The effect of the menstrual cycle on collagen metabolism, growth hormones and strength in young physically active women

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ABSTRACT: This study aimed to investigate the effect of the menstrual cycle on strength, functioning of the GH/IGF-1 axis and collagen metabolism in physically active women. Twenty-four physically active and eumenorrheic women volunteered to participate in the study (body mass 60.3 ± 9.18 kg, age 21.8 ± 0.92 years). Blood samples were obtained between the 5th and 8th days (the follicular phase) and between the 19th and 22th days (the luteal phase) of the menstrual cycle to determine sex steroid concentrations (follicle-stimulating hormone (FSH), luteinizing hormone (LH), prolactin (PRL), oestradiol (E2) and progesterone (P)). Also insulin-like growth factor 1 (IGF-1) and collagen metabolism markers (synthesis (PICP) and breakdown (ICTP)) and maximum voluntary contraction (MVC) were measured. MVC was higher in the luteal phase 164.1 ± 34.77 [N m] (F(1,24) = 4.59; p = 0.043). The recorded collagen synthesis marker (PICP = 296.4 ± 35.61 [ng/ml]) was at the upper level of the reference range (30–300), with an insignificant decrease in the luteal phase (Z = 1.612; p = 0.107) and a significant increase in oestradiol concentration (Z = 4.286; p = 0.0001). The marker of collagen breakdown (ICTP = 4.16 ± 0.68 [µg/l]) was reduced by 6.8% in the same phase (Z = 1.764; p = 0.137). The variability of physical abilities (MVC) during the menstrual cycle showed that menstrual status should be taken into account in determination of the training loads. Increasing the load in the luteal phase seems to be favoured by a beneficial change in collagen metabolism (lower synthesis decrease, lower breakdown increase) observed in physically active women.

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INTRODUCTION

Previous studies have demonstrated that musculoskeletal system injuries are 2 to 6 times more frequent in women compared to men [1, 2, 3, 4, 5, 6]. Among the most frequently reported causes of injuries are excessive load compared to body abilities and, in women, also degenerative joint diseases [7] and variability of joint stability linked to the menstrual cycle [6, 8, 9, 10, 11].

According to some researchers, hormonal changes that occur during the menstrual cycle through the effect on the level of antagonist muscle strength can significantly modify susceptibility of anterior cruciate ligament injuries [11, 12, 13].

However, the effect of sex steroids on function of the musculoskeletal system in women, especially their passive parts, has not been fully explored yet, with the results of certain examinations being contradictory [14, 15, 16, 17]. This issue is very important because a significant decrease in strength is associated with risky biomechanical strategies that may predispose to injury [16]. Wojtys et al. [12] documented a statistically significant increase in prevalence of anterior cruciate ligament (ACL) injuries in women during ovulation (days 10–14 of the menstrual cycle), characterized by high oestrogen levels. Myklebust et al. [13] also observed less frequent injuries involving the locomotor system between the 8th and 14th day of the cycle. Furthermore, Zazulak et al. [1], based on a review of 11 studies from this area, found no significant effect of the menstrual cycle on functioning the knee joint. This study demonstrated that the frequency of injuries in this joint increases during the ovulatory and post-ovulatory stages [1].

An explanation of the changes in the musculoskeletal system observed during the menstrual cycle can be offered by the findings published by Liu et al. [18], Sciore et al. [19] and Galey et al. [20], who detected a significant correlation between the increase in oestrogen and relaxin levels and a 40% decline of the collagen type I synthesis. Collagen type I is the main protein to build tendons and...
ligaments. Therefore, the amount and quality of collagen tissues determine mechanical properties of the connective tissue that transforms force from working muscles to the skeleton [21, 22]. The reduced synthesis of this protein observed in women during ovulation can lead to frequent injuries in this phase of the menstrual cycle. In a study by Yu et al. [23], where researchers documented a decline in collagen synthesis with the increase in oestriol levels, it was also demonstrated that oestrogens inhibit proliferation of fibroblasts, cells that synthesize collagen [23]. The inhibitory effect of oestrogens on collagen production was also found in a study of women who used contraceptives. They had higher concentrations of sex steroids (oestrogens and progesterone) and lower concentrations of markers of collagen metabolism compared to women who menstruated spontaneously [24].

A marker of collagen type I synthesis is carboxyterminal propeptide of type I procollagen (PICP), whereas the level of the breakdown of this protein is indicated by plasma levels of carboxyterminal cross-linked of type I collagen (ICTP) [25]. The most important hormones that stimulate collagen synthesis and, therefore, play an important role in adaptation of the connective tissue matrix to the load are growth hormone (GH) and insulin-like growth factor (IGF-1) [22]. The factors that regulate hormone secretion of the IGF-1/GH axis also include sex steroids. Therefore, it seems that changes in oestrogens during the menstrual cycle, also through the effect on functioning of the IGF-1/GH axis, should lead to differences between resistance of the tendino-ligamentous system to load and level of strength developed in women. However, few studies have attempted to comprehensively evaluate the readiness of the musculoskeletal system for exercise in women taking into consideration changes in sex steroid levels that occur during the menstrual cycle.

Due to poorer development of the musculoskeletal system in women compared to men and the variable resistance to damage during the menstrual cycle and because of the lack of comprehensive explanation of these phenomena, the study was performed to evaluate the effects of menstrual cycle phases on strength in women. However, few studies have attempted to comprehensively evaluate the readiness of the musculoskeletal system for exercise in women taking into consideration changes in sex steroid levels that occur during the menstrual cycle.

Materials and Methods

Forty-three physically active young women aged 22 to 25 years volunteered for the study. The inclusion criteria were not using contraceptives, menstrual status (eumenorheic women) and not practising sports at a professional level. Physical activity of the participants was connected exclusively with the study programme and it amounted to 3 hours a week.

Study design

The study was divided into two stages. During the first stage, the participants (n = 43) measured basal body temperature (BBT) for three consecutive menstrual cycles. In the last cycle, when thermal observation was performed in two selected moments, i.e. between the 5th and 8th day (the follicular phase) and between the 19th and 22nd days (the luteal phase) of the cycle, blood samples were obtained to determine sex steroid levels i.e. follicle-stimulating hormone (FSH), luteinizing hormone (LH), prolactin (PRL), oestradiol (E2) and progesterone (P). They were used to evaluate menstrual status. The criterion of ovulation was progesterone concentrations in the second study moment of the cycle, exceeding 1.7 ng/ml. Normal menstrual cycles were found in 24 women and only these women were qualified for the second stage of the study [26].

During the second stage that took into consideration one menstrual cycle, blood samples were obtained on the same days as at the first stage and body composition and biomechanical measurements were performed. In addition to sex steroid levels, GH, IGF-1 and collagen metabolism markers (PICP, ICTP) levels were evaluated.

Each assessment was performed in the morning. Days of blood sampling and measurements were determined individually for each woman depending on the length of the cycle. The examination procedures were started from blood sampling and then body composition was determined, followed by measurements of the muscle torques in ankle joint flexors at various angular positions.

The study was approved by the Ethics Board and the subjects were informed of the benefits and risks of the investigation prior to signing an institutionally approved informed consent document to participate in the study.

Anthropometric measurements

Measurements of basic anthropometric parameters were performed: body height (bh), with accuracy of 0.5 cm, and body weight (bw), with accuracy of 0.5 kg. Body composition, i.e. fat mass (FM), fat-free mass (FFM) and total body water (TBW), was evaluated using bioimpedance analysis (BIA) by means of a bioimpedance device (Tanita Co., Japan). Normal fat mass was adopted as 21–33%.

Table 1 presents the results of anthropometric measurements of the women studied in both phases of the menstrual cycle. Body height was measured once, in the follicular phase. Its mean value for all the participants was 168.6 ± 6.24 cm.

It was found that the women were characterized by a slim body. Both values of body mass index (BMI) and fat mass in female university students who participated in the study were normal. No significant changes in anthropometric parameters were found between cycle phases.

Biochemical measurements

Blood for biochemical tests was taken from the antecubital vein. Levels of pituitary hormones (FSH, LH and PRL) and ovarian hormones (E2 and P) were examined using the Roche test and the electrochemiluminescence immunoassay method by means of the Cobas apparatus. Sensitivity of the method for each test was: 0.100 mIU/ml for FSH, 0.100 mIU/ml for LH, 1.00 µIU/ml for PRL, 5 pg/ml for E2, and 0.05 ng/ml for P. Levels of GH and IGF-1 were determined using the chemiluminescence immunoassay (CLIA) method by means of the Immulite 2000 XPI apparatus. Sensitivity
of the tests was 0.01 ng/ml for GH and 20 ng/ml for IGF-1. The results were interpreted while adopting GH reference levels of up to 8 ng/ml for the female population and IGF-1 reference levels of 116–358 ng/ml for the adults aged 20–25 years [27].

Determination of the concentrations of synthesis markers (PICP) and collagen breakdown (ICTP) was performed using the radioimmunoassay (RIA) method by means of the Immunotech sets and Beckman Gamma Camera. Sensitivity of the method for individual measurements was 6.225 ng/ml for PICP, with a coefficient of variation of 0–12%, for ICTP 6.5–7.2%. The results were interpreted by adopting the reference values of 30–300 ng/ml for PICP and 2.1–5.6 ug/l for ICTP [28].

Muscle force measurements
Muscle force was evaluated as muscle torques of the ankle flexors in maximal voluntary contraction (MVC). The muscle torques for the ankle flexors were measured under isometric conditions using the specially designed measurement stand (Fig. 1). In this study, the measurements of muscle torques during plantar flexion of the foot were taken. The participants were stabilized by belts and supports.

Maximum voluntary contraction (MVC) was measured three times during a short (5 s) isometric contraction at two positions of the lower limbs that changed the length of the triceps surae muscle. The first measurement was performed at a specific angle of $\alpha = 90^\circ$ for all joints of the lower limb (hip joint, knee joint, ankle joint). It is assumed that with this position, muscle length is the resting length at which muscles have physiologically the best potential to generate maximal force. During the second measurement, the conditions were changed by changing the angle in the ankle joint ($\alpha = 60^\circ$). Through changing the angle, changes in the length of the triceps surae muscle were obtained. As a consequence off this effect, the total force was due to both the active and passive components dependent on the elastic properties [29].

![FIG 1. Scheme of the equipment used to measure the muscle torques of ankle joint plantar flexors.](image)

Isometric muscle contraction with duration of 5 s was repeated three times, with 10 s rests. Results of measurements were recorded online using dedicated MAX6v0 software. The median MVC from the middle three seconds of the contraction was computed for each trial to assess the repeatability. For the basic analysis, averaged values from three performed trials were taken.

Statistical analysis
In order to check the normality of the obtained results, the Shapiro–Wilk test was performed. No normal distribution was demonstrated for the biochemical variables. The Wilcoxon pairwise test was used to compare the phases of the menstrual cycle. Moreover, the concentrations of hormones were compared to the accepted norms for the individual phases of the menstrual cycle. Muscle torque results were analysed using $2 \times 2$ ANOVA for repeated measures with ankle joint angle and the phase of the menstrual cycle as within-subject factors. The assumption of sphericity was verified using the Mauchly test. In the case of significant violations,
range and there was no significant change in the luteal phase of the cycle (Table 3). Mean values of the IGF-1 concentrations, both in the first and in the second phase of the cycle, were above the upper limit of the reference range. Also for GH, no significant increase of this hormone was observed in the luteal phase ($Z = 1.671; \ p = 0.095$).

Markers of collagen synthesis and breakdown were within the reference range. However, it should be emphasized that PICP was in both cycle phases at the upper level of the reference range, whereas ICTP concentrations were at the lower level of the reference range. No significant effect of cycle phases on collagen metabolism markers was found. Analysis of the ratio of breakdown to synthesis of collagen (ICTP/PICP) showed that its value decreased (by 4%) in the luteal phase of the menstrual cycle. Although the change was not significant, it could indicate a greater role of the synthesis process than breakdown in the collagen metabolism.

**Biochemical indices**

It was found that FSH concentrations in both analysed phases of the menstrual cycle were within the reference range for this hormone, whereas LH concentrations were at the upper level of the reference range (Table 2). A significant decline in FSH concentrations was observed in the second phase of the cycle ($Z = 3.314; \ p < 0.001$), but there was no significant change in LH concentrations ($Z = 0.114; \ p = 0.909$). However, it should be emphasized that the changes were substantial (48%). The LH/FSH ratio was also computed. In normal menstrual cycles, this ratio in the follicular phase is 0.8–1. The mean value of the LH/FSH ratio in the study group was higher ($1.21 \pm 0.85$). In the second phase, this ratio was substantially higher ($2.18 \pm 1.58$), both with respect to the norm and the ratio recorded in the first phase ($Z = 3.514; \ p < 0.001$).

Oestradiol concentrations in each phase of the cycle were normal (Fig. 2A). In the luteal phase, the mean value of this hormone was over 200% higher compared to the follicular phase. Progesterone concentrations were also normal, with the mean value in the second phase significantly higher ($Z = 4.143; \ p < 0.001$) than that recorded for the follicular phase (Fig. 2B).

Mean values of PRL concentrations in both phases were within the reference range, whereas the observed decline in the luteal phase was not statistically significant ($Z = 1.143; \ p = 0.253$).

GH levels in both phases of the cycle were within the reference range and there was no significant change in the luteal phase of the cycle (Table 3). Mean values of the IGF-1 concentrations, both in the first and in the second phase of the cycle, were above the upper limit of the reference range. Also for GH, no significant increase of this hormone was observed in the luteal phase ($Z = 1.671; \ p = 0.095$).

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**Correlation analysis**

With the above results, the analysis of correlations between strength abilities and biochemical variables took into consideration MVC measured at the angle of $90^\circ$. The results of the relationships were presented in correlation maps for both phases of the menstrual cycle.

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**TABLE 2.** Mean values ± SD of sex hormones and prolactin (PRL) concentrations in both menstrual cycle phases in the studied women

| Participants (n = 24) | Follicular phase | Luteal phase | Z       | p     |
|----------------------|-----------------|--------------|---------|-------|
| FSH (mIU/ml)         | 6.5 ± 1.38      | 4.3 ± 2.45   | 3.314   | < 0.001|
| reference range      | (3.5 – 12.5)    | (1.7 – 7.7)  |         |       |
| LH (mIU/ml)          | 7.65 ± 5.65     | 11.4 ± 15.38 | 0.114   | 0.909 |
| reference range      | (2.4 – 12.6)    | (1.0 – 11.4) |         |       |
| LH/FSH               | 1.21 ± 0.85     | 2.18 ± 1.58  | 3.514   | < 0.001|
| reference range      | 0.82            |             |         |       |
| PRL mlU/l            | 391 ± 336.9     | 368 ± 293.5  | 0.076   | 0.785 |
| reference range      | (102 – 496)     |             |         |       |

FSH – follicle-stimulating hormone, LH – luteinizing hormone, PRL – prolactin
The menstrual cycle and collagen metabolism, growth hormones, strength

The correlated variables were divided into four groups: anthropometric, sex steroids, growth hormones, and markers of collagen breakdown and synthesis (Fig. 4). The correlation map contains only statistically significant relationships (p < 0.05).

Significant correlations between MVC and body composition variables (bw and FFM) were found in the follicular phase. A correlation was also observed between MVC and secretion of progesterone (r = -0.468; p < 0.05). However, this correlation was negative. No direct correlations with MVC were found for other variables. However, significant correlations were found between biochemical variables. Among sex steroids, correlations were detected between progesterone and IGF-1 (r = 0.588; p < 0.05) and the collagen synthesis marker PICP (r = 0.440; p < 0.05). Furthermore, significant correlations were found for collagen markers and IGF-1 (r = 0.441; p < 0.05 and 0.465; p < 0.05, respectively).

**TABLE 3.** Mean values ± SD of the concentration of growth hormone (GH/IGF-1) and collagen synthesis (PICP) and degradation (ICTP) markers in two phases of the menstrual cycle

| Participants (n = 24) | Follicular phase | Luteal phase | Z     | p       |
|-----------------------|------------------|--------------|-------|---------|
| GH ng/ml              | 1.57 ± 3.33      | 3.24 ± 5.08  | 1.671 | 0.095   |
| reference range       | (0 – 8)          |              |       |         |
| IGF-1 ng/ml           | 243 ± 63.7       | 262 ± 76.3   | 1.643 | 0.101   |
| reference range       | (43 – 209)       |              |       |         |
| PICP ng/ml            | 296 ± 35.6       | 291 ± 17.6   | 1.612 | 0.107   |
| reference range       | (30 – 300)       |              |       |         |
| ICTP µg/l             | 4.4 ± 0.58       | 4.1 ± 0.68   | 1.764 | 0.078   |
| reference range       | (2.1 – 56)       |              |       |         |
| ICTP/PICP             | 0.0149 ± 0.002   | 0.0143 ± 0.003 | 1.486 | 0.137   |

**FIG. 2.** Mean values + SD of estradiol (left) and progesterone (right) concentrations in both menstrual cycle phases in the studied women.

**FIG. 3.** Mean values ± SD of the muscle torque (MVC) obtained for different muscle length (two different ankle joint: 90 and 60°) in two phases of the menstrual cycle.
FIG. 4. Correlation maps between MVC and biochemical and body composition variables in each phase of the menstrual cycle (statistically significant – p < 0.05).

DISCUSSION

Little research has been done into correlations of the levels of female sex steroids and collagen metabolism markers with variations in muscle strength of young active women. This problem is very important due to the need to reduce the high frequency of injuries in the group of women compared to men [2, 5, 6, 30]. This is especially important because the number of women involved in either professional or recreational sports has been significantly rising in recent years [31, 32, 33]. In the present study, the analysis concerned strength abilities taking into consideration GH/IGF-1 axis hormone levels and collagen metabolism during the menstrual cycle in women involved in moderate-intensity physical activity.

Many researchers have demonstrated that high-intensity physical exercise can be a direct factor that disturbs cycles through increase in energy deficits [34]. More frequent disturbances in the luteal phase were also observed at lower oestradiol levels [34, 35]. It was found that for the examined women, the intensity of their physical activity did not affect the quality of their cycle.

Moreover, women who participated in the present study were characterized by a lean body. Mean values of body composition did not exceed the adopted norms and remained at nearly the same level over the menstrual cycle. It is difficult to conclude unequivocally whether this was due to physical exercise. This effect cannot be excluded, especially because the time of regular activity was over one year.

However, it was demonstrated that the women were characterized by higher MVC in the luteal phase (Fig. 3). Therefore, the influence of the menstrual cycle on strength abilities of women was proved. Despite the lack of significant correlations between growth hormone, oestradiol levels and MVC in both phases of the menstrual cycle, in other studies the GH/IGF-1 axis hormones have been demonstrated to have an impact on increased bone and muscle mass [36] and increased collagen synthesis [24, 37]. Kraemer et al. [38] and Wildman et al. [39] observed, the significant strength of this correlation in male groups. The result of the current research is most likely related with a more complicated hormonal system in women. and the necessity of taking into consideration changes in hormonal secretion of the reproductive axis, since multiple studies have found a significant correlation of elevated oestrogen and relaxin levels, and reduced collagen synthesis [18, 19, 20]. It was found that the increase in oestradiol leads to the reduction in proliferation of fibroblasts, which is the cause of reduced collagen synthesis and consequently reduced strength properties of the ligaments [23]. Shultz et al. [24] demonstrated that such changes are observed on days around the ovulatory phase in the menstrual cycle. In the luteal phase, the content of the markers of collagen synthesis and breakdown increases to the level observed during the first days of the menstrual cycle. The results of the present study did not confirm these observations due to the PICP secretion in both phases being maintained at a similar level. Furthermore, the recorded concentration of the collagen synthesis marker (PICP) was at the upper limit of the reference range, with a minimal reduction in its concentration in the luteal phase (1.7%) at substantially higher oestradiol levels. It was also observed that the marker of collagen breakdown (ICTP) was reduced by 6.8% in the same phase. We believe that the factor that masks the negative effect of oestrogens on collagen synthesis is physical activity of women and the related high level of IGF-1 secretion. The increase in growth factor during exercise is explained by its para- and autocrine effects in the area of the muscle. It was demonstrated that one of the IGF-1 isoforms is produced in the muscle tissue during active muscle excitation (stretch), and, therefore, is termed a mechanical growth factor, and consequently an increase in this factor in the human body can be expected following physical exercise [35]. It is considered that excitation of the nervous system with physical
exercise stimulates increased secretion and the effect of IGF-1 on tissues, which is explained by presence of a high number of the hormone receptors in the nervous system [40]. Furthermore, many researchers have also indicated that prolonged physical exercise stimulates collagen synthesis, which was confirmed by higher PICP plasma levels observed 72 hours following the exercise [25].

IGF-1 plays an important role in regulation of collagen synthesis [22], but it also prevents its excessive breakdown [24]. Therefore, the ICTP/PICP ratio was adopted as a precondition for maintaining fascia elasticity and opportunities for developing strength in a stretched muscle. In the second phase of the menstrual cycle, this index was lower, which indicated higher synthesis compared to collagen breakdown. This reverse correlation between IGF-1 and ICTP/PICP ratio was also found by Doessing et al. [22]. This is likely to have been the cause of significantly higher MVC values women in the luteal phase.

This study does not entirely explain the problems discussed, but it demonstrates the complexity and multifaceted nature of such problems. Functioning of women cannot be analysed in isolation from hormonal changes and the effect of external factors. It seems that the statistical analysis of this study could be underpowered. Therefore, conclusions can only be generalized with caution. For that reason, one should continue the examinations connected with in-depth analysis of individual biochemical variables that modify biomechanics of functioning of women over the menstrual cycle and compare the results to athletes characterized by higher exercise intensity and women who are physically inactive.

CONCLUSIONS

The results suggest that the level of physical activity contributed to the higher concentration of IGF-1 (upper limit of reference range) during the entire menstrual cycle. Consequently, with the effect of the increased oestradiol concentration in the luteal phase a substantial reduction in collagen synthesis was not observed, but a significant increase in strength abilities was observed in the luteal phase.

The variability of physical abilities (MVC) during the menstrual cycle showed that menstrual status should be taken into account in determination of the training loads. Increasing the load in the luteal phase seems to be favoured by a beneficial change in collagen metabolism observed in physically active women.

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Conflict of interest

The authors declare no conflict of interest.

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