EFFECTS OF MENSTRUAL PHASE–DEPENDENT RESISTANCE TRAINING FREQUENCY ON MUSCULAR HYPERTROPHY AND STRENGTH

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ABSTRACT
Sakamaki-Sunaga, M, Min, S, Kamemoto, K, and Okamoto, T. Effects of menstrual phase–dependent resistance training frequency on muscular hypertrophy and strength. J Strength Cond Res 30(6): 1727–1734, 2016—The present study investigated how different training frequencies during menstrual phases affect muscle hypertrophy and strength. Fourteen eumenorrheic women performed 3 sets of arm curls (8–15 repetitions) until failure for 12 weeks. Depending on the menstrual cycle phase, each subject trained each arm separately after either a 3- or a 1-d wk–1 training protocol during the follicular phase (FP-T) and a 3- or 1-d wk–1 training protocol during the luteal phase (LP-T). Cross-sectional area (CSA), 1 repetition maximum, and maximum voluntary contraction significantly increased 6.2 ± 4.4, 36.4 ± 11.9, and 16.7 ± 5.6%, respectively (p ≤ 0.05 vs. before training), in the FP-T group and 7.8 ± 4.2, 31.8 ± 14.1, and 14.9 ± 12.7%, respectively (p ≤ 0.05 vs. before training), in the LP-T group. Changes in CSA between the FP-T and the LP-T groups significantly and positively correlated (r = 0.54, p ≤ 0.05). There were no major differences among the different training protocols with regard to muscle hypertrophy and strength. Therefore, we suggest that variations in female hormones induced by the menstrual cycle phases do not significantly contribute to muscle hypertrophy and strength gains during 12 weeks of resistance training.

KEY WORDS menstrual cycle, estrogen, progesterone

INTRODUCTION
Diminishing muscle mass and strength in postmenopausal women can lead to sarcopenia (23,25). This is considered to be a result of declining serum levels of various hormones with aging, including estrogen and progesterone. Several studies have found a correlation between estrogen and skeletal muscle development (2,10,12,24). Estrogen promotes the proliferation and differentiation of skeletal myoblasts (12) and affects the release of growth hormone (GH), insulin-like growth factor-1 (IGF-1), and insulin, which apparently correlate positively with muscle mass (15). Thus, estrogen might be important in the regulation of muscle growth processes. In contrast, few studies have investigated the effects of progesterone on skeletal muscle, although they are supposed to be catabolic (18,19). However, progesterone replacement does not affect the cross-sectional area of the pubococcygeus muscle in rats (20). Other studies with regard to the role of progesterone on skeletal muscle have been inconclusive.

Blood concentrations of female hormones in premenopausal women change significantly throughout monthly menstrual cycles, being low and high during the early follicular phase (FP) and luteal phase (LP), respectively. Blood concentrations of both estrogen and progesterone are high during the LP. Whether female hormones affect muscle hypertrophy induced by resistance training is unclear. Furthermore, some studies have found that the degree of muscle strength changes during the menstrual cycle (5,29,30,34). These findings suggest a correlation between strength and the estrogen concentration. The effects of the number of repetitions during resistance training should be considered if strength changes according to the phases of the menstrual cycle.

Our previous comparison of muscle hypertrophy during the FP and LP after 6 days of low-intensity blood flow restriction training in eumenorrheic women showed increased muscle hypertrophy and strength gains during the LP compared with the FP (33). However, because the training model comprised low-intensity resistance training combined with blood flow restriction, the relevance to high-intensity resistance training protocols remained unclear.

Therefore, the present study aimed to determine the effects of different frequencies of high-intensity resistance training on skeletal muscle during the menstrual cycle. We thus compared the outcomes of training frequencies that were changed according to menstrual phases.
METHODS

Experimental Approach to the Problem

The menstrual cycle is believed to affect the physical condition of female athletes. Therefore, we hypothesized that it is important to design a resistance training program taking into consideration the menstrual cycle phases. This study was to investigate the effects of the menstrual cycle on resistance training–induced muscle hypertrophy and strength gains in eumenorrheic women. The subjects performed a 12-week resistance training program differing by frequency depending on the menstrual phase. One repetition maximum (1RM) and maximum voluntary contraction (MVC) were assessed before the beginning of the training (baseline) and every 4 weeks (weeks 4, 8, and 12). The elbow flexor cross-sectional area (CSA) was measured at baseline, week 8, and week 12.

Subjects

Fourteen physically active eumenorrheic women (age, 21.2 ± 1.9 years; age range, 19–25 years; height, 162.9 ± 3.4 cm; weight, 57.1 ± 5.4 kg) who had not participated in any regular resistance training for at least 1 year were enrolled in the present study. Table 1 shows the characteristics of the subjects. Body weight, body mass index, and body fat (percentage) did not significantly change throughout the 12-week training program. The average length of the menstrual cycle was 29.0 ± 1.0 days (calculated from the first day of menses).

All of them were non-smokers, nulliparous, not taking any oral contraceptives or other medications, and were free of anatomical and metabolic disorders according to their medical histories and routine medical examinations. All of them provided written informed consent before participating in the study, which was approved by the Ethics Committee of the Nippon Sport Science University and proceeded according to the Declaration of Helsinki. The study conforms to the Code of Ethics of the World Medical Association (approved by the ethics advisory board of Swansea University) and required players to provide informed consent before participation.

Menstrual Cycle Determination

Menstrual cycle phase was determined using basal body temperature mapping technique (6). The subjects measured their basal body temperature orally for 20 seconds, immediately upon awakening in the morning, using a digital thermometer (C-531; TERUMO Co. Ltd., Tokyo, Japan) with scale steps of 0.01°C. Basal body temperatures were recorded in a logbook from 1 month before the study until its completion. We confirmed the biphasic characteristics of basal body temperature.

One Repetition Maximum and Training Protocol

The 1 repetition maximum (1RM) was tested 1 week before the training started (baseline) and in weeks 4, 8, and 12. Briefly, each subject warmed up by performing 3–5 one-arm dumbbell curls with a moderate load. The load was initially increased to 80% of the predicted 1RM and then by 5% after each lift until the load could not be lifted through the entire range of motion. About 5 trials were usually required to complete a 1RM test. The subjects rested for about 2 minutes between trials. We measured the 4-, 8-, and 12-week 1RM same time of day as the basal 1RM.

The training intensity was selected so that the subjects reached failure between 8 and 15 repetitions per set and with a recovery period of 2 minutes between sets. Three sets of

| Table 1. Physical characteristics of subjects during training period.*† |
|-----------------------------|-----------------------------|-----------------------------|-----------------------------|
| Age (y)                    | 21.2 ± 1.9                  | 21.5 ± 1.8                  | 21.4 ± 1.6                  |
| Height (cm)                | 162.9 ± 3.4                 | 162.9 ± 3.4                 | 162.9 ± 3.4                 |
| Weight (kg)                | 57.1 ± 5.4                  | 57.0 ± 5.2                  | 56.7 ± 4.8                  |
| BMI (kg·m⁻²)               | 21.5 ± 1.8                  | 21.5 ± 1.7                  | 21.4 ± 1.6                  |
| Body fat (%)               | 26.8 ± 3.9                  | 26.6 ± 3.5                  | 27.9 ± 3.2                  |
| *BMI = body mass index.   |
| †Values are mean ± SD.    |
1-arm standing dumbbell curls were performed until failure for each set. If a subject could complete >15 repetitions with a given weight, the weight was increased for subsequent sets. The subjects were familiarized with the training procedures before the experiment started, and training sessions were supervised by professional trainers. The subjects reported to the laboratory whenever they were available, and the training protocol consisted of 1-arm standing dumbbell biceps curls. The curls proceeded without bouncing the weights and with a supinated grip. All training procedures were identical. Detailed instructions were provided before the exercise sessions. Each subject performed a warm-up of 5 repetitions at dumbbell curls using a moderate load.

The training frequency was changed depending on the phase of the menstrual cycle (Figure 1). The arms of each subject were randomly assigned to be trained during the follicular phase (FP-T) or luteal phase (LP-T). The FP-T and LP-T arms were trained for 3 and 1 d wk$^{-1}$ during the FP and LP, respectively. Before training started, the subjects were assigned to one of the resistance training protocols depending on their menstrual cycle phase. Among the 14 subjects, 6 individuals started in the FP and 8 individuals started in the LP. After 2 weeks of one resistance protocol with one arm, the subjects switched to the remaining resistance protocol using their other arm. The menstrual cycle was 4 weeks; the subjects exercised on Monday/Wednesday/Friday (3 d wk$^{-1}$) and on Wednesday (1 d wk$^{-1}$), and the 1RM, CSA, and MVC were completed on weekends. All subjects completed all training sessions (Figure 2).

The training volume was calculated by multiplying the weight of the dumbbell by the number of repetitions (weight \times repetitions). Data were collected during the end of each FP and LP cycle over 3 menstrual cycles.

**Maximum Voluntary Contraction**
The MVC of the elbow flexor was measured in both arms (Biodex System 3 dynamometer; Sakai Medical, Co. Ltd., Tokyo, Japan) in a seated position with the elbow joint positioned at an angle of 90° (0° at full extension). After a brief explanation of the testing procedures and a warm-up with submaximal contractions, each participant performed 2 bouts of maximal isometric contractions, and the maximal value was adopted.

**Muscle Cross-Sectional Area**
The upper arms were assessed by magnetic resonance imaging (MRI) using a standard body coil and a 0.3-T scanner (AIRIS; Hitachi Medical Corporation, Tokyo, Japan). T1-weighted, spin-echo, axial plane sequences were acquired with 460-millisecond repetitions and an echo time of 26 milliseconds. The subjects rested quietly in the magnet bore with their arms extended in a supine position. The lateral epicondyle of the humerus served as the origin, and continuous transverse images with 1.0 cm slice thickness were acquired from the lateral epicondyle of the humerus to the acromial process of the scapula. All MRI data were transferred to a personal computer for analysis using custom-designed image

![Figure 2](image-url)
The skeletal muscle tissue CSA was digitized for each slice. The elbow flexor muscle (brachial and biceps brachii muscles) CSAs of 3 continuous slices from the muscle belly (same number of slices from the origin) were averaged for statistical analysis. The coefficient of variation for this CSA measurement was <1%.

Energy and Nutrient Intake

Energy and nutrient intake was determined from 3-day dietary records that included the date when training started (baseline) and ended (12 weeks), using nutritional analysis software (ImageJ ver. 1.43; National Institutes of Health, Bethesda, MD, USA). The skeletal muscle tissue CSA was digitized for each slice. The elbow flexor muscle (brachial and biceps brachii muscles) CSAs of 3 continuous slices from the muscle belly (same number of slices from the origin) were averaged for statistical analysis. The coefficient of variation for this CSA measurement was <1%.

Table 3. Changes in muscle size and strength after 12 weeks of resistance training.

<table>
<thead>
<tr>
<th></th>
<th>FP-T</th>
<th>LP-T</th>
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<tr>
<td><strong>Baseline</strong></td>
<td>6.8 ± 1.4</td>
<td>6.5 ± 1.6</td>
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<tr>
<td><strong>4 wk</strong></td>
<td>7.9 ± 1.5</td>
<td>7.6 ± 1.5</td>
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<tr>
<td><strong>8 wk</strong></td>
<td>8.5 ± 1.4</td>
<td>8.1 ± 1.3</td>
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<td><strong>12 wk</strong></td>
<td>8.8 ± 1.3</td>
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| **CSA (cm²)** | 370 ± 60 | 387 ± 74 |
| **1RM (kg)** | 412 ± 65 | 412 ± 65 |
| **MVC (N·m)** | 41.2 ± 6.5 | 41.2 ± 6.5 |

*FP-T = follicular phase training; LP-T = luteal phase training; CSA = cross-sectional area; 1RM = 1 repetition maximum; MVC = maximum voluntary contraction.
†p < 0.01 vs. baseline.
‡p < 0.01 vs. 4 weeks.
§p < 0.01 vs. 8 weeks.

Figure 3. Time course of changes in elbow flexor muscle CSA. CSA = cross-sectional area. Values are shown as mean ± SD. ***p < 0.001 vs. baseline, #p ≤ 0.05 vs. 8 weeks.

Figure 4. Correlations between changes in CSA during FP-T and LP-T (n = 14). Changes in CSA during FP-T and LP-T correlate (r = 0.54, p ≤ 0.05). CSA = cross-sectional area; FP-T = follicular phase training; LP-T = luteal phase training.
software (Healthy Maker Pro 501; Japan Mushroom Soft Co. Ltd., Okayama, Japan).

Statistical Analyses
Data were statistically analyzed using a 2-way analysis of variance (ANOVA) with repeated measures (group [FP-T and LP-T] × time [baseline, 4, 8, and 12 weeks]) to evaluate the training effects of all dependent variables. When appropriate, within-group changes were assessed using the post hoc Bonferroni test. All baseline characteristics and percent changes in anthropometric variables, skeletal muscle volume, and muscular strength were compared between the groups using a 1-way ANOVA. Nutritional data were compared between baseline and after 12 weeks of training using paired t-tests. Data were analyzed using SPSS ver. 19.0 (IBM Co. Ltd., Tokyo, Japan), and the results were expressed as mean and SD for all variables. Statistical significance was set at $p \leq 0.05$.

RESULTS
Energy and Nutrient Intake
Table 2 shows the energy and nutrient intake determined from the 3-day records. The intake of energy, carbohydrates, fats, and proteins did not significantly differ between (baseline) and after 12 weeks the training period. The protein, fat, and carbohydrate intake ratios did not significantly differ between baseline and 12 weeks ($P$: 13.2 ± 2.6%, $F$: 31.3 ± 7.3%, $C$: 52.5 ± 8.8% vs. $P$: 12.7 ± 0.8%, $F$: 28.4 ± 5.6%, $C$: 55.7 ± 5.9%, respectively).

Muscle Size and Strength
Table 3 shows changes in muscle size and strength after 12 weeks of resistance training. The elbow flexor muscle CSA increased from 11.1 ± 1.9 to 11.8 ± 2.0 cm$^2$ after FP-T and from 11.0 ± 1.9 to 11.9 ± 2.1 cm$^2$ after LP-T, with no significant differences between the 2 phases. Figure 3 shows the time course of changes in elbow flexor muscle CSA. The increase was significant at the end of the FP-T ($6.2 \pm 4.4\%$, $p < 0.01$) and LP-T ($7.8 \pm 4.2\%$, $p < 0.001$). Changes in the CSA of FP-T and LP-T significantly correlated ($r = 0.54$, $p \leq 0.05$, Figure 4) and were quite similar. Arm curl 1RM and MVC significantly increased in both trials (Table 3), with no significant differences between FP-T and LP-T. Arm curl 1RM increased from baseline by 16.7 ± 5.6% and 14.9 ± 12.7% in the FP-T and LP-T, respectively (Figure 5A). The MVC of the elbow flexors increased from baseline by 16.7 ± 5.6% and 14.9 ± 12.7% in the FP-T and LP-T, respectively (Figure 5B), whereas 1RM and MVC did not significantly change in both trials. The CSA change in the FP-T and LP-T significantly correlated (Figure 4; $r = 0.54$, $p \leq 0.05$). The training volumes remained essentially unchanged regardless of menstrual phase (147,194 ± 43,409 and 14,824 ± 40,659 kg in the FP and LP, respectively).

DISCUSSION
The present study examined the effects of menstrual phase-dependent training frequency on muscle hypertrophy. To investigate those effects, we used a study design, including different training frequencies for each arm of the same subject. Several studies have reported that voluntary unilateral training produces modest increases in contralateral strength (22,27). However, previous research has indicated that neural adaption is one factor affecting increased strength of the contralateral arm. In addition, the muscle cross-sectional area showed no evidence that unilateral strength training causes contralateral muscle adaptations (3). Therefore, we believe that this study design is an appropriate tool to examine the influence of the menstrual cycle phase on muscle hypertrophy.

Muscle hypertrophy requires increased synthesis and decreased breakdown of skeletal muscle protein. Some reports indicate that protein catabolism increases more during the LP than the FP of the menstrual cycle (17,21).
On the other hand, one study found that myofibrillar protein synthesis similarly increases between FP and LP at 24 hours after an acute bout of 1-legged exercise (26). The net balance of skeletal muscle protein is largely affected by exercise and nutrient intake (1,16). We did not measure protein synthesis, but nutritional data (energy, carbohydrate, protein, and fat intake) did not differ between baseline and 12 weeks, indicating that the nutrient composition did not affect muscle hypertrophy induced by training.

Skeletal muscle expresses functional estrogen receptors, suggesting that skeletal muscle is sensitive to estrogen (12,32). Estrogen regulates skeletal muscle mass in developing rats (13,35). Estradiol concentrations in the mid-FP and mid-LP correlate with changes in CSA in the quadriceps muscle of humans ($r = 0.85$, $p = 0.05$) (31). However, we did not find a correlation between estradiol concentrations and changes in the CSA of the elbow flexor muscle ($r = 0.16$) (33). Concentrations of estradiol and progesterone were not measured in the present study, which is an important limitation. However, records of basal body temperature and menstrual cycles served as an indicator of the normal fluctuations in female hormones in all our subjects. Our findings suggest that changes in female hormones caused by the menstrual cycle did not strongly affect muscle hypertrophy induced by resistance training.

Several studies have associated GH and IGF-1 with muscle hypertrophy (4,8,15), and GH in women is associated with elevated estrogen levels both at rest (9) and during exercise (14). Levels of GH secretion and IGF-1 increase during the preovulatory phase compared with the early FP (7,28). However, the increased estrogen levels during the last 1–2 days of the late FP do not greatly influence muscle hypertrophy. Conversely, resistance exercise can induce an increase in GH during the LP (14) when female hormone concentrations remain elevated for about 1–2 weeks, indicating a possible effect. However, a recent study has identified a weak correlation between systemic GH responses induced by acute exercise and changes in fiber CSA induced by resistance training, but strength did not change (36,37). The increase in the elbow flexor muscle CSA after 12 weeks of resistance training did not significantly differ between FP-T and LP-T in the present study. Changes in the blood concentrations of IGF-1 and GH were associated with those of estrogen during the FP and LP. Large differences between estrogen concentrations during FP and LP might be affected by the menstrual cycle. However, this was not measured in the present study. Further studies are needed to examine the relationships between IGF-1, estrogen, and muscle hypertrophy.

Previous studies of the menstrual cycle and muscle strength found that MVC increases during the FP and strength decreases during ovulation (30,34). Phillips et al. also suggested that estrogen has a strengthening effect on skeletal muscle (30). In contrast, another study found no changes in strength throughout the menstrual cycle and no correlation between estrogen concentration and strength (11,34). Thus, the effects of the menstrual cycle on muscle strength are not clear. The load set in the training program is very important to effectively improve myofunction during resistance training. The relative load should be changed during the menstrual phases if maximum strength is affected by the menstrual cycle. Maximum strength supposedly varies according to the menstrual cycle, and the difference is considered to affect the training volume. However, we found no different effects of training volumes between FP and LP. Therefore, our data do not support the notion that the menstrual cycle influences repetition and load. Changes in the CSA were subject to large individual differences in the present study, and such differences in training volume or hormonal output among individuals might have been involved.

Reis et al. compared the effects of altering the frequency of “menstrual cycle-triggered training (MCTT)” and “regular training (RT)” over a period of 8 weeks (31). The training-induced maximal isometric strength was greater for MCTT than RT, indicating a possible benefit of increasing training frequency during the FP. However, the muscle CSA did not significantly differ between the 2 training programs. On the other hand, we compared myofunction in low-intensity arm curls with blood flow restriction once daily for 6 days during the FP and LP (33). Muscle volume and maximum isometric strength increased more in the LP than the FP, whereas the present findings indicated that muscle hypertrophy and strength gain improved to the same extent in FP-T and LP-T, which differed from our previous results. These differences are probably because of variations in muscle groups, training intensity, and methodology.

The limitations of this study are as follows. We did not measure blood hormone concentrations, but we did determine menstrual phases by noninvasively measuring basal body temperature. The design of different training frequencies between arms within the same individuals did not exclude a potential confounding systemic effect. Because only arm curls were assessed, we cannot imply that the results would be similar for other muscle groups. Further study is required to address these issues.

In conclusion, muscle CSA and strength similarly changed between the FP-T and LP-T. These results suggest that hormone variations during the menstrual cycle are not significantly involved in muscle hypertrophy and strength gains during 12 weeks of resistance training. Further studies are needed to confirm the effects of the menstrual cycle on various training programs.

**Practical Applications**

The menstrual cycle affects the mental and physical conditions of female athletes, and female hormones influence the skeletal muscle metabolism. We postulated that changes in female hormones induced by the menstrual cycle also affect the outcomes of resistance training. We did not find any differences in muscle functions after
training at several frequencies under same conditions depending on menstrual cycle phase. Therefore, we suggest that training frequency can be selected independent of menstrual cycle phase without any consequences for female athletes.

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**References**


