Resting and exercise ventilatory chemosensitivity across the menstrual cycle

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MacNutt MJ, De Souza MJ, Tomczak SE, Homer JL, Sheel AW. Resting and exercise ventilatory chemosensitivity across the menstrual cycle. J Appl Physiol 112: 737–747, 2012. First published December 15, 2011; doi:10.1152/japplphysiol.00727.2011.—We hypothesized that resting and exercise ventilatory chemosensitivity would be augmented in women when estrogen and progesterone levels are highest during the luteal phase of the menstrual cycle. Healthy, young females (n = 10; age = 23 ± 5 yrs) were assessed across one complete cycle: during early follicular (EF), late follicular (LF), early luteal, and mid-luteal (ML) phases. We measured urinary conjugates of estrogen and progesterone daily. To compare values of ventilatory chemosensitivity and day-to-day variability of measures between sexes, males (n = 10; age = 26 ± 7 yrs) were assessed on 5 nonconsecutive days during a 1-mo period. Resting ventilation was measured and hypoxic chemosensitivity assessed using an isocapnic hypoxic ventilatory response (iHVR) test. The hypercapnic ventilatory response was assessed using the Read rebreathing protocol and modified rebreathing tests. Participants completed submaximal cycle exercise in normoxia and hypoxia. We observed a significant effect of menstrual-cycle phase on resting minute ventilation, which was elevated in the ML phase relative to the EF and LF phases. Compared with males, resting end-tidal CO2 was reduced in females during the ML phase as well as the EF and LF phases but not in the LF phase. We found that iHVR was unaffected by menstrual-cycle phase and was not different between males and females. The sensitivity to chemical stimuli was unaffected by menstrual-cycle phase, meaning that any hormone-mediated effect is of insufficient magnitude to exceed the inherent variation in these chemosensitivity measures. The ventilatory recruitment threshold for CO2 was generally lower in women, which is suggestive of a hormonally related lowering of the ventilatory recruitment threshold. We detected no effect of menstrual-cycle phase on submaximal exercise ventilation and found that the ventilatory response to normoxic and hypoxic exercise was quantitatively similar between males and females. This suggests that feed-forward and feed-back influences during exercise over-ride the effects of naturally occurring changes in sex hormones.

breathing control; hypercapnic ventilatory response; hypoxic ventilatory response; sex steroidal hormones

THE CONTROL OF VENTILATION in humans and other mammalian species is known to be influenced by variations in both ovarian and testicular hormones. The effects of the menstrual cycle, pregnancy, estrogen, testosterone, and progesterone on resting ventilation have each been studied [for review, see (4, 5, 14)]. However, the effects of sex hormones on the human ventilatory responses to hypoxia and hypercapnia are equivocal. Hypoxic chemosensitivity, under resting conditions, has been found to be greater (22, 51) and lower (1, 30, 47) in males relative to females. Furthermore, a large number of human studies report no difference between males and females for hypoxic chemosensitivity (10, 18, 23, 24, 29, 40, 41). Two factors likely contribute to the disparity among studies. The first contributing factor is that few reports of carefully characterized menstrual-cycle stages are available. As such, the ovarian hormone environment is generally unknown and is likely to vary among studies. Moreover, direct measures of ovarian hormones and methods of assessing menstrual-cycle phase are inconsistently reported and poorly executed. As such, between-study comparisons of hormonally induced ovarian alterations in the control of breathing are difficult if not impossible. A second contributing factor to the divergent results reported is a myriad approaches used to determine hypoxic chemosensitivity. Hypoxic tests can be progressive, step, steady-state, isocapnic, poikilocapnic, or iso-oxy hypoxia combined with hypercapnia. Methods for assessing the hypercapnic ventilatory response (HCVR) are also variable among studies, and chemosensitivity to CO2 has been shown to be higher in males (51) relative to females or not different (1, 36).

Hypoxia and hypercapnia are powerful ventilatory stimulants. However, the largest ventilatory adjustment made by humans is in response to dynamic whole-body exercise. Despite the importance of exercise hyperpnea and necessity for precise ventilatory regulation, the modulating effects of sex hormones on human exercise ventilation are poorly understood. Ventilation during sea-level exercise has been reported to be both higher (25, 42, 52) and equivalent (6, 7, 9, 12) during the luteal phase (when female sex hormones are at highest circulating levels) relative to the follicular phase (when female sex hormones are lowest). Few studies have assessed the control of exercise ventilation during hypoxic conditions across the menstrual cycle, but there is reason to suggest that it may be altered. First, under resting conditions, peripheral chemosensitivity to hypoxia may be increased with progesterone, and the effect appears to be potentiated by estrogen (48). Tatsumi and colleagues (48) compared resting ventilation and ventilatory responses with hypoxia and hypercapnia in awake cats before and after ovariecotomy. Lower hypoxic ventilatory and carotid sinus nerve responses to hypoxia in ovariecotomized compared with intact cats suggest that endogenous female hormones specifically affect the inputs from the carotid body. Second, the gain (i.e., increased receptor output for the same stimulus input) of the carotid chemoreceptors in response to hypoxia is higher during exercise than at rest (2, 50). Weil et al. (50) had healthy male subjects perform mild-to-moderate exercise (19%, 26%, and 34% maximum) and determined the hypoxic ventilatory drive by plotting minute ventilation (V̇E) and the partial pressure of alveolar O2. Low levels of exercise produced an enhancement of hypoxic ventilatory drive, and this effect was augmented as the severity of exercise increased. Whereas physiologically relevant levels of female hormones
have been shown to act peripherally to raise carotid body hypoxic chemosensitivity at rest, the interaction between sex hormones and the independent effects of exercise on carotid body chemosensitivity are not known. Based on the above brief summary, the purpose of this study was to characterize the effects of biological sex and menstrual-cycle phase on both resting and exercise ventilation and chemosensitivity. To this end, we used a rigorous assessment of menstrual-cycle phase and daily approximation of circulating estrogen and progesterone via assessment of urinary conjugates, as well as a comprehensive evaluation of resting and exercise ventilation, chemosensitivity to O2 and CO2 during rest, and a measure of hypoxic sensitivity during exercise. In addition, identical methods were used to evaluate the effects of repeatedly assessing resting and exercise chemosensitivity in males. We hypothesized that when estrogen and progesterone levels were increased during the luteal phase, relative to the follicular phase, resting and exercise ventilatory chemosensitivity would be augmented.

**METHODS**

**Study design.** Participants visited the Health and Integrative Physiology Lab at the University of British Columbia to attend an orientation session and on five additional occasions, to evaluate ventilatory chemosensitivity during rest and exercise. Females were assessed at five time points to evaluate chemosensitivity across the menstrual cycle. To compare ventilatory chemosensitivity and the day-to-day variability of measures between sexes, males were also assessed on 5 nonconsecutive days during a 1-mo period. Participants were instructed to avoid alcohol for 12 h, as well as food, caffeine, and exercise for 4 h prior to each visit. Each participant gave informed, written consent, and all experimental procedures received institutional ethical approval and conformed to the Declaration of Helsinki.

**Participants.** Healthy, nonsmoking males and females, aged 19–40 years with no history of cardiorespiratory disease (including sleep apnoea), were recruited to participate. Competitive swimmers, breathhold divers, and individuals who had traveled above 3,000 m in the previous 6 mo were excluded. Participants were permitted to use multivitamins but not other types of nutritional supplements. Females were excluded if they had a menstrual cycle that was not of normal length (26–32 days; based on a self-reported menstrual history for the past 12 mo) or used any form of hormonal contraceptives in the previous 6 mo. In addition, due to known influences on circulating ovarian hormones (11), women were excluded if they performed more than 4 h of vigorous exercise/wk, had a body mass index <18 kg/m2, or had experienced a weight change of ±2 kg in the previous 6 mo.

**Orientation session.** During the orientation session, study procedures were explained, and participants gave written, informed consent. Participants completed a medical and physical activity questionnaire, and females provided a detailed menstrual-cycle history. Height and weight were recorded. Forced vital capacity (FVC), forced expiratory volume in 1 s (FEV1,0), FEV1,0/FVC, and maximum voluntary ventilation were measured using a portable spirometer (Spirolab II, Applied Electrochemistry, Pittsburgh, PA), measured at a port located in a mixing chamber. Inspiratory flow was measured using a calibrated pneumotachograph (Model 3813, Hans Rudolph). Heart rate was recorded every 30 s using a commercially available heart-rate monitor (Sp610i, Polar Electro, Kempele, Finland). Throughout the test, participants wore pulse oximeters at the ear and finger (Model 504, Criticare Systems, Waunakee, WI). If there was a discrepancy between the two oximeter readings, the higher oxygen saturation (S\textsubscript{O2}) value was recorded. Participants were then familiarized with the sensation of breathing hypoxic gas. During 10 min of seated rest, participants breathed a humidified gas mixture [fraction of inspired O2 (\text{FIO2}) = 0.15] through a face mask attached to a nonbreathing valve. Lastly, female participants were instructed how to monitor their menstrual cycles for the following 1–2 mo before starting the testing cycle (see below).

**Menstrual-cycle evaluation.** To determine that menstrual cycles were ovulatory and of normal length, females were monitored for 1–2 complete menstrual cycles, immediately prior to their test cycle. Participants kept a daily log of basal body temperature (BBT) and menstrual symptoms. They also used an over-the-counter ovulation-prediction kit (First Response Easy-Read Ovulation Test, Church & Dwight, Princeton, NJ) to determine the timing of a surge in luteinizing hormone (LH), which immediately precedes ovulation. During monitoring cycles, daily logs and ovulation-prediction kits were used to determine the timing of testing sessions during the test cycle. Targeted time points included the early follicular (EF1), late follicular (LF), early luteal (EL), and mid-luteal (ML) phases of one menstrual cycle, as well as the EF phase of the subsequent cycle (EF2). Testing during EF1 and EF2 phases occurred 2–4 days after onset of menses, when estrone-1 glucuronide (E1G) and pregnanediol glucuronide (PdG) should both be low. LF-phase testing targeted a time with peak E1G but low PdG, ~2 days before the LH surge. EL- and ML-phase testing occurred ~4 and ~8 days after the LH surge, when both E1G and PdG should be elevated.

The hormone profile of each test cycle was determined post hoc by measuring the conjugates of estrogen and progesterone in daily urine samples. This allowed retrospective confirmation of normal cycles and assessment of whether the desired cycle phases were targeted successfully for testing. Throughout the test cycle, first-morning urine was collected daily into a sterile vessel and frozen immediately. Participants transported the urine samples to the Health and Integrative Physiology Lab on ice, where they were prepared in 2-mL aliquots and shipped on dry ice to the Women’s Exercise and Bone Health Laboratory at the University of Toronto. Here, daily urinary E1G and PdG were measured using competitive enzyme immunoassays, as described previously (13). These conjugates are highly stable (32) and unlikely to be affected by storage and transport of samples.

**Experimental procedures.** During each testing session, resting ventilation was measured and hypoxic chemosensitivity assessed using a progressive, isocapnic hypoxic ventilatory response (iHVR) test. The HCVR was assessed using a traditional Read rebreathing protocol (HCVR1) (35) and the modified rebreathing tests (HCVR2 and HCVR3) developed by Duffin and colleagues (17). These methods have been performed previously in our laboratory and are described in detail elsewhere (27). All tests were conducted in a slightly darkened room with subjects listening to quiet, arrhythmic music to minimize distraction. Body position is known to affect resting chemosensitivity (53), and although it varied among different tests, it remained constant across all participants when assessing hypoxic (supine) and hypercapnic (seated) chemosensitivity. Participants then completed a brief, submaximal exercise test in normoxia, followed by the same exercise test in hypoxia. All tests were separated by at least 10–20 min normoxic rest. Testing time, test order, and duration of...
resting intervals were held constant across data collection sessions for each participant.

Resting measures. Participants were fitted with a face mask, heart-rate monitor, and pulse oximeters. Heart rate, $S_{\text{O}2}$, and $V_i$ were measured as during the VO$_{2\text{max}}$ test, and gases were sampled at the mouth to provide measures of end-tidal CO$_2$ ($P_{e\text{-CO}_2}$) and end-inspiration O$_2$. Resting measures were averaged across the final 5 min of a 10-min period of supine rest.

$h\text{HVR}$. Following the period of supine rest, 100% N$_2$ was gradually titrated into the inspired gas mixture, progressively lowering the F$O_2$ from 0.21 to 0.05 over 5–10 min. The test was terminated when $S_{\text{O}2}$ reached 80%. Isocapnia was maintained at resting $P_{e\text{-CO}_2}$ levels by adding CO$_2$ to the inspiratory circuit as needed. The iHVR (L · min$^{-1}$ · %$^{-1}$) was calculated as the absolute slope of the line of best fit when $V_i$ (L/min) was plotted against $S_{\text{O}2}$ (%).

HCVR1. All rebreathing tests were performed in the seated position and with a similar setup. All ventilatory variables were measured as above, but instruments were interfaced with a personal computer using custom-made software (LabVIEW 7.0, National Instruments, Austin, TX). Participants wore nose clips and breathed through a mouthpiece attached to a three-way rebreathing valve, which was connected to a rebreathing bag. Participants were instructed to relax and breathe ad libitum until the test was terminated when $P_{e\text{-CO}_2}$ reached 60 mmHg or until subjects became too uncomfortable to continue. Before assessing HCVR1, participants sat quietly at rest, breathing room air through the mouthpiece. After 5 min, participants were instructed to exhale completely and then rapidly switched to the rebreathing bag, containing 7% CO$_2$, balance O$_2$. After three large breaths to equilibrate gases between the lungs and the rebreathing bag, participants were instructed to breathe ad libitum until the test was terminated. HCVR1 (L · min$^{-1}$ · mmHg$^{-1}$) was calculated as the slope of the line of best fit for $V_i$ (L/min) vs. $P_{e\text{-CO}_2}$ (mmHg).

HCVR2. Before beginning the second rebreathing test, participants were coached to reduce their $P_{e\text{-CO}_2}$ to 19–25 mmHg by hyperventilating for 5 min. Following a full exhalation, participants were switched to the rebreathing bag, this time containing 6% CO$_2$, 24% O$_2$, balance N$_2$. As $P_{e\text{-CO}_2}$ rose throughout the test, the rebreathing bag was maintained iso-oxic at 200 mmHg using a computer-controlled valve. A custom-designed computer program was used to calculate the CO$_2$ threshold (T; mmHg) and sensitivity (S; L · min$^{-1}$ · mmHg$^{-1}$). “T” represents the $P_{e\text{-CO}_2}$ above which ventilation begins to increase in response to increased CO$_2$; “S” represents the slope of the line of best fit when above-threshold values of $V_i$ (L/min) are plotted against $P_{e\text{-CO}_2}$ (mmHg). T2 and S2 were calculated for each HCVR2 test.

HCVR3. Determination of HCVR3 was similar to HCVR2 except that it assessed T and S in hypoxic, rather than hyperoxic, conditions. The procedure was identical, except that the rebreathing bag contained 6% CO$_2$, 4.5% O$_2$, balance N$_2$, and isooxia was maintained throughout the test at 50 mmHg. T3 and S3 were calculated for each HCVR3 test as above.

Submaximal exercise. Participants completed 15 min of cycle exercise at 40% of maximum power output while breathing room air. After a 10-min rest period, the submaximal cycle test was repeated with participants breathing hypoxic gas (F$O_2$ = 0.15). Cadence was held constant between tests and across days for each individual. Throughout exercise, heart rate, $S_{\text{O}2}$, $V_i$, and $P_{e\text{-CO}_2}$ were measured continuously and averaged over the final 10 min of each exercise test.

Data analysis. Lung volumes and metabolic rates and variability in O$_2$ and CO$_2$ sensitivity are known to vary in proportion with body size (22, 37). As such, resting ventilation and indices of chemosensitivity were corrected for body surface area (BSA), as per Dubois and Dubois (16). Paired $t$-tests were used to evaluate the effect of practice by comparing measures from the first and last testing day for all participants. Repeated measures (RM) ANOVA procedures were used to determine the effect of cycle phase on each variable. Given a significant F-ratio, paired $t$-tests were used to test planned comparisons among cycle phases. Mean responses across all testing days were calculated for each participant. Mean data from males were compared with data from females in each cycle phase and to mean female data using unpaired $t$-tests. Mean values from EF1 and EF2 were used to represent the EF phase in analyses. Data were missing for two females from the LF phase. Missing values were replaced with the group mean value to complete RM ANOVA. However, data were excluded case-wise from all post hoc comparisons among LF and EF, ML, and males. Pearson product moments were also calculated to determine correlations among each outcome measure and E1G, PdG, and the E1G:PdG ratio. Correlation coefficients were determined for individual and pooled data. Statistical significance was satisfied at $P < 0.05$.

RESULTS

Participants. Twelve males and 13 females were enrolled in the study. Two males and one female withdrew because of the large time commitment of study participation. In monitoring their menstrual cycles, one female presented with a 40-day cycle and was excluded from the study. Another female failed to detect a LH surge during one of her monitoring cycles and was also excluded. Of the 10 females who completed a testing cycle, menstrual-cycle profiles indicated that no elevation in PdG occurred during the luteal phase for two participants [female subject F03 and F11], and another participant showed no marked increase in E1G throughout the cycle, despite a normal PdG profile (subject F08; see Fig. 1). A fourth participant demonstrated a normal profile for both E1G and PdG (F13), but the ML-phase testing occurred too late to capture the increase in PdG. All analyses were completed on two data sets: $n = 6$ females with “normal” menstrual cycles and the complete data set for all 10 females. Results from both sets of analyses were very similar, and since these menstrual-cycle abnormalities are common in the general population (11), data from all 10 females were included in the analyses and figures presented. Characteristics of participants can be found in Table 1.

Confirmation of menstrual phase at testing points. Hormone status on each testing day is shown in Table 2. Comparisons with T1 indicate that as expected, E1G was elevated during LF, EL, and ML compared with the EF phase and that PdG was elevated at EL and ML phases only. There were no differences in either E1G or PdG between EF1 and EF2 phases. Figure 1 further confirms that most females were tested successfully during the EF, LF, and ML phases of their cycles. However, females were also targeted for testing in the EL phase. Although circulating levels of both estrogen and progesterone should be similar between the EL and ML phases, at EL, progesterone has increased recently, whereas in ML, levels of progesterone have been elevated for several days. Females were tested at both time points in an attempt to make the first assessment of the effect of cumulative exposure to progesterone on chemosensitivity and ventilatory control during rest and exercise. However, post hoc assessment indicated that PdG levels were consistently higher at ML rather than EL phases, suggesting that tests in targeting the EL phase had occurred too early. As such, meaningful comparisons between EL and ML phases were not possible, and all data collected during the EL phase were excluded from further analyses.

BBT did not prove to be a reliable method for determining onset of ovulation. Two women provided insufficient BBT data to decipher temperature trends. Three women showed an ele-

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vated BBT throughout the second one-half of their cycles, but there was a clear rise that corresponded to the measured LH surge in only one participant.

Resting respiratory function. A comparison of normoxic resting respiratory function between males and females in EF, LF, and ML phases is shown in Fig. 2. There was a significant effect of cycle phase on $V_i$ in females, with $V_i$ elevated in ML above both EF and LF phases. There was no effect of cycle phase on either breathing frequency ($F_B$) or tidal volume ($V_T$). The effect of cycle phase on $P_{\text{ETCO}_2}$ approached but did not reach statistical significance ($P = 0.06$). $F_B$ was greater in females relative to males but only in the ML phase. Although absolute $V_T$ and $V_i$ were significantly lower in females than males across all testing points ($V_T$: 640 ± 30 vs. 830 ± 26 mL, $P = 0.0003$; $V_i$: 8.40 vs. 10.0 ± 2.0 L/min, $P = 0.0004$), these differences were nonsignificant when values were corrected for BSA. $P_{\text{ETCO}_2}$ was reduced significantly in females compared with males in the EF and ML phases but not in the LF phase.

In females, both $V_i$ and $P_{\text{ETCO}_2}$ were significantly correlated with PdG but not with E1G or E1G:PdG ($r = 0.47$ and 0.50, respectively; both $P < 0.01$). Individual correlation coefficients ranged dramatically, from −0.78 to 0.70 for $V_i$, and from −0.98 to 0.52 for $P_{\text{ETCO}_2}$.

$\text{iHVR}$. BSA-corrected iHVR for males and females is shown in Fig. 3. There was no significant effect of cycle phase and no difference between males and females for iHVR, and iHVR was not significantly correlated with any measure of sex hormones in females.

$\text{HCVR}$. The threshold and sensitivities associated with three assessments of HCVR are shown in Fig. 4. For HCVR1, there was no effect of cycle phase on CO2 sensitivity, but females were significantly less sensitive to CO2 than males across all phases of the cycle. For HCVR2 and HCVR3, CO2 sensitivity was again unaffected by cycle phase. In these tests, sensitivity was lower in females than males, only during the LF phase and only for HCVR2. Cycle phase had a significant effect on the
threshold for HCVR2 only. This threshold was reduced in ML compared with LF but not compared with the EF phase. Thresholds for HCVR2 and HCVR3 were all significantly lower in females compared with males, except for the threshold for HCVR2, measured during the LF phase. In females, there was no correlation between measured hormone and slope or threshold for any HCVR measurement.

Exercise. Ventilation during cycle exercise, corrected for both BSA and power output, is shown in Fig. 5. There was no effect of cycle phase on normoxic or hypoxic exercise ventilation or on the change in ventilation from normoxia to hypoxia. Interindividual variability in corrected $V_i$ was much greater in females than males, and although corrected exercise $V_i$ tended to be higher in females in both normoxia and hypoxia, these values did not reach statistical significance. The percent change from normoxia to hypoxic condition ($\Delta V_i$) was not different between males and females at any cycle phase. However, the effect of cycle phase on exercise $P_{ET}CO_2$ was significant in normoxia ($P = 0.02$) and nearly so in hypoxia ($P = 0.10$). In both conditions, $P_{ET}CO_2$ was lowest during the ML phase. Exercise $P_{ET}CO_2$ were consistently lower in females than males in both normoxia and hypoxia, with significant differences in both the EF and ML phases. In the LF phase, the difference between males and females only approached significance in both normoxia ($P = 0.11$) and hypoxia ($P = 0.10$). As with $V_i$, data there was no effect of cycle phase and no difference between males and females for $\Delta P_{ET}CO_2$.

There was no effect of cycle phase on exercise $S_{p}O_2$ in normoxia or hypoxia, nor in $\Delta S_{p}O_2$. Neither males nor females desaturated significantly during normoxic exercise, but on average, $S_{p}O_2$ fell to 91 ± 5% (females) and 88 ± 3% (males) during hypoxic exercise. Females desaturated significantly less than males, with $S_{p}O_2$ significantly higher and $\Delta S_{p}O_2$ significantly lower during the EF and LF phases. Although large interindividual variability prevented a significant difference in $S_{p}O_2$ between males and females during ML ($P = 0.09$), the $\Delta S_{p}O_2$ was significant ($P = 0.03$). Ventilation and $S_{p}O_2$ during exercise were not correlated with measured sex hormones in females.

Repeatability of measures. In males, the interday coefficients of variation (CVs) ranged from 3% to 4% for HCVR thresholds and $P_{ET}CO_2$ to 37% for iHVR and 55% $\Delta V_i$ from normoxic to hypoxic exercise (see Table 3). In females, the repeatability of measures from EF1 to EF2 phases was similar: lowest for HCVR thresholds and $P_{ET}CO_2$ (2–6%) and highest for sensitivities to $O_2$ and $CO_2$ (15–38%). There was also substantial intra-individual variability in E1G and PdG between EF1 and EF2, with mean CVs of 25% and 43%, respectively. CVs calculated between EF1 to EF2 were lower than those calculated for males for iHVR and T2 but were otherwise similar between males and females. For females, variability across all testing sessions was greater than variability between EF1 and EF2, only for measures of E1G, PdG, and iHVR. All other measures of resting and exercise ventilation and chemosensitivity were similarly variable both among and within menstrual phases.

**DISCUSSION**

There is a history of inconsistent results in studies that have sought to characterize the effects of endogenous fluctuations in ovarian hormones on human ventilatory control. Accordingly, the purpose of this study was to determine the magnitude of the ovarian-related changes in both resting and exercise measures of ventilatory chemosensitivity. A strength of our study was the daily assessment of hormonal status combined with multiple methods of quantifying ventilatory chemosensitivity.

**Main findings.** The principal findings of this study are four-fold. First, there was a significant modulatory effect of cycle phase on resting $V_i$ and $P_{ET}CO_2$. When $V_i$ was corrected for body size, there were no differences between college-aged men and women. Second, we found that BSA-corrected iHVR was unaffected by menstrual-cycle phase and was not different between males and females at any point. Third, we found that alterations to sex hormones have little or no effect on the sensitivity to $CO_2$. We further observed that the ventilatory recruitment threshold for $CO_2$ was always lower in women, but there were no sex differences for $CO_2$ sensitivity. Lastly, we detected no effect of menstrual-cycle phase on submaximal exercise ventilation and found that the ventilatory response to normoxic and hypoxic exercise was quantitatively similar between males and females. The effects of sex hormones were more consistent when considered separately from cycle phase.

**Effect of menstrual-cycle phase on resting ventilation.** We found a significant effect of cycle phase on $V_i$. Specifically,

**Table 1. Characteristics of male and female participants**

<table>
<thead>
<tr>
<th>Measure</th>
<th>Males (n = 10)</th>
<th>Females (n = 10)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y)</td>
<td>23.1 ± 4.6</td>
<td>25.7 ± 6.9</td>
<td>NS</td>
</tr>
<tr>
<td>Height (m)</td>
<td>1.81 ± 0.06</td>
<td>1.68 ± 0.08</td>
<td>0.001</td>
</tr>
<tr>
<td>Mass (kg)</td>
<td>76.9 ± 12.1</td>
<td>62.0 ± 11.9</td>
<td>0.01</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>23.4 ± 2.6</td>
<td>219 ± 2.5</td>
<td>NS</td>
</tr>
<tr>
<td>FEV1.0 (L)</td>
<td>4.7 ± 0.7</td>
<td>3.3 ± 0.5</td>
<td>0.0001</td>
</tr>
<tr>
<td>% predicted</td>
<td>98 ± 11</td>
<td>96 ± 16</td>
<td></td>
</tr>
<tr>
<td>FVC (L)</td>
<td>5.5 ± 1.0</td>
<td>3.8 ± 0.7</td>
<td>0.0004</td>
</tr>
<tr>
<td>% predicted</td>
<td>103 ± 12</td>
<td>94 ± 9</td>
<td></td>
</tr>
<tr>
<td>FEV1.0/FVC (%)</td>
<td>27 ± 0.04</td>
<td>86 ± 6.3</td>
<td>NS</td>
</tr>
<tr>
<td>% predicted</td>
<td>85 ± 4</td>
<td>100 ± 3</td>
<td></td>
</tr>
<tr>
<td>MVV (L/min)</td>
<td>178 ± 44</td>
<td>123 ± 27</td>
<td>0.005</td>
</tr>
<tr>
<td>VO2max (mL · kg⁻¹ · min⁻¹)</td>
<td>55.6 ± 6.5</td>
<td>43.4 ± 9.5</td>
<td>0.006</td>
</tr>
<tr>
<td>Peak power (W)</td>
<td>288 ± 56</td>
<td>196 ± 63</td>
<td>0.006</td>
</tr>
</tbody>
</table>

Values are presented as mean ± SD. NS, not significant; BMI, body mass index; FEV1.0, forced expired volume in 1 s; FVC, forced vital capacity; MVV, maximal voluntary ventilation; VO2max, maximal oxygen consumption. 

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**Table 2. Urinary conjugates of estrogen and progesterone for T1–T5**

<table>
<thead>
<tr>
<th>Menstrual phase</th>
<th>E1G (ng/mL)</th>
<th>PdG (gg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>EF1</td>
<td>25.5 ± 8.0</td>
<td>1.4 ± 1.0</td>
</tr>
<tr>
<td></td>
<td>(11.4 – 35.6)</td>
<td>(0.5 – 3.4)</td>
</tr>
<tr>
<td>LF</td>
<td>52.7 ± 22.3*</td>
<td>1.5 ± 1.0</td>
</tr>
<tr>
<td></td>
<td>(23.8 – 82.9)</td>
<td>(0.4 – 3.0)</td>
</tr>
<tr>
<td>EL</td>
<td>45.9 ± 8.1†</td>
<td>3.6 ± 2.2*</td>
</tr>
<tr>
<td></td>
<td>(33.4 – 56.7)</td>
<td>(0.8 – 6.8)</td>
</tr>
<tr>
<td>ML</td>
<td>46.2 ± 15.6*</td>
<td>7.6 ± 5.8*</td>
</tr>
<tr>
<td></td>
<td>(27.1 – 64.7)</td>
<td>(1.0 – 20.4)</td>
</tr>
<tr>
<td>EF2</td>
<td>29.7 ± 13.5</td>
<td>2.4 ± 2.9</td>
</tr>
<tr>
<td></td>
<td>(13.0 – 58.6)</td>
<td>(0.4 – 9.3)</td>
</tr>
</tbody>
</table>

Values represent mean ± SD (range). T1–T5, thresholds 1–5; E1G, estrone-1 glucuronide; PdG, pregnanediol glucuronide; EF1, early follicular 1st cycle; LF, late follicular; EL, early luteal; ML, mid-luteal; EF2, early follicular 2nd cycle. Significant differences from hormonal status on EF1 are indicated with *P < 0.05 and †P < 0.01.
BSA-corrected $V_t$ was higher during the ML phase relative to EF and LF phases. We also found that absolute $V_t$ (L/min) was lower in women than men across all time points. However, once corrected for BSA, there were no differences in resting $V_t$ between males and females. There was a nearly significant effect of cycle phase on PETCO$_2$ ($P \approx 0.06$), but the pattern did not parallel changes in $V_t$ as expected. Instead, PETCO$_2$ was lower in females than males during the EF and ML phases but not during LF. Our findings under resting conditions show that despite between-subject variation in hormonal levels (see below), there is a menstrual-cycle effect on $V_t$. Although both $V_t$ and PETCO$_2$ correlated with PdG as expected, these relationships were far from consistent. These observations extend the findings of some other investigations (6, 38, 51) and serve to emphasize that ventilatory control is indeed hormonally altered. Specifically, our findings show that when considering basal values for ventilation (i.e., eupnea) between males and females, it is necessary to take hormonal status into account. We consider this to be an important finding if we and others are to determine how other ventilatory stimuli (hypoxia, hypercapnia, exercise) interact with naturally occurring hormonal changes (i.e., not pharmacologically induced changes). However, basal metabolic has been shown to decrease at menstruation, fall to its lowest point 1 wk prior to ovulation, and then increase until the beginning of the next menstrual cycle (45). It is possible that naturally occurring alterations in basal metabolism could have influenced our ventilatory measures, and we cannot exclude this possibility.

Resting chemosensitivity. To characterize comprehensively the effects of cyclical changes in sex hormones on ventilatory control, we evaluated chemosensitivity in several ways. First, we found that the iHVR did not vary across the menstrual-cycle phase or correlate with quantified hormone status nor was it different between males and females. The iHVR is a test designed to determine the hypoxic sensitivity of the carotid body. As such, our findings suggest that under isocapnic conditions, peripheral hypoxic chemosensitivity is not altered by cyclical changes in female sex hormones [for review, see (4, 49)]. For example, carotid body neural-output responsiveness to hypoxia is increased with estrogen and progesterone in castrated male cats.
It is difficult to reconcile the differences between human and animal studies, but it may be related to the measurement of chemosensitivity via measures of spontaneous (and variable) ventilation in humans vs. carotid sinus-nerve activity in anesthetized animals.

In the present study, we carefully controlled the laboratory environment (see METHODS) to minimize the introduction of external stimuli, which could affect respiration. However, despite using a rigorous approach to measuring chemosensitivity, it is known that iHVR is variable when measured in conscious humans. The CV for iHVR was 31% for all female tests and was 15% between EF1 and EF2 phases, which approximates current and previous reproducibility values for males in our laboratory [CV = 27% (26)] and is lower than other studies that have evaluated iHVR in women [CV = 63% (6)]. We cannot exclude the possibility that despite using a controlled environment and minimizing behavioral influences, the inherent variation in iHVR obscured any potential hormonally related effects.

We also used a modified rebreathing method (17) to determine central and peripheral ventilatory chemoreflex sensitivity. The PETCO2, at which ventilation increases with progressive increases in PETCO2, is considered the recruitment T. The slope of the linear relationship between ventilation and PETCO2 above T is considered an estimate of chemoreflex S. Under both hyperoxic (HCVR2) and hypoxic (HCVR3) conditions, we found that S was unaffected by menstrual-cycle phase, did not correlate with measured hormone levels, and was only lower than male values during the LF for HCVR2. Slatkovska et al. (44) found that S was not different between the luteal and follicular phase, where progesterone (5–58 mmol/L) and 17β-estradiol (105–780 pmol/L) were highly variable among subjects. There was also no significant difference in CO2 sensitivity between pre- and postmenopausal women (34). We interpret our findings, in conjunction with those of others, to mean that normally occurring alterations in sex hormones have little to no effect on the sensitivity to CO2 in either hypoxia or hyperoxia. Our interpretation is also supported by the lack of effect of menstrual-cycle phase for HCVR1 (i.e., Read rebreathe protocol), further emphasizing that ventilatory chemosensitivity to CO2 does not change across the menstrual cycle. We do, however, report that CO2 sensitivity is lower in females than males during hyperoxic conditions.

Significant differences were observed across all cycle phases for HCVR1, but differences only reached significance during LF for HCVR2. Conversely, there was no difference in S between males and females when hypercapnia was combined with hypoxia. Although Slatkovska et al. (44) found no difference in the CO2 recruitment T between follicular and luteal phases, others have found that T is reduced when female sex hormones are elevated in pre- vs. postmenopausal women (34). We found a significant effect of menstrual-cycle phase on T during the hyperoxic trial (HCVR2) only. However, although T was lowest during ML, there was actually no measurable difference between EF and ML, and T did not correlate with measured PdG or E1G, suggesting that the effect of sex hormones on T is minimal. On the other hand, T was consistently lower in females than males, with a significant sex difference in all cases except during LF for HCVR2. The mechanisms by which hormones might alter T are not known, but our finding of a male-female difference in recruitment threshold warrants additional investigation.
Ventilation during exercise. We found no effect of menstrual-cycle phase on \( V_i \) (corrected for BSA and power output) during submaximal exercise in either normoxia or hypoxia (see Fig. 5). The lack of a cycle-phase effect on exercise ventilation is consistent with other work, which shows that submaximal exercise ventilation is not increased at sea-level or simulated high altitude (hypobaric chamber; 4,300 m), despite significant changes to hormonal levels (6). Other reports are in accordance with our finding that ventilation during exercise is unaltered by phase of menstrual cycle (7, 12, 20), whereas others show significant increases in exercise ventilation in the ML phase (15, 42, 52). Specific comparisons among studies are complicated by the use of different levels of exercise intensity and modes (run vs. cycle), as well as limited information about hormonal values. Moreover, in one study that reports an increase in exercise ventilation at submaximal intensities (55–85% \( \text{VO}_2_{\text{max}} \)) in the ML phase, there was an increase in whole-body \( \text{VO}_2 \) (52). As such, the rise in ventilation, which they observed, may have been secondary to the increased \( \text{VO}_2 \), rather than hormonally mediated.

We interpret the findings from the present study—where hormonal values were monitored daily in conjunction with the observations of others—to mean that cyclical hormonal changes have no or very modest effects on overall ventilation during submaximal normoxic and hypoxic exercise. The fact that we observed no effect on ventilation speaks to the complexities of exercise ventilatory control in humans. Maintaining the appropriate level of pulmonary ventilation is a complex and highly regulated task. If ventilation is too little, then the systemic oxygen needs are not met. If ventilation is too high, then there is an associated increased mechanical work and oxygen cost of breathing. As such, the magnitude of effect of circulating hormones is likely insufficient to disrupt the balance between oxygen needs and the mechanical work and cost of breathing during exercise. It may be that exercise-induced changes to ventilatory drive (both feed-forward and/or feedback mechanisms) are dominant and mask any possible hormone-mediated ventilatory effects.

Despite the above, it is necessary to comment that although we did not detect any change to \( V_i \), there was a lower \( P_{\text{ET}CO_2} \) during exercise in women during the EF and ML phases, which is consistent with an increase in alveolar ventilation relative to metabolism. This is also supported by the consistent but nonsignificant \((P = 0.07)\) trend of higher \( S_\text{O}_2 \) (~3%) during hypoxic exercise in females. This finding is surprising, given that even in normoxia, exercise-induced arterial hypoxemia...
Table 3. *Interday CVs (%) for measures of ventilation and chemosensitivity*

<table>
<thead>
<tr>
<th>Measure</th>
<th>Males</th>
<th>Females, EF&lt;sub&gt;1&lt;/sub&gt; and EF&lt;sub&gt;2&lt;/sub&gt;</th>
<th>Females, all sessions</th>
</tr>
</thead>
<tbody>
<tr>
<td>E1G</td>
<td>–</td>
<td>25 ± 17</td>
<td>36 ± 16&lt;sup&gt;*&lt;/sup&gt;</td>
</tr>
<tr>
<td>PdG</td>
<td>–</td>
<td>43 ± 33</td>
<td>82 ± 44&lt;sup&gt;*&lt;/sup&gt;</td>
</tr>
<tr>
<td>resting V&lt;sub&gt;1&lt;/sub&gt;</td>
<td>16 ± 9</td>
<td>20 ± 20</td>
<td>20 ± 13</td>
</tr>
<tr>
<td>resting P&lt;sub&gt;e&lt;/sub&gt;CO&lt;sub&gt;2&lt;/sub&gt;</td>
<td>4 ± 1</td>
<td>2 ± 3</td>
<td>4 ± 3</td>
</tr>
<tr>
<td>iHVR</td>
<td>37 ± 15</td>
<td>15 ± 15&lt;sup&gt;†&lt;/sup&gt;</td>
<td>31 ± 18&lt;sup&gt;*&lt;/sup&gt;</td>
</tr>
<tr>
<td>HCVR S1</td>
<td>33 ± 12</td>
<td>22 ± 17</td>
<td>22 ± 14</td>
</tr>
<tr>
<td>HCVR T2</td>
<td>3 ± 2</td>
<td>6 ± 6†</td>
<td>5 ± 3</td>
</tr>
<tr>
<td>HCVR S2</td>
<td>30 ± 12</td>
<td>26 ± 21</td>
<td>23 ± 13</td>
</tr>
<tr>
<td>HCVR T3</td>
<td>3 ± 2</td>
<td>2 ± 2</td>
<td>3 ± 1</td>
</tr>
<tr>
<td>HCVR S3</td>
<td>19 ± 15</td>
<td>23 ± 22</td>
<td>30 ± 142</td>
</tr>
<tr>
<td>normoxic exercise V&lt;sub&gt;1&lt;/sub&gt;</td>
<td>7 ± 4</td>
<td>7 ± 6</td>
<td>7 ± 4</td>
</tr>
<tr>
<td>hypoxic exercise V&lt;sub&gt;1&lt;/sub&gt;</td>
<td>7 ± 2</td>
<td>6 ± 4</td>
<td>5 ± 5</td>
</tr>
<tr>
<td>ΔV&lt;sub&gt;e&lt;/sub&gt;, from normoxic to hypoxic exercise</td>
<td>55 ± 32</td>
<td>38 ± 27</td>
<td>64 ± 70</td>
</tr>
<tr>
<td>normoxic exercise P&lt;sub&gt;e&lt;/sub&gt;CO&lt;sub&gt;2&lt;/sub&gt;</td>
<td>4 ± 5</td>
<td>4 ± 2</td>
<td>5 ± 3</td>
</tr>
<tr>
<td>hypoxic exercise P&lt;sub&gt;e&lt;/sub&gt;CO&lt;sub&gt;2&lt;/sub&gt;</td>
<td>6 ± 7</td>
<td>5 ± 3</td>
<td>4 ± 2</td>
</tr>
<tr>
<td>ΔP&lt;sub&gt;e&lt;/sub&gt;CO&lt;sub&gt;2&lt;/sub&gt; from normoxic to hypoxic exercise</td>
<td>35 ± 18</td>
<td>51 ± 27</td>
<td>46 ± 35</td>
</tr>
<tr>
<td>normoxic exercise S&lt;sub&gt;O&lt;/sub&gt;2</td>
<td>1 ± 1</td>
<td>2 ± 2</td>
<td>2 ± 2</td>
</tr>
<tr>
<td>hypoxic exercise S&lt;sub&gt;O&lt;/sub&gt;2</td>
<td>5 ± 4</td>
<td>2 ± 1</td>
<td>2 ± 2</td>
</tr>
<tr>
<td>ΔS&lt;sub&gt;O&lt;/sub&gt;2, from normoxic to hypoxic exercise</td>
<td>38 ± 24</td>
<td>48 ± 29</td>
<td>52 ± 29</td>
</tr>
</tbody>
</table>

Values for males were calculated across 5 testing days. Values for females were calculated for measurements from the 2 sessions conducted in the EF<sub>1</sub> and EF<sub>2</sub> and for measurements collected at all sessions. V<sub>e</sub>, minute ventilation; P<sub>e</sub>CO<sub>2</sub>, end-tidal CO<sub>2</sub>; iHVR, isocapnic hypoxic ventilatory response; HCVR S<sub>1</sub>–S<sub>3</sub>, hypercapnic ventilatory responses, sensitivity 1–3; Δ, percent change; S<sub>O</sub>2, oxygen saturation. *Differences between coefficients of variation (CVs) calculated for females in 2 ways are indicated. Significant differences (†P < 0.05) between CVs for males and females are denoted.

appears to occur more frequently in untrained females than their male counterparts (18, 39). Although there seems to be a correlation between the degree of desaturation in normoxia and hypoxia in males (28), no such comparison has been made for females. It remains possible that subtle differences in human ventilatory control are not detectable by measuring total ventilation and that by determining effective alveolar ventilation and variables associated with pulmonary gas exchange, greater insight can be obtained. We emphasize that further studies with direct arterial blood sampling are required to corroborate our pulse oximeter and end-tidal observations.

**Menstrual-cycle phase and perspectives.** An important aspect of the present study was the determination of hormonal levels on a daily basis. Most studies consider the menstrual cycle as a series of discrete phases, but this is an oversimplification, and it is more appropriate to consider that some days are intermediate to different phases. The inability to time and categorize menstrual cycles by the EF, LF, or ML phase likely contributes to the difficulty in detecting differences among phases. For example, Fig. 1 shows that testing time points were 1–2 days late for subjects F09 and F13 for testing to occur during ML when PdG was highest. Furthermore, testing occurred 1–2 days too early to observe the increase in E1G for subject F03 during the LF. These observations serve to illustrate the point that controlling for menstrual-cycle phase requires more than simply counting the days since onset of menses. The use of BBT to determine ovulation is not reliable. Cycles should be confirmed to be ovulatory using an ovulation-prediction kit and ideally, circulating hormones assayed at least on the day of testing, if not throughout the entire cycle.

We monitored ovarian steroid hormones in a thorough and systematic fashion. We assayed daily urine in subjects with a known (prescreened) history of menstrual cycles that were both ovulatory and of normal length. Our approach to determining the ovarian steroid profile via daily urine collection was based on four factors. First, daily hormonal measures can be made, because the urine is self-collected and is noninvasive. Second, the samples are stable and are easy to store. This is an important consideration, because the first-morning urine was collected from subjects and immediately frozen. Third, hormonal values represent a pooled value over time rather a single time point. Lastly, the validity of the urinary technique, as representative of the pattern of E1G and PdG excretion, has been reported by others (31), and the secretion of these metabolites in the urine parallels serum concentrations of the parent hormones. As such, our method is justifiable, because it permits daily sampling, is noninvasive, and has been validated.

We assessed ventilatory and exercise measures at specific time points across the menstrual cycle, which have been previously reported to coincide with ventilatory changes. In our study, we report considerable between-subject variation in hormonal levels at predetermined times (see Fig. 1 and Table 2). Within the context of our ventilatory measures, several salient points merit discussion. First, although our subjects were homogenous with respect to aerobic fitness, routine ovulation, and cycle history, the hormonal response was heterogeneous, implying that few women have a normal, cyclical hormonal profile. Second, measures of chemosensitivity are highly variable among and within individuals and both among and within menstrual-cycle phases. This suggests that despite the high variability, the differences that we observed for indices of ventilation and chemosensitivity are physiologically robust. Lastly, we further suggest that it is important to consider the effects of sex hormones on ventilatory control in females, and future studies should make efforts to include females when examining ventilatory control during exercise or at high altitude by properly controlling for cycle phase whenever possible. Although experimentally challenging, this is much preferred to excluding women from such studies.

**Summary.** The present study supports the hypothesis that menstrual-cycle phase has effects on resting ventilation and P<sub>e</sub>T<sub>CO</sub>2. We found no effect of menstrual-cycle phase or difference between men and women for iHVR or the sensitivity to CO<sub>2</sub> in normoxia or hypoxia. We found that sensitivity to
chemical stimuli (hypoxia, hypercapnia) was unaffected by menstrual-cycle phase or that any hormone-mediated effect is of insufficient magnitude to exceed the inherent variation in these measures. The ventilatory recruitment threshold for CO₂ was always lower in women, which is suggestive of a hormonally related lowering of the ventilatory recruitment threshold. The full physiological relevance remains to be elucidated, but these observations may be relevant to our understanding of sleep-disordered breathing following the onset of menopause (8) and the effects of hormone replacement therapy on ventilatory control (33). We observed no effect of menstrual-cycle phase or male-female differences in the ventilatory response to submaximal exercise in normoxia or hypoxia. We interpret this to mean that feed-forward and the many feed-back influences over-ride any possible modulating effects of naturally occurring changes in sex hormones.

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We are indebted to our research participants for their patience, commitment, and enthusiastic participation in this study.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

Author contributions: M.J.M., M.J.D.S., and A.W.S. conception and design of research; M.J.M., S.E.T., and J.L.H. performed experiments; M.J.M., S.E.T., and J.L.H. analyzed data; M.J.M., M.J.D.S., S.E.T., J.L.H., and A.W.S. interpreted results of experiments; M.J.M. prepared figures; M.J.M. and A.W.S. drafted manuscript; M.J.M. and A.W.S. edited and revised manuscript; M.J.M., M.J.D.S., S.E.T., J.L.H., and A.W.S. approved final version of manuscript.

REFERENCES