Cardiorespiratory Responses to Endurance Exercise Over the Menstrual Cycle and With Oral Contraceptive Use

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Abstract
Barba-Moreno, L, Cupeiro, R, Romero-Parra, N, Janse de Jonge, XA, and Peinado, AB. Cardiorespiratory Responses to Endurance Exercise Over the Menstrual Cycle and With Oral Contraceptive Use. J Strength Cond Res 33(6): 815;2019—Female steroid hormone fluctuations during the menstrual cycle and exogenous hormones from oral contraceptives may have potential effects on exercise performance. The aim of this study was to investigate the effects of these fluctuations on cardiorespiratory responses during steady-state exercise in women. Twenty-three healthy endurance-trained women performed 40 minutes of running at 75% of their maximal aerobic speed during different phases of the menstrual cycle (n = 15; early follicular phase, midfollicular phase, and luteal phase) or oral contraceptive cycle (n = 8; hormonal phase and nonhormonal phase). Ventilatory parameters and heart rate (HR) were measured. Data were analyzed using a mixed linear model. For the eumenorrheic group, significantly higher oxygen uptake (\( p = 0.049 \)) and percentage of maximum oxygen uptake (\( p = 0.035 \)) were observed during the midfollicular phase compared with the early follicular. Heart rate (\( p = 0.004 \)), oxygen ventilatory equivalent (\( p = 0.042 \)), carbon dioxide ventilatory equivalent (\( p = 0.017 \)), and tidal volume (\( p = 0.024 \)) increased during luteal phase in comparison with midfollicular. In oral contraceptive users, ventilation (\( p = 0.030 \)), breathing frequency (\( p = 0.018 \)), oxygen ventilatory equivalent (\( p = 0.032 \)), and carbon dioxide ventilatory equivalent (\( p = 0.001 \)) increased during the hormonal phase. No significant differences were found for the rest of the parameters or phases. Both the eumenorrheic group and oral contraceptive group showed a significant increase in some ventilatory parameters during luteal and hormonal phases, respectively, suggesting lower cardiorespiratory efficiency. However, the lack of clinical meaningfulness of these differences and the nondifferences of other physiological variables, indicate that the menstrual cycle had a small impact on submaximal exercise in the current study.

Key Words: female, oral contraceptives, estrogen, progesterone

Introduction
Women experience steroid hormone fluctuations during the menstrual cycle. These hormonal variations especially in estrogen and progesterone may have significant effects on exercise responses and athletic performance (13,20,30,42). The existing research on potential changes over the menstrual cycle in metabolic and cardiorespiratory variables during exercise has shown conflicting findings. Most studies have reported no changes over the menstrual cycle in oxygen uptake (\( \dot{V}_{\text{O}_2} \)), ventilation (\( V_E \)), and heart rate (HR) during maximal or submaximal exercise (1,11,21,30,38). However, other studies have found higher \( \dot{V}_{\text{O}_2} \), HR, or \( V_E \) during the luteal phase indicating a higher cardiopulmonary strain (14,20,21,30,35,42).

Progesterone levels increase significantly during the luteal phase as compared to the follicular phase in the ovulatory cycle. High progesterone levels during the luteal phase have been associated with an increase in \( V_E \) in both animal (2,40) and human studies (21,35,42). Research suggest that progesterone may lower the threshold of the medullary respiratory center, increasing its excitability (25,42) and that this respiratory response (increased ventilation) to progesterone is mediated by an estrogen-dependent progesterone receptor through a central effect in the hypothalamus (2). Another factor associated with an increase in \( V_E \) in humans is the increase of body temperature (10,41). During the luteal phase of the menstrual cycle, progesterone’s thermogenic effect changes the body’s thermoregulatory set-point (6,9), which leads to an increased body temperature (18,37), which may also explain the increase in HR reported during the luteal phase (14,35). Consequently, these hormonal mechanisms could explain the changes in cardiorespiratory responses to exercise over the menstrual cycle.

Use of oral contraceptives (OC) has increased significantly over the years in both the general and athletic population (3). Besides for contraceptive reasons, OC are used to reduce cycle-length variability and premenstrual symptoms and allow for cycle manipulation (postponing or skipping bleeding) (5). The exogenous hormones in the OC suppress the natural fluctuations in the endogenous hormones to avoid ovulation. Research to date over the potential effects of the exogenous hormones in OC on cardiorespiratory responses to exercise has shown conflicting results.
Some authors reported a significant decrease in peak oxygen uptake (\(\text{VO}_{2\text{peak}}\)) during the hormonal phase of a contraceptive cycle in active, trained, or athletic women (7,12,23,39), whereas other authors reported no differences for \(\text{VO}_{2\text{peak}}\) (24,32). Despite this, none of these aforementioned studies showed significant differences for \(V_E\) or \(HR\) between OC phases.

The different types of OC used (monophasic, biphasic, or triphasic) and different testing days chosen by each study may explain some of these conflicting findings, but further research is needed into the potential effect of the OC on exercise responses.

In an attempt to address some of the methodological issues encountered so far, the current study focused on only active endurance-trained women to ensure practical implications of the findings for female athletes. The eumenorrheic group completed cardiorespiratory testing at 3 time points, which coincides with different hormonal levels throughout the menstrual cycle.

In addition, this study is the first one to date analyzing the novel comparison between early follicular and midfollicular phases, providing a more complete picture about the possible influence of different hormonal profiles on cardiorespiratory variables.

A higher HR, \(\text{VO}_2\), and \(V_E\) observed in a specific phase of the menstrual cycle for the same intensity and volume may indicate a reduced respiratory efficiency. However, these findings are still controversial, and further research is needed before obtaining specific recommendations for the optimization of training programs.

The hypothesis of this study was that increased progesterone levels during the luteal phase would cause a greater ventilatory drive accompanied by higher \(\text{VO}_2\) and HR. For the OC group, the higher ovarian activity during the nonhormonal phase would produce a higher cardiorespiratory response. Therefore, the purpose of this study was to investigate whether the ventilatory and cardiorespiratory responses to submaximal endurance exercise are affected by acute endogenous hormone fluctuations throughout the normal menstrual cycle and exogenous hormone levels from the monophasic oral contraceptive cycle in well-trained endurance athletes.

### Methods

**Experimental Approach to the Problem**

The current study is an observational, randomized, and controlled study conducted by a collaboration of different specialists. Eumenorrheic women and OC users participated in this study. A repeated-measures design was used to determine if there were differences in cardiorespiratory variables depending on menstrual cycle phases. Subjects performed the same exercise protocol in 3 different phases of the menstrual cycle for the eumenorrheic group (early follicular, midfollicular, and luteal) and in 2 phases of a monophasic oral contraceptive cycle for the OC group (nonhormonal and hormonal). The exercise protocol consisted of endurance exercise based on 40 minutes of continuous running at 75% of maximal aerobic speed. Estradiol and progesterone were measured to confirm menstrual cycle phase.

### Subjects

Twenty-three healthy endurance-trained women, eumenorrheic (\(n = 15\); age = 35.6 ± 4.2 years; age range: 28-40 years; height = 163.9 ± 5.9 cm; body mass = 58.1 ± 5.2 kg; \(\text{VO}_{2\text{peak}} = 50.3 ± 3.6 \text{ml.min}^{-1}	ext{kg}^{-1}\)) or taking OC (\(n = 8\); age = 30.1 ± 4.8 years; age range: 24-40 years; height = 164.3 ± 9.5 cm; body mass = 59.3 ± 6.0 kg; \(\text{VO}_{2\text{peak}} = 51.7 ± 3.9 \text{ml.min}^{-1}	ext{kg}^{-1}\)) were recruited for this study. No baseline variables (height, body mass, and \(\text{VO}_{2\text{peak}}\)) differed between groups except for age (\(p = 0.05\)). This study was approved by an Institutional Review Board, specifically by the Research Ethics Committee of the Polytechnic University of Madrid. Subjects were informed of the risks and benefits of the study before any data collection, and an institutionally approved informed consent document was signed by each subject.

Inclusion criteria were (a) healthy female between 25 and 40 years; (b) regular menstrual cycles for last year for the eumenorrheic group or taking monophasic contraceptive pill for the OC group for at least 6 months; (c) participating in endurance sports (athletics or triathlon) between 5 and 12 hours per week; (d) free of iron deficiency (serum ferritin >20 \(\mu\text{g.L}^{-1}\), hemoglobin >115 \(\mu\text{g.L}^{-1}\), and transferrin saturation <16%); (e) no thyroid problems (TSH levels between 0.550 and 4.780 \(\mu\text{UI.ml}^{-1}\)); (f) nonsmokers; (g) not consuming medication or dietary supplements that alter vascular function (e.g., tricyclic antidepressants, \(\beta\)-blockers, etc.); (h) not pregnant; and (i) no ovarietomy. Eumenorrheic menstrual cycles were defined as regularly occurring menstrual cycles ranging from 24 to 35 days in length (22).

### Procedures

**Oral Contraceptive Pill.** Subjects used different monophasic OC preparations during the research, including different doses of exogenous hormones. These preparations were Loette diario (100-\(\mu\text{g}\) levonorgestrel, 20-\(\mu\text{g}\) ethinylestradiol; \(n = 1\)); Donabel (2,000-\(\mu\text{g}\) dienogest, 30-\(\mu\text{g}\) ethinylestradiol; \(n = 1\)); Droserule (3,000-\(\mu\text{g}\) drosperronel, 30-\(\mu\text{g}\) ethinylestradiol; \(n = 1\)); Drospurelle (3,000-\(\mu\text{g}\) drosperronel, 20-\(\mu\text{g}\) ethinylestradiol; \(n = 1\)); Yasmin (3,000-\(\mu\text{g}\) drosperronel, 30 \(\mu\text{g}\) ethinylestradiol; \(n = 1\)); STADA Genéricos (100-\(\mu\text{g}\) levonorgestrel, 20-\(\mu\text{g}\) ethinylestradiol; \(n = 2\)); Gestinyl (75-\(\mu\text{g}\) gestodene, 20-\(\mu\text{g}\) ethinylestradiol; \(n = 1\)). All these brands included 3 weeks of 21 exogenous hormone pills and 1 week of 7 placebo pills with no hormonal load (0-\(\mu\text{g}\) dose for both estradiol and progestogen during the nonhormonal phase). During the hormonal phase test (between days 15 and 21), the mean daily dose was either 20 \(\mu\text{g}\) (\(n = 5\)) or 30 \(\mu\text{g}\) (\(n = 3\)) of ethinylestradiol, and 100 \(\mu\text{g}\) of levonorgestrel (\(n = 3\)), 2,000 \(\mu\text{g}\) of dienogest (\(n = 1\)), 3,000 \(\mu\text{g}\) of drosperronel (\(n = 3\)) or 75 \(\mu\text{g}\) of gestodene (\(n = 1\)) for progestogen.

**Study Design.** The subjects of both eumenorrheic and OC groups performed the same experimental protocol on a treadmill in each hormonal phase. The eumenorrheic group participated in 3 laboratory sessions corresponding to the early follicular phase (day 3 ± 1), midfollicular phase (day 8 ± 1), and luteal phase (day 21 ± 2). The OC group performed 2 laboratory sessions corresponding to the nonhormonal phase during the inactive pill week (day 5 ± 2) and in the hormonal phase during the second week of the active pill (day 16 ± 2). Specifically, the different phases of the menstrual cycle of this study were selected to meet the following criteria: low progesterone and estrogen levels in the early follicular phase (days 2–5), low progesterone levels, and mid-high estrogen concentrations in the midfollicular phase (days 7–10) to analyze different estrogen values with similar progesterone levels and elevated levels of both progesterone and estrogen in the luteal phase (days 19–21).

All eumenorrheic subjects were asked to complete information about the length of their last 4 menstrual cycles (number of days from the cycle onset to the next one) prior to the beginning of the project to determine the average cycle length. Day 1 of the menstrual cycle was characterized by the onset of menstrual bleeding.
The early follicular and midfollicular phases’ testing sessions were conducted a standardized number of days from the first day of menstruation, while midluteal phase testing was undertaken 7–9 days after predicted ovulation, which was calculated as the middle day of the cycle, based on cycle length of the previous 4 cycles.

In addition, blood samples were collected for determination of female steroid hormones in each menstrual phase for both groups to confirm subjects were performing the tests in the correct phase. Oral contraceptives group phases were based on OC ingestion with day 1 of the OC cycle coinciding with the first inactive pill consumption (7 days of nonhormonal phase) followed by 21 days of active pill consumption (hormonal phase). During the hormonal phase, each OC subject was required to standardize pill consumption, taking the pill at the same time every day. All testing sessions were identical. The order of testing for subjects was counterbalanced not to affect the response to exercise.

All subjects completed a 72-hour diet record around the first testing session (during the day before of the test, the testing day, and the day after the test). They were asked to replicate this diet for each of the testing sessions.

**Screening Protocol.** Initial testing involved screening and measurement of \( \dot{V}O_2 \text{peak} \). All initial testing was performed during the early follicular phase (between days 2 and 5 of a normal cycle) for the eumenorrheic group or during the nonhormonal phase for the OC group (days 3–7). Resting blood samples were obtained in an early morning fasted state. The gynecologist of the project verified that none of the subjects showed signs of inflammation, iron deficiency, thyroid problems, or menstrual cycle dysfunction.

Body composition, as well as height and body mass (Beurer GmbH, Ulm, Germany) were measured. A dual-energy X-ray absorptiometry (DEXA) scanner (GE Lunar Prodigy apparatus; GE Healthcare, Madison, WI) was used to determine the following variables: body fat mass (%), total body fat mass (kg), and free fat mass (kg). DEXA scans were analyzed using GE Encore 2002 software (v 6.10.029).

A maximal graded exercise test to exhaustion was completed with a computerized treadmill (H/P/COSMOS 3 PW 4.0; H/P/Cosmos Sports & Medical, Nussdorf-Traunstein, Germany) to determine each subject’s \( \dot{V}O_2 \text{peak} \). Subjects began with a warm-up of 3 minutes at 6 km h\(^{-1}\). The speed was then increased by 0.2 km h\(^{-1}\) every 12 seconds. A slope of 1% was set throughout the test. Expired gases were measured breath-by-breath using a gas analyzer Jaeger Oxycon Pro (Erich Jaeger; Viasys Healthcare, Würzburg, Germany). Heart response was continuously monitored with a 12-lead ECG.

\( \dot{V}O_2 \text{peak} \) was determined as the mean of the 3 highest and continuous 15-second interval \( \dot{V}O_2 \) measurements in the incremental test to exhaustion, as previously reported (8). The maximal aerobic speed was recorded as the minimum speed required to elicit \( \dot{V}O_2 \text{peak} \). For the submaximal test, the speed equivalent to 75% of the maximal aerobic speed was calculated.

**Experimental Protocol.** Subjects attended the laboratory at 07:00 AM on the testing day. They had breakfast 2 hours before the test and were instructed to abstain from alcohol, caffeine, and exercise for 24 hours before each test. Before the test, weight and blood pressure were measured, and blood samples were taken for later confirmation of the cycle phase. After this, the steady-state running test protocol was performed. This consisted of a 5-minute warm-up at 60% of maximal aerobic speed followed by 40 minutes of continuous running at 75% of maximal aerobic speed. The same absolute velocity was used for each of the testing sessions. Each test finished with a 5-minute recovery at 30% of maximal aerobic speed. The testing protocol has been previously selected by other studies (36). The mean \( \pm SD \) speed that subjects ran on the treadmill was 11.2 \( \pm 1.1 \) km h\(^{-1}\) for the eumenorrheic group and 11.7 \( \pm 0.9 \) km h\(^{-1}\) for the OC group. Our subjects were trained in endurance sports or prolonged running.

During exercise, oxygen uptake (\( \dot{V}O_2 \)), carbon dioxide production (\( \dot{V}CO_2 \)), HR, ventilation (\( \dot{V}E \)), tidal volume (\( \dot{V}T \)), breathing frequency (BF), ventilatory equivalent for oxygen (\( E\dot{V}O_2 \)), and ventilatory equivalent for carbon dioxide (\( E\dot{V}CO_2 \)) were measured continuously using the same apparatus as mentioned for the maximal test. The percentage of \( \dot{V}O_2 \text{peak} \) was calculated as \( \dot{V}O_2/\dot{V}O_2 \text{peak} \times 100 \). For statistical analysis, all the variables described above were averaged over 5 minutes during the 40 minutes at 75% of maximal aerobic speed starting at minute 10.

**Blood Sample Analysis.** All blood samples were obtained in a rested and fasted state by venipuncture into a vacutainer containing clot activator. After inversion and clotting, the whole blood was centrifuged (Biosan LMC-3000 version V.5AD) for 10 minutes at 3,000 rpm and transferred into Eppendorf tubes and stored frozen at \(-80^\circ\) C until further analysis. Within 1–15 days after testing, the serum samples were delivered to the clinical laboratory for determination of sex hormones to verify menstrual cycle phase. Total estradiol 17-beta, progesterone, follicle-stimulating hormone, and luteinizing hormone were measured through ADVIA Centaur solid-phase competitive chemiluminescent enzymatic immunoassay (Siemens, Munich, Germany).

Coefficients of variation reported by the laboratory were 7.74 for FSH, 10.77 for LH, 7.48 for E2, and 14.11 for progesterone.

**Statistical Analyses**

Data are expressed as mean \( \pm \) SEM. Independent t-test was performed to examine whether any baseline variables (age, height, body mass, and \( \dot{V}O_2 \text{peak} \)) differed between groups of study (eumenorrheic and OC group). A repeated-measures analysis of variance was used to analyze differences for sex hormones between phases. The rest of statistical analyses were performed using the mixed linear model to account for the multiple observations on each subject (i.e., cycle phases and time throughout test) for cardiorespiratory responses. A separate model was used for each ventilatory parameter and for each sample population (eumenorrheic and OC). For each model, the fixed effects were menstrual cycle phase (i.e., early follicular, midfollicular, and luteal phase for the menstrual cycle group or hormonal and nonhormonal phase for OC group) and time (average of every 5 minutes during the 40 minutes of the speed corresponding to 75% of \( \dot{V}O_2 \text{peak} \), starting at minute 10). A random intercept was used at the subject level, using a variance component structure, and the residuals of the repeated observations were modeled using a first-order autoregressive structure. The restricted maximal likelihood estimation procedure was used. For each fixed effect that showed a significant mean effect or interaction effect, Bonferroni-adjusted post hoc tests were performed to examine the pairwise comparisons. Statistical significance for each model was set at \( p \leq 0.05 \).

Cohen’s \( d \) was calculated to verify the magnitude of the mean differences between cycle phases. It was interpreted based on the
following criteria: <0.2 = trivial, 0.2–0.6 = small effect, 0.6–1.2 = moderate effect, 1.2–2.0 = large effect, and >2.0 = very large effect (15). The 95% confidence interval (CI) was also calculated. SPSS version 22 (IBM, Armonk, NY) was used to perform the statistical analyses.

Results

Subjects
There were no statistically significant differences between groups for any baseline variables (height, body mass, and VO2peak; p > 0.05) except for the age (35.6 ± 4.2 and 30.1 ± 4.8 years for eumenorrheic and OC groups, respectively; p > 0.05). Nevertheless, the groups of study were analyzed separately.

Hormonal Concentrations
Hormone levels are presented as mean ± SD. The estrogen levels were significantly lower during the early follicular phase (39.4 ± 18.4 pg·ml⁻¹) compared to the midfollicular phase (82.7 ± 51.3 pg·ml⁻¹; p < 0.05) and the luteal phase (110.7 ± 33.6 pg·ml⁻¹; p < 0.001), as expected. The progesterone levels during the luteal phase (10.43 ± 5.88 ng·ml⁻¹) were higher compared to the midfollicular phase (0.91 ± 0.79 ng·ml⁻¹; p < 0.001) and the midfollicular phase (0.53 ± 0.31 ng·ml⁻¹; p < 0.001), as expected. All eumenorrheic subjects met the minimum progesterone limit of 3 ng·ml⁻¹ set to verify occurrence of a regular luteal phase (17). For the OC group, natural fluctuations in endogenous estrogen were suppressed as demonstrated by the low estrogen (13.6 ± 3.7 pg·ml⁻¹) in the hormonal phase compared with the nonhormonal phase (36.0 ± 27.6 ng·ml⁻¹; p < 0.05). Finally progesterone levels remained low in hormonal (0.45 ± 0.19 pg·ml⁻¹) and nonhormonal phases (0.45 ± 0.21 pg·ml⁻¹; p > 0.05).

Cardiorespiratory Variables

Eumenorrheic Group. A significant main effect for menstrual cycle phases was presented for VO2 (p = 0.044), %VO2peak (p = 0.028), HR (p = 0.005), VT1 (p = 0.03), EqO2 (p = 0.014), and EqCO2 (p = 0.021). Despite these results suggesting a significant influence of menstrual phases over some cardiorespiratory variables, the ES on these phases was small for all the variables presenting significant differences (Table 1), showing a low clinical effect. The main effect of menstrual cycle phase was not significant (p > 0.05) for VO2peak and BF. The fixed-factor time (data not shown) was significant in all variables except in VO2peak and %VO2peak. However, no significant interactions for menstrual cycle phase and time were found for any of the variables (all Fs < 1). Specifically, the pairwise comparisons for the factor menstrual cycle phase are presented in Figure 1.

Oral Contraceptives Group. The main effect of the fixed-factor cycle phases was significant for VT1 (p = 0.03), BF (p = 0.018), EqO2 (p = 0.032), and EqCO2 (p = 0.001). The cycle phase was not significant (p > 0.05) for VO2peak, %VO2peak, VO2peak, HR, and VT1. The pairwise comparisons for the factor cycle phase are presented in Figure 2. The obtained ES showed a small effect of hormonal phase for VT1, BF, and EqO2, whereas the effect for EqCO2 was moderate (Table 2). Significant effects were found for

<table>
<thead>
<tr>
<th>Variable</th>
<th>ES (95% CI)</th>
<th>p</th>
<th>Chances of being positive/trivial/negative</th>
<th>Qualitative inference</th>
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<tbody>
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<td>VO2</td>
<td>Early vs. mid 0.26 (0.10, 0.43)</td>
<td>0.049</td>
<td>77.1/22.9/&lt;0.01</td>
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<td>%VO2peak</td>
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<td>3.1/95.9/1.1</td>
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<td>2.7/97.3/&lt;0.01</td>
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<td>4.7/95.3/&lt;0.01</td>
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<td>0.4/99.5/&lt;0.01</td>
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<td>HR</td>
<td>Early vs. mid –0.16 (~0.39, 0.07)</td>
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<td>0.1/63.2/36.7</td>
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<td>8.3/91.5/0.1</td>
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<td>10.9/89.1/&lt;0.01</td>
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<td>Mid vs. luteal 0.04 (~0.17, 0.24)</td>
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<td>5.6/93.2/1.2</td>
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<td>EqO2</td>
<td>Early vs. mid –0.35 (~0.54, –0.16)</td>
<td>0.081</td>
<td>0.0/5.6/94.4</td>
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<td>3.6/95.1/1.3</td>
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<td>Mid vs. luteal 0.37 (0.13, 0.62)</td>
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<td>0.3/99.6/10.1</td>
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<td>Early vs. luteal 0.29 (0.08, 0.51)</td>
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<td>0.017</td>
<td>97.2/2.8/&lt;0.01</td>
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</tbody>
</table>

*p CI = confidence interval; Early = early follicular phase; Mid = midfollicular phase; Luteal = luteal phase; VO2 = oxygen uptake; %VO2peak = percentage corresponding to VO2peak; VO2peak = carbon dioxide production; HR = heart rate; VE = ventilation; VT1 = tidal volume; BF = breathing frequency; EqO2 = ventilatory equivalent for oxygen; EqCO2 = ventilatory equivalent for carbon dioxide; †Results expressed as effect size (ES), statistical significance (p), and magnitude-based inference. Bold represents a significant difference, p < 0.05.
the fixed-factor time (data not shown) for HR, \( V_\text{O}_2 \), BF, EqO2, and EqCO2. Nevertheless, we did not find any statistical interaction of cycle phase and time factor in any of the variables (all \( F < 1 \)).

**Discussion**

Significant phase effects for \( V_\text{O}_2 \), \%\( V_\text{O}_2\)peak, HR, EqO2, EqCO2, and \( V_t \) were presented for the eumenorrheic group. In addition, the OC group presented significant changes in \( V_{E} \), EqO2, and EqCO2 over a monophasic OC cycle.

The eumenorrheic group reported a significant increase in \( V_\text{O}_2 \) and \%\( V_\text{O}_2\)peak during the midfollicular phase compared with the early follicular phase. Most previous research has reported no effect of the menstrual cycle phases on \( V_\text{O}_2\) during submaximal exercise (1,11,14,21,30,38). However, other studies found significantly higher \( V_\text{O}_2 \) in the luteal phase during exercise at 80% of \( V_\text{O}_2\)max (42) and at 70% of \( V_\text{O}_2\)max (14). These studies suggested a higher cardiovascular strain explained by the thermogenic effect of progesterone and therefore a higher body temperature during the luteal phase.

Surprisingly, in the current study, the highest \( V_\text{O}_2 \) and \%\( V_\text{O}_2\)peak occurred during the midfollicular phase when progesterone values were considerably lower (0.53 ng·ml\(^{-1}\)) in relation to those concentrations found in the luteal phase (10.43 ng·ml\(^{-1}\)). This novel comparison between early and midfollicular phases has highlighted that it is perhaps not progesterone, but more likely estrogen and potentially the ratio between estrogen and progesterone that may explain the higher \( V_\text{O}_2 \) in the midfollicular phase.

In addition, increases in body temperature and progesterone concentrations during the luteal phase have been associated with elevated \( V_E \) at rest and during exercise (20,26,35,42). Bayliss and Millhorn (1992) reported that the respiratory response to progesterone is mediated at hypothalamic sites through an estrogen-dependent progesterone receptor-mediated mechanism. Williams and Krahenbuhl (1997) found a significantly higher \( V_E \) in the midluteal phase during running on a treadmill at 55 and 80% of \( V_\text{O}_2\)max and associated these changes with the stimulatory effect of progesterone on ventilation. This finding disagrees with our results because no significant differences were reported for \( V_E \) between phases (Figure 1F), which is supported by several studies (11,27,38). It has been suggested that the exercise intensity may mask the progesterone effect on \( V_E \) because the increase in \( V_E \) due to exercise would be greater than the increase associated with progesterone (19). Moreover, a significant higher ventilatory drive for EqO2, EqCO2, and \( V_t \) was reported during the luteal phase that is supported by other studies (11,35). The subjects in this study may have exhibited a typical response to the stimulatory effect of progesterone. Our findings suggest that a higher ventilatory drive for the same oxygen consumption during the luteal phase may reduce the ventilatory efficiency during exercise. Similarly and supported by the literature during submaximal exercise (14,30,35), a significant increase in HR was reported for the luteal phase in this study. It has been suggested that the greater HR response during the luteal phase could be due to the increased body temperature of approximately 0.3–0.5° C (16) during that time.

On a practical level, the small increase found in the current study for \( V_\text{O}_2 \), HR, or the ventilatory drive in a specific menstrual phase compared to other phases lacks significant impact on...
submaximal exercise, although it may suggest a slight deleterious influence at a physiological level. Despite the statistically significant differences found for these variables, a small and moderate effect (between 0.2 and 0.6) was reported for all of them, indicating a low clinical application. It is important to consider that an effect size calculated from a very large sample would be more accurate than one calculated from a small sample, as it is the case for both study groups in the current study. The positive aspect of our results is the correct approach to the problem using a strong methodology regarding menstrual cycle, which has allowed us to find significant results for many variables despite the sample size limitation. Therefore, a larger sample size in further research would be interesting to show clinical applications of our results.

In relation to sex hormones, the verification of the actual menstrual cycle phase at the time of testing is a very important factor. Some authors suggest a conservative minimum serum progesterone concentration of greater that 6.0 ng ml\(^{-1}\) during the luteal phase to confirm a regular ovulatory cycle (33). Others, however, have suggested that progesterone concentrations lower than 3.0 ng ml\(^{-1}\) represent a luteal phase deficient or anovulatory cycle (17). In the current study, 11 subjects met the conservative minimum limit of 6.0 ng ml\(^{-1}\), while 4 subjects had lower luteal phase progesterone concentrations (between 4.18 and 5.72 ng ml\(^{-1}\)), which still met the 3.0 ng ml\(^{-1}\) minimum. Despite hormonal measurements being applied to confirm the menstrual phases in the current study, the method for ovulation estimation is considered a limitation of this study. Hence, to improve the timing of menstrual cycle phase testing to coincide better with the different hormonal environments, recent research has suggested that urinary ovulation prediction tests can be used in combination with serum hormone measurements (34). Emphasis on improved methodology to ensure testing in and verification of the different hormonal environments is of utmost importance for future menstrual cycle research to clarify the current inconsistencies in findings.

Regarding the OC group, significantly higher EqO\(_2\) and EqCO\(_2\), as well as VE and BF, were reported during the hormonal phase. The endogenous progesterone levels were very low in both phases of the OC cycle, so it is likely that the exogenous hormones in the OC may have played a role in this finding. Our results agree with Rechichi et al. (2008), who studied 13 well-trained cyclists performing a cycle endurance test at the highest power output throughout 60 minutes of cycling. They reported higher levels for V\(_E\) and EqO\(_2\) during monophasic OC consumption compared with the withdrawal phase. These findings may suggest a lower ventilatory efficiency and a worse aerobic capacity during the active pill phase. The increase in V\(_E\) and BF in the current study may result in a higher rating of perceived exertion during this phase, such as several authors reported (29). Rating of perceived exertion was not included in this study and would be an important factor to assess in future research to support or reject the actual findings about the influence of OC on aerobic performance. The significant increase in V\(_E\) and BF was not associated with changes in V\(_{O2}\) and %V\(_{O2}\)peak between OC phases, which is supported by most of the studies using monophasic OC in trained females.
(31) and untrained women (24). Only one study found changes in exercising V̇O₂ throughout a monophasic OC cycle, showing lower V̇O₂ during the hormonal phase (12). However, these authors attributed these differences in V̇O₂ to biomechanical rather than metabolic factors, due to the lack of significant differences in HR or ventilatory parameters over the OC cycle.

Other studies conflict with our results reporting no differences in V̇O₂ or EqO₂ between OC phases (12,28); however, these studies were conducted in 1983 using an earlier OC generation (ethinodrol 1 mg; significantly higher steroid dose) or with untrained subjects, which may partly explain the differences between studies. Furthermore, the different types of monophasic OC agents used, containing different progesterone formulations and hormonal doses, may influence the results between studies. This highlights the importance of the current study finding significant differences in V̇E, EqO₂, and EqCO₂ between the non-hormonal and hormonal phase of current generation monophasic OC in trained women.

For the OC group, the nondifferences found in HR over the OC cycle may be explained by the low and similar concentrations of progesterone during both OC phases. This finding agrees well with other studies reporting no differences in HR over the OC cycle using low-dose monophasic OC agents (12,24) or triphasic OC (23,39). Moreover, the effect size was trivial and moderate for the variables presenting significant differences (EqO₂, EqCO₂, and V̇E) in the OC group indicating a low practical application of our results. Nevertheless, as aforementioned earlier for the eumenorrheic group, the sample size limitation in this study, especially for this group (OC; n = 8) may have notoriously limited the meaningfulness of these data.

The hormonal concentrations in the current study were higher during the nonhormonal phase for estrogen and progesterone. Ethinylestradiol is detectable in the body for up to 2 days after discontinuation, whereas some progestogens are detectable for up to 5 days. For this reason, the progesterone levels in the current study were similar in both phases (hormonal and nonhormonal phase). To see differences between endogenous and exogenous hormones (especially for estrogen), it would be more interesting to conduct the test in the late stage of the nonhormonal phase, when the hypothalamic-pituitary-ovarian axis activity starts again (as it occurred in the current study). However, it is important to highlight that the estrogen from monophasic OC (ethinylestradiol) is different to the endogenous estrogen (estradiol 17-beta), and therefore, the effects on cardiorespiratory responses may be different. Future studies should explore the different effects of exogenous and endogenous estrogens and progesterone.

Finally, it would be important to point out that the controversy between studies could be due to several factors such as interindividual variability or sample size but very important to the measurement error as the multilevel models cannot account for measurement error of the device itself (4). Therefore, it would be possible that the real effects of OC or menstrual phases could be higher or lower than this study reports depending on the measurement error.

**Practical Applications**

Based on the findings of this research, there is some indication that the response of women to submaximal exercise may be dependent on their menstrual cycle phase. Specifically that a less effective cardiorespiratory response might occur in submaximal exercise during the luteal phase when progesterone levels are significantly high. However, our results are not strong and clear enough to confirm that exercise programs should be timed in accordance with the menstrual cycle. Given the lack of strong evidence in support of a harmful role of progesterone against submaximal exercise in this study, it seems likely that menstrual phase will have small impact on cardiorespiratory variables.

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Sexual Hormones Influence on Athletic Performance


