

Adiponectin and osteocalcin responses to rowing exercise, and the relationship to substrate oxidation in female rowers

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This study investigated the effects of acute exercise and menstrual phase on adiponectin and osteocalcin concentrations, and the possible role of these biomarkers in exercise-induced substrate oxidation in rowers. Thirteen female rowers (19.3 ± 2.3 years; height: 172.7 ± 3.9 cm; body mass: 66.5 ± 7.9 kg) performed 1-h rowing ergometer exercise at 70% of maximal oxygen consumption (VO_2max) during follicular phase and luteal phase of the menstrual cycle. Oxygen consumption (VO_2), total energy expenditure (EE), carbohydrate EE, and lipid EE were assessed during the exercise. Venous blood samples were collected before and after ergometer exercise. No differences ($p > 0.05$) were observed in substrate oxidation values during exercise across menstrual cycle. Exercise resulted in an acute rise in osteocalcin and no changes in adiponectin at both menstrual cycle phases. Adiponectin and osteocalcin were not related across phase or time ($r < 0.211$; $p > 0.05$). Post-exercise adiponectin was related ($p < 0.05$) to mean VO_2 ($r = 0.459$) and total EE rate ($r = 0.598$), while post-exercise osteocalcin was correlated ($p < 0.05$) with mean total ($r = 0.411$) and lipid ($r = 0.557$) EE rates. In conclusion, menstrual cycle phase had no effect on substrate oxidation, and adiponectin and osteocalcin responses to acute exercise. It appears that adiponectin and osteocalcin may serve as signals for metabolic reaction to the energy cost of the acute exercise in female rowers.

Keywords: female athletes, menstrual cycle, endurance exercise, substrate oxidation, adipocytokines, osteokines

Introduction

Substrate utilization during acute endurance exercise has been well studied. The relative utilization of carbohydrates and lipids as fuel sources during substrate oxidation in athletes can be influenced by diet (2), training status (13), exercise intensity (29), prolonged strenuous exercise repeated over consecutive days in a multi-day sport event (24), and also by a relative hormonal milieu during exercise (2). For example, it has been suggested that females utilize more lipids to fuel aerobic exercise than males (29). The possible gender difference in relative fuel oxidation during aerobic exercise is due to differences in circulating estrogen (4). Furthermore, certain data demonstrate that there may be a greater lipid oxidation and a lower carbohydrate oxidation during submaximal exercise [$<70\%$ of maximal oxygen consumption (VO_2max)] in the luteal phase (LP) compared with the follicular phase (FP) of the menstrual cycle (31), while other studies report that there are only small differences in substrate metabolism during aerobic exercise due to the endogenous ovarian hormone fluctuations across the normal menstrual cycle (2, 4). In accordance with this, we have

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previously demonstrated no effect of menstrual cycle phase on substrate oxidation during acute rowing exercise at 70% of VO_2max in female rowers (28). However, in addition to the impact of the ovarian hormone milieu on substrate oxidation during aerobic exercise in female athletes (2, 4), other hormones that participate in the regulation of energy homeostasis may also influence lipid and carbohydrate oxidation during acute aerobic exercise in athletes (12).

The regulation of energy homeostasis is dependent on several peripheral factors that signal the status of energy stores to the hypothalamus (17). These peripheral factors are synthesized from adipose, bone, and muscle tissues, which may act as endocrine organs (6, 18). Adipose tissue produces variety of adipocytokines including leptin and adiponectin (17). While leptin is the most studied adipocytokine that is known to vary during the menstrual cycle, showing lower values during the FP and higher values during the LP in normally menstruating women (1, 21), adiponectin has not been reported to fluctuate during the menstrual cycle in female athletes (20). The relatively stable adiponectin concentrations throughout the menstrual cycle could indicate that this adipocytokine may not play a considerable role in female reproductive functions in contrast to leptin (1). However, similar to leptin, adiponectin is sensitive to the effects of prolonged exercise and could be linked to the energetic status induced by acute exercise (17). In addition to adipose tissue, bone tissue has also emerged as endocrine organ that produces several osteokines with potential effects on energy homeostasis (6). Specifically, osteocalcin that has been traditionally considered a biological marker of bone formation has effects on body mass, energy expenditure (EE), and glucose homeostasis (6, 8). It has been found that osteocalcin is not affected by menstrual cycle phase similar to adiponectin in female rowers (20). Furthermore, fasting osteocalcin and adiponectin concentrations were inter-related and it was suggested that these measured peripheral markers of adipose and bone tissues could be used to characterize overall energy homeostasis in female athletes (20). However, after extensive literature research, we were unable to find any investigations that describe the influence of a single exercise session that utilizes carbohydrate and lipid oxidation as fuel source during acute exercise on the possible interaction between adipocytokines and osteokines at different menstrual cycle phases in female athletes.

The evaluation of the response of different adipocytokines and osteokines to acute exercise is of great interest due to their implications in the regulation of acute energy homeostasis in female athletes. The hormonal response to a single exercise session may actually be a response to the acute energy deficit (18). Furthermore, differences in estrogen and progesterone concentrations during the FP and LP of the menstrual cycle suggest that glucose and fat metabolism may also vary between different phases in female athletes (4). Accordingly, the purposes of the current investigation were to: (1) examine adiponectin and osteocalcin responses to the aerobic exercise session at FP and LP of the menstrual cycle and (2) determine whether post-exercise adiponectin and osteocalcin are related to exercise substrate oxidation in female athletes. It was hypothesized that adiponectin and osteocalcin would increase in response to a single exercise session and post-exercise changes in these hormones would be related to exercise-induced substrate metabolism values. Furthermore, elevated levels of the ovarian hormones in the LP of the menstrual cycle could cause higher substrate oxidation and total EE as a result of the aerobic exercise session in the LP compared with the FP of the menstrual cycle. Accordingly, this in turn may result in higher post-exercise values of adiponectin and osteocalcin concentrations in the LP compared with the FP of the menstrual cycle in female rowers.

Materials and Methods

Participants

Thirteen college-level eumenorrheic female rowers (19.3 ± 2.3 years; height: 172.7 ± 3.9 cm; body mass: 66.5 ± 7.9 kg; $27.7 \pm 4.5\%$ body fat) with normal menstrual cycles participated in this study. The subjects were drug and medication free, and were not taking any medications as determined by health history questionnaire. None of the rowers smoke or used any supplements. Participants were required to have menstrual cycle duration of 24–35 days, with at least 6 months of documented menstrual cycles, and were not using the oral contraceptive pills for at least 6 months preceding the study (2, 23, 27, 28). The study was conducted during the preparatory period for the competitive rowing season. The main goal of training during the preparatory period was to increase the aerobic base through aerobic extensive endurance training sessions. The training intensity was below anaerobic threshold for approximately 90% of the entire training time (17). The study design, purpose, and possible risks were explained to the participants and written informed consent was obtained from the participants prior to the investigation. The study protocol was approved by the Medical Ethics Committee of the University of Tartu.

Experimental design

Rowers completed two experimental testing sessions during the FP (determined as days 7–11 from onset of menstruation, mean day 9 ± 2 for the main experiment) and the LP (determined as days 18–22 from onset of menstruation, mean day 20 ± 2 for the main experiment) of the menstrual cycle (2, 26, 28, 29). Information about previous menstrual cycles was used to identify the phases of the menstrual cycle (3, 21). The length of the menstrual cycle was calculated from the first day of the menses to the day preceding the next menses. Menstrual cycle phases were later confirmed by estradiol and progesterone concentrations from the fasting blood samples (28, 29). The accepted concentration ranges for the ovarian hormones during both menstrual cycle phases were 85–220 pmol/l for estradiol and <3 nmol/l for progesterone during the FP, and 230–750 pmol/l for estradiol and >16 nmol/l for progesterone during the LP (27, 28). Therefore, the resting level of progesterone, higher than 16 nmol/l, was required to confirm LP (22).

Two identical testing sessions were conducted in both menstrual cycle phases. Preliminary tests included incremental rowing ergometer test that was followed by body composition measurements. One-hour endurance rowing ergometer session was conducted on the following day after the incremental rowing ergometer test (28). Test order was balanced with respect to the cycle phase and test time was standardized between 4.00 p.m. and 6.00 p.m. On the day before the exercise tests, no physical activities were allowed (15, 18, 19). Over the testing period, participants were asked to maintain a regular and constant volume and intensity of training (15, 18, 19). Participants were in a post-absorptive state having eaten a meal for about 2 h before each physical test (15, 18, 19). They were asked to document what they ate before the first test and were asked to replicate as exactly as possible for the subsequent sessions, in order to maintain a diet of similar composition and to reach nearly identical nutritional intake (4, 12, 28). In addition, rowers were instructed by a dietician, and their daily nutritional intake consisted of a high-carbohydrate diet with the composition remaining stable throughout the training session (28).

Body composition

Height (Martin Metal Anthropometer) and body mass (A&D Instruments Ltd., Oxfordshire, UK) of the participants were measured to the nearest 0.1 cm and 0.05 kg, respectively. Body composition was assessed via dual-energy X-ray absorptiometry. Scans of the whole body were performed on each of the subjects using Lunar DPX-IQ Densitometer (Lunar Corporation, Madison, WI, USA) and analyzed for fat mass and fat-free mass. The coefficient of variations (CVs) for body composition measurements were less than 2% (16).

Incremental rowing ergometer test

A stepwise incremental rowing ergometer test was performed on a wind resistance-braked rowing ergometer (Concept II, Morrisville, VT, USA) to determine VO_2max and target heart rate (HR) values for 1-h endurance exercise protocol (10). The rowers were fully familiarized with the use of the apparatus. Participants were equipped with the instruments and sat quietly for 1 min on the ergometer before starting to exercise at 40 W. Workload was increased by 15 W every minute until maximal voluntary exhaustion. Power and stroke rate were recorded continuously on the computer display of the rowing ergometer. The test was designed to reach the maximum in approximately 15 min in each participant (10). Subjects were strongly encouraged to achieve maximal performance. HR was recorded every 5 s during the test using Sporttester Polar 725X (Polar Electro Oy, Kempele, Finland). Respiratory gas exchange variables were measured throughout the test in a breath-by-breath mode and data were stored in 10 s intervals by a portable open circuit spirometry system (MetaMax 3B, Cortex Biophysic GmbH, Germany). All data were processed by means of computer analysis using standard software (MetaSoft, Cortex Biophysic GmbH, Germany) and the system for HR analysis (10). To establish that VO_2max was reached, the attainment of a plateau in oxygen consumption (VO_2) with increasing work rate was used as a criterion. When this plateau in VO_2 was not observed, a respiratory exchange ratio (RER) exceeding 1.1 and theoretical maximal cardiac frequency were used as a criterion (10).

Main endurance exercise protocol

The exercise test consisted of rowing on a rowing ergometer for 1 h at the intensity of 70% VO_2max (18, 19, 28). Target HR was set at the level obtained from the incremental test using a practical set ± 2 bpm of 70% VO_2max (18, 19, 28). Rowers were asked to increase the exercise intensity smoothly and the requested HR was achieved after the first 5 min. The participants were instructed to maintain the target HR steady state for the entire exercise session and to reduce exercise intensity to accommodate the required HR steady state as needed (18, 19, 28). Respiratory gas exchange variables were measured throughout the test in a breath-by-breath mode using a portable open circuit spirometry system (MetaMax I, Cortex, Germany) during the 1-h exercise session as described above. Fat and carbohydrate oxidation and EE were estimated from the RER using stoichiometric equations (7), with the assumption that urinary nitrogen excretion rate was negligible (29). These equations have previously been used in females to assess submaximal exercise substrate oxidation, during which the RER was < 1 (4, 25, 29).

Blood analysis

A 10-ml blood sample was obtained before (PRE), immediately after (POST), and after the first 30 min (POST-30') of the 1-h endurance exercise session from the antecubital vein with the participant in the upright position (15, 18, 19). The blood plasma was separated and

frozen at $-20\text{ }^{\circ}\text{C}$ for subsequent analysis. All blood samples were analyzed at the same time. Total adiponectin was assessed via commercially available radioimmunoassay (RIA) kit (Linco Research, St. Charles, MO, USA). The intra- and inter-assay CVs were $<7\%$. Osteocalcin, estradiol and progesterone were determined on Immulite 2000 (DPC, Los Angeles, CA, USA). The intra- and inter-assay CVs for osteocalcin were $<7\%$. The intra- and inter-assay CVs for estradiol were 5.3% and 6.5%, and for progesterone 5.4% and 3.4%, respectively.

Statistical analysis

Statistical analyses were performed using SPSS software for Windows (SPSS Inc., Chicago, IL, USA). Means (\pm SD) were determined. A two-way analysis of variance and least significant difference post hoc analysis tests were used to evaluate differences between measured variables. Pearson correlation analysis was used to examine relationships between adiponectin and osteocalcin, as well as the relationships of adiponectin and osteocalcin with variables of substrate metabolism during exercise. In addition, Pearson correlation analysis was used to assess the bivariate relationships between the change in adiponectin and osteocalcin from PRE to POST, and from POST to POST-30'. The level of significance was set at $p < 0.05$.

Results

Female rowers were weight stable throughout the study period, with no differences ($p > 0.05$) in measured body composition and aerobic capacity values over the days preceding the FP and LP of the menstrual cycle (Table I). Circulating estradiol (FP: 116.7 ± 60.4 pmol/l; LP: 462.4 ± 106.4 pmol/l) and progesterone (1.6 ± 0.6 nmol/l; LP: 28.4 ± 9.8 nmol/l) concentrations confirmed the menstrual cycle phases, with a 4-fold increase in estradiol ($p < 0.05$) and an 18-fold increase in progesterone ($p < 0.05$) in the LP compared with the FP.

Table I. Mean (\pm SD) body composition and aerobic capacity characteristics in studied female rowers ($n = 13$)

| Variable | FP | LP |
|------------------------------------|-----------------|-----------------|
| Body mass (kg) | 66.5 ± 7.9 | 66.9 ± 7.7 |
| Body fat (%) | 27.7 ± 4.5 | 27.7 ± 4.6 |
| Fat mass (kg) | 17.6 ± 3.8 | 17.8 ± 3.7 |
| Fat-free mass (kg) | 47.0 ± 5.6 | 47.7 ± 5.5 |
| VO ₂ max (l/min) | 3.09 ± 0.49 | 3.08 ± 0.42 |
| VO ₂ max/kg (ml/min/kg) | 46.7 ± 7.9 | 46.2 ± 4.9 |

Covered distance, HR and VO₂ during 1-h rowing ergometer exercise were not different ($p > 0.05$) among menstrual cycle phases (Table II). On the average, rowers exercised at $68.6 \pm 4.1\%$ and $68.4 \pm 4.4\%$ of VO₂max during the FP and LP, respectively. In addition, no differences between menstrual cycle phases were observed ($p > 0.05$) for total EE (FP: 610 ± 104 kcal; LP: 627 ± 95 kcal) of the 1-h endurance test. During the exercise, at least 70% of the

energy used was derived from carbohydrate sources (FP: 75.6%; LP: 72.7%) and also no significant menstrual cycle phase effect was observed either (Table II). Body mass was reduced ($p < 0.05$) after the exercise at FP (from 66.5 ± 7.9 to 66.0 ± 8.0 kg) and LP (from 66.9 ± 7.7 to 65.7 ± 7.9 kg) with no significant menstrual cycle phase effect ($p > 0.05$). Changes in plasma volume during rowing ergometer exercises were small and did not significantly differ ($p > 0.05$) between FP ($-0.3 \pm 1.3\%$) and LP ($-0.4 \pm 2.1\%$). Therefore, no adjustments were made in measured concentrations of plasma constituents.

Table II. Mean (\pm SD) measured physiological and EE values during 1-h rowing ergometer exercise ($n = 13$)

| Variable | FP | LP |
|--------------------------|-----------------|-----------------|
| Distance (km) | 11.9 ± 1.0 | 12.0 ± 1.0 |
| Heart rate (bpm) | 150.5 ± 7.1 | 150.3 ± 7.7 |
| VO ₂ (l/min) | 2.2 ± 0.5 | 2.1 ± 0.4 |
| RER | 0.90 ± 0.06 | 0.93 ± 0.05 |
| CHO EE rate (kcal/min) | 7.7 ± 1.7 | 7.6 ± 1.8 |
| Lipid EE rate (kcal/min) | 2.5 ± 1.5 | 2.8 ± 1.7 |
| EE rate (kcal/min) | 10.4 ± 1.3 | 10.8 ± 1.6 |

No menstrual cycle phase effect ($p > 0.05$) was observed for baseline and post-exercise adiponectin and osteocalcin concentrations (Table III). Adiponectin was not changed ($p > 0.05$) as a result of 1-h rowing ergometer exercise, while osteocalcin was increased immediately after the exercise and remained elevated ($p < 0.05$) during the first 30-min post-exercise (Table III).

Table III. Mean (\pm SD) blood biochemical variables during 1-h rowing ergometer exercise ($n = 13$)

| Variable | Menstrual phase | PRE | POST | POST-30' |
|---------------------------|-----------------|-----------------|-------------------|-------------------|
| Adiponectin (μ g/ml) | FP | 11.2 ± 3.1 | 11.0 ± 4.1 | 11.4 ± 3.7 |
| | LP | 11.1 ± 3.9 | 10.7 ± 3.9 | 11.0 ± 3.5 |
| Osteocalcin (ng/ml) | FP | 36.0 ± 13.9 | $40.3 \pm 17.5^*$ | $40.1 \pm 15.8^*$ |
| | LP | 37.4 ± 13.3 | $41.5 \pm 14.8^*$ | $42.5 \pm 15.6^*$ |

*Significantly different from PRE; $p < 0.05$

Correlational analysis demonstrated that adiponectin and osteocalcin concentrations were not significantly related across phase or time ($r < 0.211$; $p > 0.05$), although baseline adiponectin and osteocalcin values demonstrated some trend for significance ($r = 0.366$; $p = 0.066$). Changes in adiponectin were not related to changes in osteocalcin across time. However, adiponectin measured immediately after 1-h rowing ergometer exercise was related to mean exercise VO₂ (l/min) ($r = 0.459$; $p = 0.018$; Fig. 1) and total EE rate (kcal/min)

($r = 0.598$; $p = 0.001$; Fig. 2). In addition, osteocalcin measured immediately after the exercise was related to lipid EE rate (kcal/min) ($r = 0.557$; $p = 0.001$; Fig. 3) and total EE rate (kcal/min) ($r = 0.411$; $p = 0.037$; Fig. 4). Other estimates of exercise substrate metabolism were not related to adiponectin and osteocalcin concentrations ($r < 0.321$; $p > 0.05$).

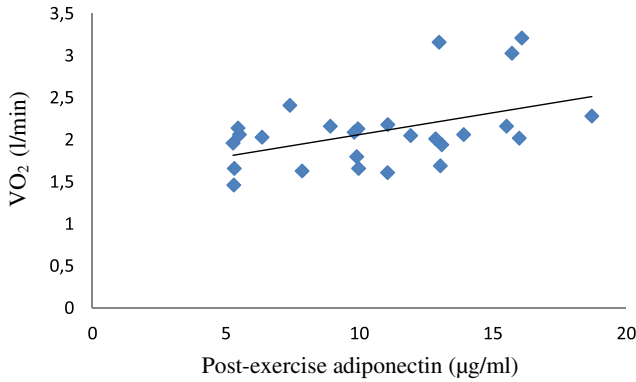


Fig. 1. Relationships between post-exercise adiponectin concentration and mean VO_2 during submaximal rowing ergometer exercise performed at 70% of $\text{VO}_{2\text{max}}$ in college-level female rowers ($r = 0.459$; $p < 0.05$)

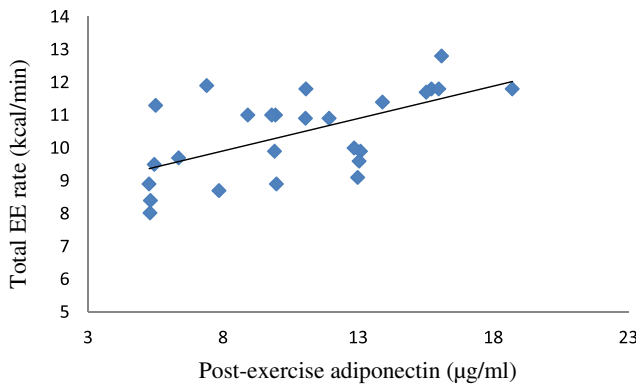


Fig. 2. Relationships between post-exercise adiponectin concentration and mean total EE rate measured during submaximal rowing ergometer exercise performed at 70% of $\text{VO}_{2\text{max}}$ in college-level female rowers ($r = 0.598$; $p < 0.05$)

Discussion

The present study investigated adiponectin and osteocalcin responses to aerobic exercise at different phases of the menstrual cycle and determined possible associations of these markers of EE to substrate metabolism in college-level female rowers. In this study, a rowing exercise was used, during which relatively large muscle mass is used and higher EE is produced in comparison with other endurance events (14). The 1-h rowing ergometer exercise was performed slightly below 70% of $\text{VO}_{2\text{max}}$ (28) and provided a valid reflection of sport-specific endurance capacity in rowers (14). To the best of our knowledge, this is the first study to determine adiponectin and osteocalcin responses to prolonged, steady-state exercise in different phases of the menstrual cycle in female athletes. The main finding of this investigation was that there appeared to be no interaction between adiponectin and osteocalcin, and menstrual cycle phase had no effect on both blood biochemical marker responses

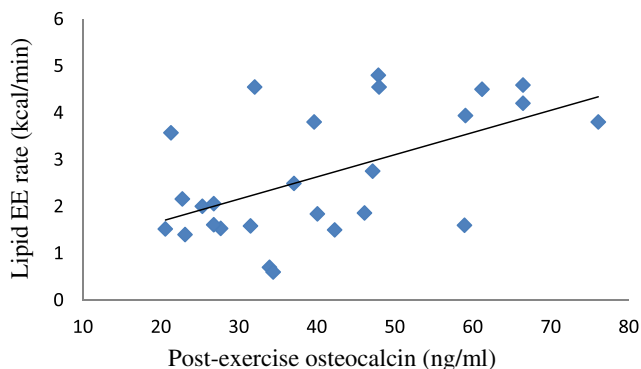


Fig. 3. Relationships between post-exercise osteocalcin and mean EE from fat (lipid EE rate) measured during submaximal rowing ergometer exercise performed at 70% of VO_2max in college-level female rowers ($r = 0.557$; $p < 0.05$)

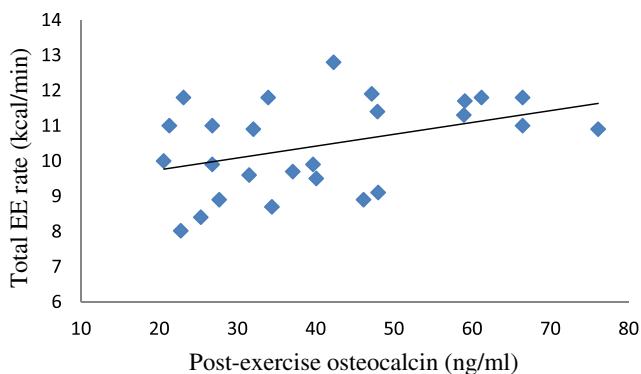


Fig. 4. Relationships between post-exercise osteocalcin and mean total EE rate measured during submaximal rowing ergometer exercise performed at 70% of VO_2max in college-level female rowers ($r = 0.411$; $p < 0.05$)

to acute exercise. Adiponectin was not changed and osteocalcin was increased as a result of acute exercise in both trials (see Table III). Similar to other studies (28), no menstrual phase (FP vs. LP) effects were also observed for measured substrate oxidation variables either (see Table II) and thus the data for the two menstrual cycle phases were combined for subsequent analysis (12). Some associations of post-exercise adiponectin and osteocalcin concentrations with substrate-level metabolism values were found, which may indicate that circulating adiponectin and osteocalcin concentrations could be factors influencing substrate selection and availability during acute rowing ergometer exercise in college-level female rowers.

The results of the present investigation demonstrated that the measured physiological and EE values during 1-h submaximal rowing ergometer exercise slightly below 70% of VO_2max were not substantially influenced by the menstrual cycle phase (see Table II). This is in line with other studies (2, 4, 21, 23, 28). In addition, the percentage of EE derived from carbohydrate and lipid oxidation was similar in both rowing ergometer trials at different menstrual cycle phases, which is also similar to other studies (11, 28). There are studies that have reported greater lipid oxidation and lower carbohydrate oxidation in LP compared with FP of the menstrual cycle in physically active healthy women (9, 31). However, Wenz et al. (30) reported greater lipid oxidation during cycle ergometer exercise at the lower intensities (30% and 50% of VO_2max), but not during cycle ergometer exercise at the intensity of 70% of VO_2max in healthy eumenorrheic women. In accordance, our results show that lipid oxidation is similar at both menstrual cycle phases, when prolonged acute exercise is performed at higher subintensity of VO_2max .

The present investigation found that 1-h rowing ergometer exercise with an average EE just over 600 kcal (FP: 610 kcal; LP: 627 kcal) was not sufficient to cause significant increase in circulating adiponectin concentrations. It has been demonstrated that acute exercise has no effect on adiponectin concentration as 1-h cycle ergometer exercise at the intensity of 65% of VO_2max did not change adiponectin concentration in a group of healthy males and females (5). In contrast, our previous studies with male rowers have suggested that the increased post-exercise adiponectin concentration could be regarded as a signal of metabolic reaction to acute rowing exercise (17). Specifically, post-exercise adiponectin concentration was increased after 30 min of on-water sculling at the intensity of 75% of VO_2max (15) and after 2 h of on-water sculling at the intensity of 70% of VO_2max (18). Assuming that the negative energy balance drives the specific hormonal response (17), it is conceivable that the measured post-exercise adiponectin concentration is related to the exercise-induced metabolic parameters. In accordance with this, the current study demonstrated significant ($p < 0.05$) association of post-exercise adiponectin concentration with mean exercise VO_2 (l/min) ($r = 0.459$; see Fig. 1) and total EE rate (kcal/min) ($r = 0.598$; see Fig. 2) in female rowers.

While the EE during 1-h rowing ergometer exercise was not high enough to cause significant post-exercise increase in adiponectin concentration, post-exercise osteocalcin was increased and remained elevated during the first 30 min of recovery period (see Table III). Post-exercise osteocalcin was similarly related to mean total EE rate (kcal/min) of the rowing ergometer exercise ($r = 0.411$; $p < 0.05$), demonstrating that circulating osteocalcin could also be used as a biological marker of exercise stress after acute rowing exercise in female rowers. Furthermore, lipid EE rate (kcal/min) measured during submaximal rowing exercise was related to post-exercise osteocalcin ($r = 0.557$; $p < 0.05$) (see Fig. 4). It could be speculated that the increase in osteocalcin may have resulted from the activation of lipolysis, thereby increasing lipid EE rate. These results are in line with the findings that osteocalcin is an osteoblast-derived protein acting as a hormone that stimulates EE (6).

This study has some limitations. While participants were encouraged to replicate the same diet for both testing sessions, caloric intake was not directly measured. In addition, substrate utilization was collected via indirect calorimetry but not measured more precisely using labeled isotopes. Finally, blood samples were only collected before and after exercise but not during exercise.

In conclusion, the results of the present study demonstrated no effect of menstrual cycle phase on acute exercise substrate oxidation similarly as observed in many previous studies. While EE was high enough to increase post-exercise osteocalcin, post-exercise adiponectin was not increased as a result of acute exercise. However, post-exercise adiponectin and osteocalcin levels were found to be related to markers of substrate metabolism indicating that both these markers may serve as signals for metabolic reaction to the energy cost of the acute exercise in female rowers.

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