128 women presented to the clinic between December 2019-2020. One hundred eight women were excluded due to reasons given in Figure 1. Twenty women aged 22.4±0.9 years were finally enrolled. The average level of physical activity was 1162.2±189.1 MET-min/week. The average menstrual cycle was 30.3±0.5 days.

Average body mass index was 20.7±0.3 kg/m². Body fat ratios were calculated as (13.3±0.4) and (18.3±0.7) % according to Yuhazs and Siri formulas, respectively. The mean body fat ratio calculated by averaging these two results was (15.0±0.5) %. Mean waist circumference was 70.2±1.3 cm and mean hip circumference was 94.1±1.2 cm.

Among the multiple performance tests of physical fitness parameters, menstrual cycle phase was not found to be statistically (p>0.05) (Table 1-3). We found that only ‘minimum power’ in the Wingate test was significantly higher in the midluteal phase (p=0.048) (Table 2).

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**Table 1. Flexibility, dynamic balance, agility, and vertical jump findings**

<table>
<thead>
<tr>
<th>Test/phase</th>
<th>Follicular phase</th>
<th>Luteal phase</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sit-and-reach test (cm)</td>
<td>25.9±1.6</td>
<td>25.2±1.9</td>
<td>0.587</td>
</tr>
<tr>
<td>Y Balance total score (cm)</td>
<td>101.7±1.7</td>
<td>101.9±2.3</td>
<td>0.191</td>
</tr>
<tr>
<td>Hexagon agility test (s)</td>
<td>29.5±1.8</td>
<td>29.5±1.1</td>
<td>0.911</td>
</tr>
<tr>
<td>Vertical jump distance (cm)</td>
<td>29.5±0.8</td>
<td>29.5±1.1</td>
<td>0.911</td>
</tr>
</tbody>
</table>

Results as median±standard error (Med±SE); n=20

**Table 2. Aerobic capacity, anaerobic power and capacity findings**

<table>
<thead>
<tr>
<th>Test/phase</th>
<th>Follicular phase</th>
<th>Luteal phase</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>VO₂max (ml/min/kg)</td>
<td>23.7±0.3</td>
<td>23.8±0.3</td>
<td>0.507</td>
</tr>
<tr>
<td>Anaerobic power (kgm/s)</td>
<td>89.4±14</td>
<td>89.1±18</td>
<td>0.911</td>
</tr>
<tr>
<td>Wingate anaerobic capacity test (W/kg)</td>
<td>Peak power</td>
<td>8.3±0.5</td>
<td>7.9±0.3</td>
</tr>
<tr>
<td>Average power</td>
<td>4.2±0.2</td>
<td>4.5±0.2</td>
<td>0.086</td>
</tr>
<tr>
<td>Minimum power</td>
<td>2.7±0.2</td>
<td>3.1±0.2</td>
<td>0.048*</td>
</tr>
<tr>
<td>Power drop</td>
<td>5.6±0.5</td>
<td>5.0±0.4</td>
<td>0.287</td>
</tr>
</tbody>
</table>

Results as Med±SE; n=20; *: p<0.05

**Table 3. Isokinetic muscle strength findings at 60°/s (IPT and TW at 180°/s)**

<table>
<thead>
<tr>
<th>Test/phase</th>
<th>Follicular phase</th>
<th>Luteal phase</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Extension</td>
<td>PT (Nm)</td>
<td>112.1±2.6</td>
<td>110.1±2.7</td>
</tr>
<tr>
<td></td>
<td>PT/BW (Nm/kg)</td>
<td>1.90±0.01</td>
<td>1.90±0.06</td>
</tr>
<tr>
<td></td>
<td>Position of peak torque (°)</td>
<td>45.6±1.5</td>
<td>45.8±1.2</td>
</tr>
<tr>
<td></td>
<td>Time to peak torque (s)</td>
<td>0.70±0.02</td>
<td>0.70±0.02</td>
</tr>
<tr>
<td></td>
<td>Time from peak to relaxation (s)</td>
<td>0.80±0.03</td>
<td>0.80±0.03</td>
</tr>
<tr>
<td></td>
<td>IPT (Nm)</td>
<td>67.8±1.9</td>
<td>68.3±1.7</td>
</tr>
<tr>
<td></td>
<td>TW (J)</td>
<td>940.7±19</td>
<td>959.6±37.2</td>
</tr>
<tr>
<td></td>
<td>PT (Nm)</td>
<td>40.6±2.2</td>
<td>42.7±1.9</td>
</tr>
<tr>
<td></td>
<td>PT/BW (Nm/kg)</td>
<td>0.70±0.05</td>
<td>0.80±0.04</td>
</tr>
<tr>
<td></td>
<td>Position of peak torque (°)</td>
<td>34.5±3.2</td>
<td>34.2±2.0</td>
</tr>
<tr>
<td></td>
<td>Time to peak torque (s)</td>
<td>0.50±0.03</td>
<td>0.50±0.03</td>
</tr>
<tr>
<td></td>
<td>Time from peak to relaxation (s)</td>
<td>0.70±0.04</td>
<td>0.70±0.04</td>
</tr>
<tr>
<td></td>
<td>IPT (Nm)</td>
<td>32.1±7.3</td>
<td>32.8±14.1</td>
</tr>
</tbody>
</table>

Results as Med±SE; n=40; PT: peak torque, BW: body weight, IPT: initial peak torque, TW: total work
DISCUSSION

Sex steroid hormone concentrations fluctuate during the menstrual cycle. Consequently, researchers argued that women’s physical performance parameters could also fluctuate throughout the menstrual cycle (20,21). Despite the long-lasting debate, existing literature does not provide a consensus on the issue. This is presumably partly due to the differences in methodologies across studies in terms of determining the phases of menstrual cycle and measuring performance (22).

Flexibility, dynamic balance, agility, vertical jump, aerobic capacity, anaerobic power, and isokinetic muscle strength in the early follicular and midluteal phases of the menstrual cycle were herein assessed. We found that the ‘minimum power’ value in Wingate test was higher during the midluteal phase. No other parameter was determined to be significantly different among different menstrual cycle phases.

Melegario et al. (23) used blood tests to determine menstrual cycle phases, and, did not find any significant phase-related differences (p>0.05) for eight different flexibility movements in 20 women. Another study evaluated musculo-tendinous stiffness and knee joint laxity with KT2000™ among 11 female netball players during early follicular, mid-follicular, ovulatory, and midluteal phases. The study did not reveal that estrogen fluctuations significantly influenced knee joint laxity (p>0.05) (24).

Another study on 37 women assessed muscle stiffness by elastography and did not detect significant differences in resting muscle stiffness between different phases. However, this study reported significantly increased stiffness during contraction in the early follicular phase compared to the ovulatory phase (25). In our study, we did not find that flexibility, as determined via the sit-and-reach test, differed significantly between different menstrual phases. Our results are consistent with the literature in that hormonal fluctuations did not clinically influence flexibility. High estrogen levels are known to cause collagen levels to decrease and fibroblast levels to be suppressed. Connective tissue laxity that results from this situation increases the risk of musculoskeletal injury 2-8 times compared men. Again, fluctuations in 17b-estradiol levels have been associated with injury risk, and this fluctuation may cause changes in tendon and muscle stiffness, increasing susceptibility to injury (25).

However, neither in this study nor in others where flexibility was evaluated, although small differences in flexibility between cycles were observed, they were not significant. In the literature examined, the number of subjects was always limited. In a study where a change in muscle stiffness was observed during contraction, muscle contractions were not evaluated quantitatively. Quantitative measurements of muscle stiffness are needed in broader studies, and during sports-specific movements in order to clearly demonstrate the relationship between the fluctuations in estrogen levels and injury.

In our review of the literature, we did not come across any study that examined the relationship between menstrual cycle phases and balance. Our results did not reveal an association between Y balance parameters and menstrual phases. Considering that studies do not report significant changes in muscle strength between phases (18,19), it may be expected that balance would not be affected by hormonal changes, either. Unchanging flexibility may be another factor that is effective in unchanging balance performance.

Kheniser et al. (26) studied agility in early follicular and midluteal phases among 10 women. They determined ovulation using urine LH testing, and agility in the T-test and reactive agility test. They reported that in the T-test, the subjects were significantly faster during the early follicular phase, but that the two phases were not significantly different in terms of the reactive agility test. The authors attributed this finding to the fact that the reactive agility test requires cognitive function, which is not affected by the menstrual cycle, and that the altered joint laxity resulted in changed T-test results.

A different study investigated agility and edema in the gastrocnemius muscle through MRI in the early follicular, follicular, ovulatory, early luteal, and late luteal phases of 13 female athletes. They reported significantly increased T2 signals in the early follicular phase compared to other phases (p<0.01). They also found that agility decreased in the early follicular phase. These results were interpreted as indicating that agility decreases with increased fluid retention (27). In our study, we did not find a significant difference between agility results in early follicular and midluteal phases. We ascribe the inconsistency of our results with the literature to having used different test methods to evaluate agility and ovulation, and the small sample size. Further studies with larger samples are needed to conclusively demonstrate a relationship between agility and different menstrual phases.

In a study by Pisapia et al. (28), vertical jump performance was evaluated in the luteal and follicular phases, yielding no significant difference. In their study of 58 female athletes, Vrublevskiy et al. (29) found that jump performance was highest during the ovulatory phase and lowest during the early follicular phase. The results of these two studies...
are consistent. In our study, we found that different estrogen levels did not influence vertical jump performance. We predicted that there might be better vertical jumping performance in the mid-luteal phase, given the positive effects of high estrogen levels on the carbohydrate metabolism.

In another study, adolescent volleyball players were divided into two groups, and their vertical jumping performance was evaluated (30). Although estrogen levels were found to be statistically significantly higher in one group, no change was observed in vertical jumping performances. Considering these studies, we can comment that there is no significant relationship between estrogen level and vertical jumping performance, unlike our estimate. In addition, it should not to be forgotten that there are variations in hormonal changes between individuals, and seasonal ones, so each individual should be evaluated individually.

Numerous studies have examined the relationship between menstrual cycle and aerobic capacity, anaerobic power, and muscle strength. Minimal (2%) decreases in aerobic capacity during the mid-luteal phase compared to the early follicular one is reported (29). In contrast, it is argued that progesterone can increase aerobic capacity due to increasing the respiratory rate during the mid-luteal phase (31). Studies investigating changes in lactate and ventilatory thresholds did not display changes in performance or threshold associations with menstrual cycle (32,33). One study showed an increased ventilation threshold in the late follicular phase (32), whereas another reported that the 4.0 mmol/l lactate threshold occurred at a significantly higher exercise intensity in the luteal phase (33).

A review of 51 studies by McNulty et al. (4) concluded that aerobic capacity decreased minimally in the early follicular phase compared with other phases. They argued that conclusive evidence does not exist about exercise performance during the menstrual cycle due to the wide variation between studies. Our review of literature revealed multiple studies that concluded that aerobic capacity did not significantly change in different menstrual phases (34,35). We did not find a significant change in aerobic capacity between the early follicular and mid-luteal phases.

In a study where the amount of glycogen in vastus lateralis biopsy material was evaluated, it was shown that the amount of intramuscular glycogen increased in the luteal phase, without contributing to aerobic performance (34). Again, it was shown in hamsters that the amount of uterine glycogen increased in the luteal phase that was not reflected in the muscle (34). When assessing studies in general, it is seen that even if the increased estrogen level increases the amount of intramuscular glycogen, this increase does not contribute to aerobic performance. Increased glycogen accumulation does not seem to make sense independent of enzyme and cofactor levels required in substrate use. Thus, the relationship between menstrual cycle phases and anaerobic performance remains controversial (34).

A study by Araz et al. (36) on 20 women aged 20-30 years did not reveal significant differences between Wingate test parameters in early follicular, ovulatory, and mid-luteal phases. Similarly, a study on 16 women did not display significant differences between anaerobic power results, as measured by a cycle ergometer, in the follicular and luteal menstrual phases (37). We found that 'minimum power' in the Wingate test was significantly higher during the mid-luteal phase. A review by McNulty et al. (3) stated that exercise performance minimally decreased during the early follicular phase. Estrogen is known to increase glucose uptake and maintain glycogen reserves. It can also act as an antioxidant and membrane stabilizer to attenuate inflammatory response (38). Moreover, estrogen may have neuro-stimulating effects that increase volitional activation. High estrogen levels in the mid-luteal phase may positively affect muscle performance and anaerobic power (3). Similarly, increased phosphocreatine and adenosine triphosphate reserves may be associated with better anaerobic performance in the luteal phase (34). These findings suggest that anaerobic power may change between the phases of the menstrual cycle.

Literature on the relationship between menstrual cycle phases and muscle strength is constantly growing. Janse de Jonge et al. (39) evaluated isokinetic knee flexion and extension muscle strengths in early follicular, late follicular, and luteal phases, and found no significant changes. Another study evaluated isokinetic knee flexor and extensor muscle strength ratios of 26 female athletes in the luteal and follicular phases. The hamstring-to-quadriceps muscle strength ratio was not different among phases in the dominant limb, but was significantly reduced during the follicular phase in the non-dominant limb (p=0.011) (40). A study by Fridén et al. (21) evaluated isokinetic knee extension and flexion muscle strength in early follicular, ovulatory, and mid-luteal phases in two consecutive menstrual cycles, and did not reveal significant changes in muscle strength between phases. In this study, we did not find a significant difference in isokinetic muscle strength parameters between the early follicular and mid-luteal phases. The variance of results in literature is partly ascribed to differences in isokinetic measurement methods, assessment parameters, and differences in evaluated menstrual cycle phases. Studies with comparable methodologies are required for more conclusive results.
The heterogeneity of methodologies becomes obvious when reviewing the literature. Discrepancies concerning the investigated parameters and menstrual phases, sample sizes, and assessment methods can influence results, making it difficult to make comparative analysis. Regardless, the common finding is that different menstrual cycle phases do not significantly affect physical performance. However, it should not be forgotten that these small changes may cause significant results in professional sports. The limitations of our study are not having included professional female athletes, and the small number of participants, which did not allow parametric testing in statistical analysis.

To conclude, physical performance is minimally affected by the different phases of menstrual cycle in the defined group. But, as tenths of a second can be critical in competitive sports, each female athlete still should be evaluated individually, and a personalized training program should be prepared, taking into account the menstrual cycle.

Acknowledgments

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Ethics Committee Approval / Etik Komite Onayı

The approval for this study was obtained from Institutional Ethics Committee of Süleyman Demirel University, Isparta, Turkey (Decision no: 323 Date: 29/11/2019).

Conflict of Interest / Çıkar Çatışması

The authors declared no conflicts of interest with respect to authorship and/or publication of the article.

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Author Contributions / Yazar Katılımları

Concept: SE; Design: SE; Supervision: ÇÇ; Materials: SE; Data Collection and/or Processing: AO, ME, TP; Analysis and Interpretation: SE; Literature Review: AO; Writing Manuscript: AO, SE; Critical Reviews: All Authors.

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