

Phasic menstrual cycle effects on the control of breathing in healthy women

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Abstract

This study examined the effects of menstrual cycle phase on ventilatory control. Fourteen eumenorrhic women were studied in the early follicular (FP; 1–6 days) and mid-luteal (LP; 20–24 days) phase of the menstrual cycle. Blood for the determination of arterial P_{CO_2} (Pa_{CO_2}), plasma strong ion difference ([SID]), progesterone ($[\text{P}_4]$), and 17β -estradiol ($[\text{E}_2]$) concentrations were obtained at rest. Subjects performed a CO_2 rebreathing procedure that included prior hyperventilation and maintenance of iso-oxia to evaluate central and peripheral chemoreflex, and nonchemoreflex drives to breathe. Resting Pa_{CO_2} and [SID] were lower; minute ventilation (\dot{V}_E), $[\text{P}_4]$ and $[\text{E}_2]$ were higher in the LP versus FP. Within the LP, significant correlations were observed for Pa_{CO_2} with $[\text{P}_4]$, $[\text{E}_2]$ and [SID]. Menstrual cycle phase had no effect on the threshold or sensitivity of the central and/or peripheral ventilatory chemoreflex response to CO_2 . Both (\dot{V}_E) and the ventilatory response to hypocapnia (representing nonchemoreflex drives to breathe) were ~ 1 L/min greater in the LP versus FP accounting for the reduction in Pa_{CO_2} . These data support the hypothesis that phasic menstrual cycle changes in Pa_{CO_2} may be due, at least in part, to the stimulatory effects of $[\text{P}_4]$, $[\text{E}_2]$ and [SID] on ventilatory drive.

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1. Introduction

The transition from the follicular (FP) to luteal phase (LP) of the normal human menstrual cycle is characterized by increased minute ventilation (\dot{V}_E) and reduced arterial Pa_{CO_2} (Pa_{CO_2}) in the resting state (Schoene et

al., 1981; Preston et al., 2001). The mechanism(s) of these changes are poorly understood. However, they may be due, at least in part, to the stimulatory effects of ovarian hormones on chemoreflex and/or nonchemoreflex drives to breathe (Bayliss and Millhorn, 1992; Tatsumi et al., 1995; Behan et al., 2003; Jensen et al., 2005b).

The LP is accompanied by significant increases in circulating progesterone ($[\text{P}_4]$) and 17β -estradiol ($[\text{E}_2]$) concentrations (Chabbert et al., 1998). Human

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pregnancy is characterized by more substantial increases in $[P_4]$ and $[E_2]$ and decreases in PaCO_2 , compared to those observed in the LP (Wolfe et al., 1998). These changes are accompanied by significant increases in central and peripheral chemoreflex sensitivity to CO_2 and hypoxia, respectively (Moore et al., 1987; Jensen et al., 2005b). Furthermore, many investigators have demonstrated significant increases in central and peripheral chemoreflex responsiveness to CO_2 and hypoxia, respectively, in the LP compared to the FP (Schoene et al., 1981; Takano, 1984; Dombrov et al., 1987; Dutton et al., 1989; Masuda et al., 2001); however, others have not (Takano et al., 1981; Regensteiner et al., 1990; Beidleman et al., 1999). Therefore, ovarian hormones may act alone, or in combination, at central and/or peripheral chemoreceptor sites to stimulate (\dot{V}_E) and reduce PaCO_2 (Bayliss and Millhorn, 1992; Behan et al., 2003).

In addition to the established effects of ovarian hormones on ventilatory drive, Preston et al. (2001) recently hypothesized that reductions in plasma strong ion difference concentration ($[\text{SID}]$), which represents the difference between the concentration of all strong cations minus the concentration of all strong anions, may contribute to respiratory adaptations in the LP. As reviewed by Jennings et al. (1994) changes in cerebrospinal fluid and arterial $[\text{SID}]$ in conscious animals, as well as human subjects, consistently predicts ventilatory regulation of PaCO_2 . Indeed, Heenan and Wolfe (2003) recently demonstrated a positive correlation between PaCO_2 and plasma $[\text{SID}]$ in the transition from the non-pregnant to pregnant state of healthy women. However, the physiological mechanism(s) by which $[\text{SID}]$ exerts its effects on respiration has not been definitively established.

This study used a modified version of Read's (1967) rebreathing technique that includes prior hypoventilation and maintenance of iso-oxia (Duffin et al., 2000) to examine the effects of menstrual cycle phase on chemoreflex and nonchemoreflex drives to breathe. The contribution of ovarian hormones and $[\text{SID}]$ to ventilatory adaptations observed across the menstrual cycle were also studied. It was hypothesized that increased $[P_4]$ and $[E_2]$, and reduced $[\text{SID}]$ would be associated with changes in central and peripheral chemoreflex, and nonchemoreflex drives to breathe, resulting in increased \dot{V}_E and reduced PaCO_2 .

2. Methods

2.1. Subjects

Subjects were 17 healthy, physically active, non-smoking, eumenorrheic women, aged 20–35, recruited via media advertisements and posted announcements. A telephone or email interview was first conducted to acquire menstrual cycle history and to ensure that subjects were taking no medications and had no significant cardiorespiratory, hematological, metabolic and/or eating disorder(s). Each subject then completed the revised physical activity readiness questionnaire (<http://www.csep.ca/forms.asp>) and general health-fitness questionnaire to ensure there were no contraindications to study participation. The study protocol and consent form were approved by the Research Ethics Board, Faculty of Health Sciences, Queen's University, and all subjects provided written consent.

Menstrual cycle phase was determined using the first day of the last menstrual cycle and average length of at least three previous menstrual cycles. The LP was assumed to be 14 days for all subjects (Chabbert et al., 1998). The FP was calculated by subtracting 14 days from the length of an average menstrual cycle for each subject. Menstrual cycle status and phase were confirmed by measurement of plasma $[P_4]$ and $[E_2]$. Three of 17 subjects were anovulatory (LP: $[P_4] < 5.3$ nmol/L; $[E_2] < 147$ pmol/L) and excluded from further analysis. Thus, a total of 14 subjects completed the study.

2.2. Experimental design

Subjects participated in laboratory testing on three separate occasions and abstained from aerobic and muscular conditioning exercise as well as caffeine and alcohol on all test days. During the first laboratory visit, subjects performed a familiarization hyperoxic CO_2 rebreathing procedure (described below). Basic physical measurements including height (cm), body mass (kg), forced vital capacity (FVC), forced expiratory volume in 1 s (FEV_{1s}), and peak expiratory flow rate (Pneumoscan, model S-301) were also obtained. Body mass index (BMI) was calculated as body height/body mass² (kg/m^2).

The second and third laboratory tests were conducted during the early-FP (1–6 days) and mid-LP (20–24 days) of the menstrual cycle in randomized

order. During each visit, subjects performed a hyperoxic and hypoxic CO₂ rebreathing procedure separated by at least 45-min. The order of hyperoxic and hypoxic rebreathing trials was randomized between subjects during the second laboratory visit, and altered for each subject during the third laboratory testing session. Rebreathing trials were followed by a 1.5-h break after which resting blood samples and ventilatory variables were collected. The second and third laboratory testing sessions were conducted at the same time of day to minimize the effects of circadian rhythm on respiratory control (Stephenson et al., 2000).

2.3. Modified rebreathing procedure

Chemoreflex and nonchemoreflex ventilatory control characteristics were measured using a modified version of Read's (1967) rebreathing procedure that includes 5-min of prior hyperventilation and maintenance of iso-oxia (Duffin et al., 2000; Jensen et al., 2005a,b). The modified rebreathing apparatus, data acquisition and analysis software has been described in detail in previous publications from this laboratory (Jensen et al., 2005a,b).

Prior to rebreathing, subjects voluntarily hyperventilated room air for 5-min using a slow and deliberate breathing pattern to reduce the body stores of CO₂ below 25 mmHg. Subjects were then switched from room air to a rebreathing bag, containing a hyperoxic-hypercapnic (24% O₂, 6% CO₂, N₂ balanced) or hypoxic-hypercapnic (4.5% O₂, 6% CO₂, N₂ balanced) gas mixture. Rebreathing began with three deep breaths, producing rapid equilibration of the P_{CO₂} in the bag, lungs and arterial blood to that of mixed venous blood. Equilibration was verified by observation of a plateau of end-tidal CO₂ tension (PET_{CO₂}) and was a prerequisite for continuing the test. Upon equilibration, subjects were asked to breathe as they felt the need. During rebreathing, PET_{CO₂} increased while iso-oxia was maintained, under computer control, at a hyperoxic (150 mmHg) or hypoxic (50 mmHg) end-tidal O₂ tension. Arterial blood O₂ saturation (SaO₂, %) and heart rate were monitored continuously with an ear oximeter (OXI, Radiometer Copenhagen, Copenhagen, Denmark). Rebreathing was terminated if $\dot{V}_E > 100$ L/min, PET_{CO₂} > 60 mmHg, SaO₂ < 70% and/or subject discomfort.

Measured volumes were corrected for body temperature (37 °C) and pressure, saturated with water vapor (BTPS). Breath-by-breath PET_{CO₂} was plotted against time and fitted with a least-squares regression line, whose slope depends on the metabolic rate of CO₂ production. The equation for this line provided a predicted value of PET_{CO₂} versus time, thereby minimizing inter-breath variability due to measurement. Thereafter, \dot{V}_E was plotted against the predicted PET_{CO₂}.

The \dot{V}_E response to hyperoxic and hypoxic CO₂ rebreathing was fitted with a model made up of the sum of two segments separated by one breakpoint (Duffin et al., 2000). The PET_{CO₂} at which \dot{V}_E increased with increases in PET_{CO₂} was identified as the ventilatory recruitment threshold to CO₂ (V_{ET}; Fig. 1). The \dot{V}_E response below the V_{ET} (basal ventilation; V_{EB}), was taken as an estimate of nonchemoreflex and/or behavioral drives to breathe (Shea, 1996; Fig. 1). The slope of the linear relation between \dot{V}_E and PET_{CO₂} above the V_{ET} was taken as a measure of chemoreflex sensitivity (V_{ES}; Fig. 1). Based on the modeling of Duffin et al. (2000) it was assumed that the V_{ET} and V_{ES} measured during hyperoxic CO₂ rebreathing experiments originate from the central chemoreflex alone, whereas the same measurements recorded during hypoxic trials

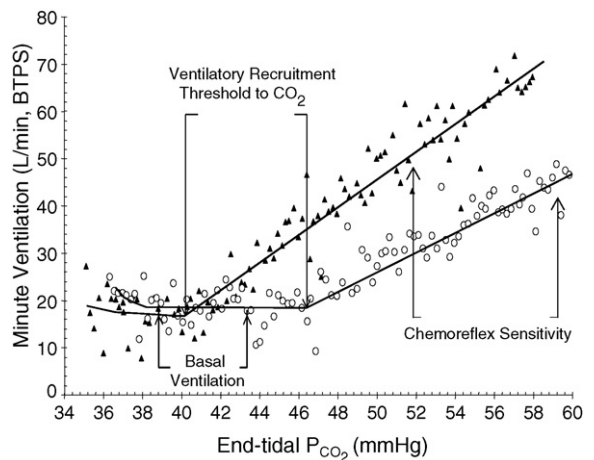


Fig. 1. An example of the ventilatory response to hyperoxic (filled diamonds) and hypoxic (open triangles) CO₂ rebreathing in a representative subject during the early follicular phase of the menstrual cycle. Note measurement of basal ventilation, the ventilatory recruitment threshold to carbon dioxide and chemoreflex sensitivity. Refer to text for details.

derive from the sum of central and peripheral chemoreflex stimulation.

2.4. Resting ventilatory and blood biochemistry data collection

Subjects were comfortably seated and an experienced nurse inserted an indwelling catheter into a dorsal hand vein situated as far from the thumb as possible. The hand and lower arm were placed in a Plexiglas box and heated by warm circulating air to promote vasodilation and allow for arterialization of venous blood. This was followed by 10-min of resting ventilatory data collection, during which blood was drawn after the 6th min. Resting \dot{V}_E , tidal volume (V_T), respiratory frequency (fR), O_2 uptake (\dot{V}_{O_2}), and CO_2 output (\dot{V}_{CO_2}) were measured on a breath-by-breath basis as previously described (Heenan and Wolfe, 2003) in 11 of 14 participants.

2.5. Blood biochemistry

Blood samples for the determination of PET_{CO_2} , arterial P_{O_2} and hydrogen ion concentration ($[H^+]$) were obtained using a heparinized syringe (Radiometer Copenhagen PICO70, Copenhagen, Denmark) and analyzed immediately using a Radiometer ABL 5 acid-base analyzer (Radiometer Copenhagen, Copenhagen, Denmark) at a standard temperature of 37 °C. The remaining blood was used for strong ion analysis (described below). Next, blood for the determination of plasma lactate concentration ($[La^-]$) was collected in a vacutainer containing potassium oxalate and sodium chloride. Finally, blood for plasma $[P_4]$ and $[E_2]$ determination was collected in a vacutainer containing no additive and stored on ice for 0.5–1.0 h to clot. Blood was then centrifuged for 10-min at 2500 revolutions/min and frozen at -80 °C for later analysis.

Plasma concentrations of sodium ($[Na^+]$), potassium ($[K^+]$), chloride ($[Cl^-]$), calcium ($[Ca^{2+}]$) and $[La^-]$ were analyzed according to previously published methods from this laboratory (Preston et al., 2001; Heenan and Wolfe, 2003). The [SID] was calculated as $([Na^+] + [K^+] + 2 [Ca^{2+}]) - ([Cl^-] + [La^-])$ (Kowalchuk and Scheuermann, 1994). $[P_4]$ and $[E_2]$ were determined by electrochemiluminescence immunoassay.

Difficulties with maintaining venous catheterization (i.e., patency of dorsal hand vein) compromised blood collection for the determination of [SID], Pa_{CO_2} and $[H^+]$ in 3 of 14 subjects. However, blood for plasma $[P_4]$ and $[E_2]$ determination were obtained via venipuncture of an arm vein in those three subjects such that these data were available for all 14 participants.

2.6. Minimum sample size estimate

A paired subject formula for the comparison of means was used to estimate minimum sample size for this study, assuming 90% power and an alpha level of $p < 0.05$. Pa_{CO_2} and both hyperoxic and hypoxic V_{ET} and V_{ES} parameters were identified as important outcome variables. Standard deviations for Pa_{CO_2} , and hyperoxic and hypoxic V_{ET} and V_{ES} parameters in healthy, eumenorrhic women were obtained from Preston et al. (2001) and Jensen et al. (2005a), respectively. The sample size capable of detecting between-phase differences of 1 mm Hg for Pa_{CO_2} was estimated to be 7. Sample sizes capable of detecting between-phase differences of 0.75 mm Hg for hyperoxic and hypoxic V_{ET} were 11 and 11, respectively. Similarly, sample sizes capable of detecting between-phase differences of 1 L/min/mmHg for hyperoxic and hypoxic V_{ET} were 5 and 7, respectively. Therefore, a sample size of 14 subjects was considered sufficient.

2.7. Statistical analyses

Paired Student *t*-statistics were used to identify significant between-phase differences of blood biochemistry measurements (SigmaStat 3.10, Systat Software Inc., Point Richmond, CA). A two-way repeated measures analysis of variance was used to detect differences between-phases (FP versus LP) and levels of iso-oxia (hyperoxia versus hypoxia) within mean rebreathing data. When significant main effects were observed, the post hoc test of Tukey (HSD) was used to identify where the differences resided.

Pearson product-moment correlation coefficients (Pearson *r*; one-tailed) were calculated for Pa_{CO_2} with $[P_4]$, $[E_2]$ and [SID], within the LP, to test the hypothesis that increased circulating $[P_4]$ and $[E_2]$ and reduced [SID] act to lower Pa_{CO_2} . Correlation coefficients were not calculated within the FP since values for $[P_4]$ and $[E_2]$ were very homogenous (Fig. 2) and any corre-

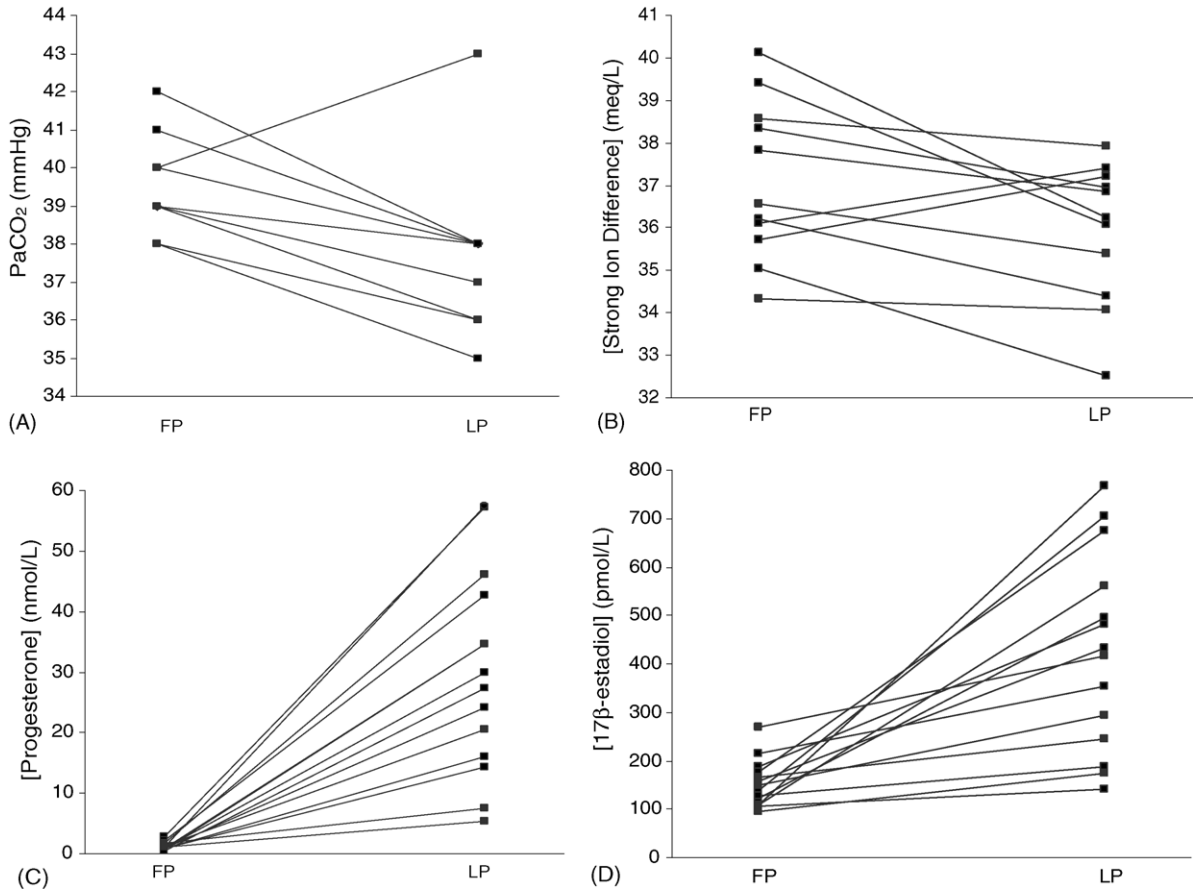


Fig. 2. Phasic menstrual cycle changes in: arterial P_{CO_2} (A); plasma strong ion difference concentration (B); plasma progesterone concentration (C); plasma 17 β -estradiol concentration (D). Data for each volunteer are plotted separately for each condition. FP, early follicular phase; LP, mid-luteal phase.

lation or lack thereof may reflect a statistical artifact. Values for all statistical tests were considered significant if $p < 0.05$. Values are presented as means \pm S.E.

3. Results

3.1. General

Physical characteristics of the 14 female subjects are described in Table 1. $[P_4]$ and $[E_2]$ were significantly higher in the LP (Table 2). $PaCO_2$ and $[SID]$ were significantly lower, and \dot{V}_E , $[P_4]$ and $[E_2]$ significantly higher in the LP compared to the FP (Table 2). All other ventilatory and blood biochemistry variables were not significantly affected by menstrual cycle phase (Table 2).

Table 1
Physical characteristics of subjects

Variable	Measurement
Age (year)	26.9 \pm 1.2
Height (cm)	166.0 \pm 1.5
Body mass (kg)	59.4 \pm 1.7
BMI (kg/m ²)	21.7 \pm 0.7
Parity	0, $n = 13$; 1, $n = 1$
Average length of the menstrual cycle (days)	29.7 \pm 0.5
FP, days from the first day of menstruation	4.4 \pm 0.4
LP, days from the first day of menstruation	23.8 \pm 0.7

Values are mean \pm S.E. BMI, body mass index; FP, early follicular phase; LP, mid-luteal phase.

Table 2
Resting ventilatory and blood biochemistry measurements

	FP	LP
Ventilatory measurements		
\dot{V}_E (L/min)	8.0 ± 0.4	9.0 ± 0.5 ^a
V_T (L)	0.52 ± 0.03	0.57 ± 0.05
fR (breaths/min)	15.9 ± 1.0	16.6 ± 1.1
\dot{V}_{O_2} (ml/min)	313 ± 15	323 ± 16
\dot{V}_{CO_2} (ml/min)	269 ± 15	286 ± 14
Peak flow (L/s)	5.5 ± 0.2	5.7 ± 0.2
FVC (L)	3.2 ± 0.2	3.1 ± 0.2
FEV ₁ (L)	2.8 ± 0.1	2.7 ± 0.1
Blood biochemistry measurements		
PaCO ₂ (mmHg)	39.8 ± 0.4	37.6 ± 0.6 ^a
[P ₄] (nmol/L)	1.2 ± 0.2	29.8 ± 4.5 ^a
[E ₂] (pmol/L)	152.1 ± 13.0	423.8 ± 54.4 ^a
[SID] (meq/L)	37.1 ± 0.6	35.9 ± 0.5 ^a
[H ⁺] (nequiv./L)	39.5 ± 0.3	39.6 ± 0.3

Values are means ± S.E. FP, early follicular phase; LP, mid-luteal phase; \dot{V}_E , minute ventilation; V_T , tidal volume; fR, respiratory frequency; \dot{V}_{O_2} , O₂ uptake; \dot{V}_{CO_2} , CO₂ output; FVC, forced vital capacity; FEV₁, forced expired volume in one second; PaCO₂, arterial P_{CO₂}; P₄, progesterone; E₂, 17β-estradiol; SID, strong ion difference; H⁺, hydrogen ion; brackets indicate concentration.

^a Significant between-phase difference ($p < 0.05$).

Significant correlations were observed for PaCO₂ with: [P₄] ($r = -0.75$, $p < 0.01$), [E₂] ($r = -0.61$, $p = 0.02$) and [SID] ($r = 0.55$, $p = 0.04$) (Fig. 3).

3.2. Rebreathing studies

Measurements of V_{EB} from both hyperoxic and hypoxic CO₂ rebreathing tests were not significantly different between the LP and FP. However, V_{EB} was significantly greater under hypoxic versus hyperoxic rebreathing conditions, independent of menstrual cycle phase (Table 3).

Hyperoxic and hypoxic V_{ET} and V_{ES} measurements were not significantly different in the LP compared to FP (Table 3). V_{ES} was significantly greater, while V_{ET} was significantly lower under hypoxic versus hyperoxic rebreathing conditions, independent of menstrual cycle phase (Table 3).

4. Discussion

A previous publication from this laboratory demonstrated that between the FP and LP of the human men-

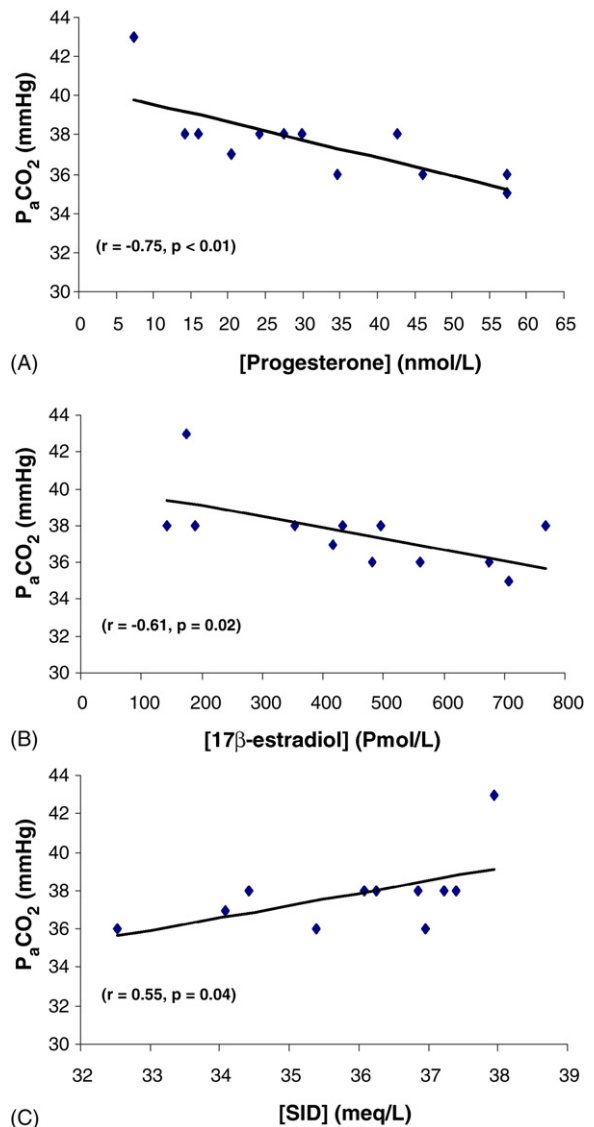


Fig. 3. Relationship of arterial P_{CO₂} with: (A) plasma progesterone concentration; (B) plasma 17β-estradiol concentration; (C) plasma strong ion difference concentration within the luteal phase of the menstrual cycle.

strual cycle there is an increase in \dot{V}_E and a decrease in PaCO₂ at rest (Preston et al., 2001). The purpose of the present study was to examine potential regulatory mechanisms to account for these ventilatory adaptations. Specifically, we measured 'respiratory' changes with respect to circulating [P₄], [E₂] and [SID], all of which have been reported to affect respiration, as

Table 3

Phasic menstrual cycle effects on basal ventilation, ventilatory recruitment threshold and ventilatory sensitivity during hyperoxic and hypoxic CO₂ rebreathing

	Hyperoxia		Hypoxia	
	FP	LP	FP	LP
V_{E_B} (L/min)	8.0 ± 1.0 ^a	9.0 ± 1.3 ^a	11.0 ± 1.5	12.3 ± 1.3
V_{E_T} (mmHg)	46.4 ± 0.8 ^a	45.9 ± 0.6 ^a	42.6 ± 0.5	41.6 ± 0.8
V_{E_S} (L/min/mmHg)	2.77 ± 0.32 ^a	3.25 ± 0.34 ^a	4.11 ± 0.51	4.30 ± 0.35

Values are mean ± S.E. FP, early-follicular phase; LP, mid-luteal phase; V_{E_B} , basal ventilation; V_{E_T} , ventilatory recruitment threshold to carbon dioxide; V_{E_S} , ventilatory chemoreflex sensitivity.

^a Significant difference between hyperoxia and hypoxia ($p < 0.05$).

reviewed in the introduction. Furthermore, this study was the first to assess the contribution of chemoreflex and nonchemoreflex drives to breathe to ventilatory adaptations across the menstrual cycle using the modified rebreathing technique of Duffin et al. (2000). This modified procedure has significant methodological and interpretative advantages (reviewed by Mohan and Duffin, 1997; Duffin et al., 2000; Duffin and Mahamed, 2003; Jensen et al., 2005a) over Read's original rebreathing procedure (1967) and the progressive isocapnic hypoxia procedure (Weil et al., 1970) used in previous studies of ventilatory control across the menstrual cycle (Schoene et al., 1981; Takano et al., 1981; Takano, 1984; Dombrov et al., 1987; Dutton et al., 1989; Regensteiner et al., 1990; Beidleman et al., 1999; Masuda et al., 2001).

As predicted, resting PaCO₂ in this study was reduced by approximately 2 mmHg in the LP relative to the FP in association with a 1 L/min increase in \dot{V}_E (Table 2). Thus, our subjects demonstrated a definitive increase in ventilatory drive. These adaptations were not due to an increase in basal metabolism, since no significant between-phase differences in resting \dot{V}_{O_2} or \dot{V}_{CO_2} were observed in this study, or in a previous report from this laboratory (Preston et al., 2001).

In the present study, reductions in PaCO₂ between the FP and LP were accompanied by increased [P₄] and [E₂], and decreased [SID] (Fig. 2). In this regard, we observed significant correlations for PaCO₂ with [P₄], [E₂] and [SID] within the LP (Fig. 3). These findings support our original hypothesis and the hypotheses described by Preston et al. (2001) and Wolfe et al. (1998), respectively, that ovarian hormones and [SID] may act alone, or in combination, to stimulate \dot{V}_E and reduce PaCO₂ in the LP of the menstrual cycle. The

interaction of ovarian hormones and [SID] with central and peripheral sites involved in ventilatory control has been well described in a number of reviews (Bayliss and Millhorn, 1992; Jennings, 1994; Tatsumi et al., 1995; Wolfe et al., 1998; Behan et al., 2003).

In late pregnancy, similar but more dramatic decreases in PaCO₂ and increases in [P₄] and [E₂], compared to those observed in the LP of this study, have been associated with changes in central chemoreflex and nonchemoreflex drives to breathe (Jensen et al., 2005b). These changes included a significantly lower V_{E_T} , and greater V_{E_S} and V_{E_B} in pregnant versus non-pregnant women.

Despite the latter observations in pregnancy, and in contrast to our expectation, central and peripheral chemoreflex drives to breathe, represented by hyperoxic and hypoxic V_{E_T} and V_{E_S} measurements, respectively, were not significantly different in the FP compared to LP of the menstrual cycle. Furthermore, V_{E_B} , an index of all neural drives to breathe independent of the chemoreflexes was not statistically different in the FP versus LP. This could be interpreted to indicate that ovarian hormones and [SID] did not stimulate breathing via direct or indirect interaction(s) with chemoreflex and/or nonchemoreflex drives to breathe across the menstrual cycle.

4.1. Critique of methods and interpretation of results

The model of ventilatory control used in the present study (Duffin, 1990; Duffin et al., 2000; Duffin and Mahamed, 2003) as well as a comprehensive critique of the modified rebreathing procedure (Mohan and Duffin, 1997; Jensen et al., 2005a) has been described in previous publications. Briefly, resting \dot{V}_E and PaCO₂

depend on both central and peripheral chemoreflexes and other, nonchemoreflex drives to breathe (all of which are assumed to be additive) and their intersection with the metabolic hyperbola (i.e., relation between alveolar ventilation and PaCO_2 at a given \dot{V}_{CO_2}) (Cunningham et al., 1986; Duffin, 1990; Duffin et al., 2000). In other words, any change in resting \dot{V}_{E} and PaCO_2 may only be accounted for by a change(s) in chemoreflex and nonchemoreflex drives to breathe and/or metabolic rate. In the present study, neither metabolic rate, chemoreflex or nonchemoreflex ventilatory drives were significantly increased in the LP versus FP. Thus, the mechanism(s) through which hyperpnea and hypocapnia occurred is not immediately evident.

In contrast to the above, it must be admitted that the statistically significant decrease in average PaCO_2 between the FP and LP in this study was small, approximately 2 mmHg. Although the criterion for setting the subject sample size in the methods allowed for significance at a 1 mmHg difference in PaCO_2 , it is possible that the between-phase difference for PaCO_2 reflects a type 1 error. On the other hand, between-phase measurements of central and peripheral chemoreflex V_{ET} and V_{ES} parameters may have been concealed by considerable within- and/or between-subject variability (Sahn et al., 1977; Zhang and Robbins, 2000). Indeed, Beidleman and colleagues (1999), using Read's rebreathing procedure (1967) and the progressive isocapnic hypoxia procedure (Weil et al., 1970), found no effect of menstrual cycle phase on central or peripheral chemoreflex responsiveness to CO_2 and hypoxia, respectively. These investigators reported within-subject, between-day coefficients of variation (CVs) of 42.7 and 63.9% for CO_2 and hypoxic chemosensitivity measurements which are considerably greater than CVs calculated for hyperoxic and hypoxic V_{ES} parameters ($21 \pm 5\%$ and $23 \pm 5\%$) in this study. Moreover, within-subject, between-day CVs for hyperoxic and hypoxic V_{ET} were quite small, $4 \pm 1\%$ and $3 \pm 1\%$. These data further support our conclusion that menstrual cycle phase had no significant effect on central or peripheral chemoreflex drives to breathe.

These data also suggest that methodological variability associated with measurement of the ventilatory response to CO_2 and hypoxia using Read's rebreathing procedure (1967) and the progressive isocapnic hypoxia procedure (Weil et al., 1970) may be greater

than that of the modified CO_2 rebreathing procedure (Duffin et al., 2000), since both past (Beidleman et al., 1999) and present studies were conducted in healthy, eumenorrheic women in the early FP and mid-LP of their menstrual cycles. Therefore, the modified rebreathing procedure is quite robust for V_{ET} and V_{ES} determination.

However, the modified rebreathing technique may be less robust for V_{EB} estimation, as its determination (or 'fit'), relative to V_{ES} , is based on fewer data points (Duffin et al., 2000). In addition, V_{EB} is influenced by the subject's level of alertness or anxiety at the time of the test (Shea, 1996) which may obscure any change(s) in V_{EB} . Although attempts were made to minimize this effect (i.e., familiarization), repeated tests were conducted approximately 20 days apart in a group of relatively inexperienced subjects. Consequently, the lack of between-phase differences for V_{EB} may be explained, at least in part, by a type 2 error that a larger sample size may have overcome. Therefore, the increased \dot{V}_{E} and reduced PaCO_2 at rest in the LP versus FP of the present study may be attributed to phasic menstrual cycle changes in V_{EB} , independent of metabolic rate and/or the ventilatory chemoreflexes.

In this regard, even a small increase in V_{EB} (i.e., upward shift of the ventilatory response curve to CO_2) would increase resting \dot{V}_{E} and reduce PaCO_2 , because at that position (i.e., point of intersection between metabolic hyperbola and normoxic ventilatory response curve to CO_2) the metabolic hyperbola is fairly flat and so small changes in \dot{V}_{E} yield relatively large changes in PaCO_2 (Cunningham et al., 1986; Duffin, 1990; Duffin and Mahamed, 2003). Indeed, V_{EB} , albeit not significant due to high measurement variability, and resting \dot{V}_{E} ($p < 0.05$) were both increased by an average of 1 L/min in the LP versus FP. In addition, resting \dot{V}_{E} was, on average, the same as hyperoxic V_{EB} in the FP and LP, respectively, suggesting that a change in V_{EB} may account, at least in part, for differences in PaCO_2 .

Studies using the modified rebreathing procedure, have consistently demonstrated that under hypocapnic or subthreshold conditions, hypoxia has no independent effect on ventilation (Mohan and Duffin, 1997; Rapanos and Duffin, 1997; Duffin et al., 2000; Jensen et al., 2005a). Contrary to these results, we found that V_{EB} measured under iso-oxic hypoxic rebreathing conditions was significantly greater than that calcu-

lated from iso-oxic hyperoxic experiments. Previous studies by Nielsen and Smith (1952) and Weil et al. (1970) also found that the acute ventilatory response to hypocapnia is increased in the presence of hypoxia. Therefore, hypoxia may stimulate ventilation, independent of CO₂. This effect likely varies between-subjects and in contrast to previous investigations (Mohan and Duffin, 1997; Rapanos and Duffin, 1997; Duffin et al., 2000; Jensen et al., 2005a), reached statistical significance in our study population.

4.2. Conclusions

The findings of this study support the hypothesis that phasic menstrual cycle changes in resting \dot{V}_E and PaCO₂ may be due, at least in part, to the stimulatory effects of progesterone, 17 β -estradiol and the strong ion difference on ventilatory drive. In addition, LP-associated increases in nonchemoreflex and/or behavioral drives to breathe may have contributed to reductions in PaCO₂. However, the interaction(s) between ovarian hormones and the strong ion difference with ventilatory control mechanisms were not revealed. Additional studies employing the modified rebreathing procedure, accompanied by serial (i.e., daily) measurements of arterial blood gases, ovarian hormone and strong ion difference concentrations in a larger number of subjects, are necessary to confidently establish the underlying mechanism(s) of ventilatory adaptations across the normal human menstrual cycle.

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References

- Bayliss, D.A., Millhorn, D.E., 1992. Central neural mechanisms of progesterone action: application to the respiratory system. *J. Appl. Physiol.* 73, 393–404.
- Behan, M., Zabka, A.G., Thomas, C.F., Mitchell, G.S., 2003. Sex steroid hormones and the neural control of breathing. *Respir. Physiol. Neurobiol.* 136, 249–263.
- Beidleman, B.A., Rock, P.B., Muza, S.R., Fulco, C.S., Forte Jr., V.A., Cymerman, A., 1999. Exercise \dot{V}_E and physical performance at altitude are not affected by menstrual cycle phase. *J. Appl. Physiol.* 86, 1519–1526.
- Chabbert, B.N., Djakoure, C., Maitre, S.C., Bouchard, P., 1998. Regulation of the human menstrual cycle. *Front. Neuroendocrinol.* 19, 151–186.
- Cunningham, D.J.C., Robbins, P.A., Wolff, C.B., 1986. Integration of respiratory responses to changes in alveolar partial pressure of CO₂ and O₂ and in arterial pH. In: Cherniack, N.S., Widdicombe, J.G. (Eds.), *Handbook of Physiology, Section 3: The Respiratory System, vol. II: Control of Breathing, part II.* American Physiological Society, Bethesda, MD, pp. 475–528.
- Dombovy, M.L., Bonekat, H.W., Williams, T.J., Staats, B.A., 1987. Exercise performance and ventilatory response in the menstrual cycle. *Med. Sci. Sports. Exerc.* 19, 111–117.
- Duffin, J., 1990. The chemoreflex control of breathing and its measurement. *Can. J. Anaesth.* 37, 933–942.
- Duffin, J., Mahamed, S., 2003. Adaptation in the respiratory control system. *Can. J. Physiol. Pharmacol.* 81, 733–765.
- Duffin, J., Mohan, R.M., Vasilliou, P., Stephenson, R., Mahamed, S., 2000. A model of chemoreflex control of breathing in humans: model parameters measurement. *Respir. Physiol.* 120, 13–26.
- Dutton, K., Blanksby, B.A., Morton, A.R., 1989. CO₂ sensitivity changes during the menstrual cycle. *J. Appl. Physiol.* 67, 517–522.
- Heenan, A.P., Wolfe, L.A., 2003. Plasma osmolality and the strong ion difference predict respiratory adaptations in pregnant and nonpregnant women. *Can. J. Physiol. Pharmacol.* 81, 839–847.
- Jennings, D.B., 1994. The physicochemistry of [H⁺] and respiratory control: roles of P_{CO2}, strong ions, and their hormonal regulators. *Can. J. Physiol. Pharmacol.* 72, 1499–1512.
- Jensen, D., Wolfe, L.A., O'Donnell, D.E., Davies, G.A.L., 2005a. Chemoreflex control of breathing during wakefulness in healthy men and women. *J. Appl. Physiol.* 98, 822–828.
- Jensen, D., Wolfe, L.A., Slatkovska, L., Webb, K.A., Davies, G.A.L., O'Donnell, D.E., 2005b. Effects of human pregnancy on the ventilatory chemoreflex response to carbon dioxide. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 288, R1369–R1375.
- Kowalchuk, J.M., Scheuermann, B.W., 1994. Acid-base regulation: a comparison of quantitative methods. *Can. J. Physiol. Pharmacol.* 72, 818–826.
- Masuda, A., Ohyabu, Y., Kobayashi, T., Yashino, C., Honda, Y., 2001. Hypoxic-hypercapnic interaction on ventilatory response and respiratory sensation in females. *Adv. Exp. Med. Biol.* 499, 393–397.
- Mohan, R., Duffin, J., 1997. The effect of hypoxia on the ventilatory response to carbon dioxide in man. *Respir. Physiol.* 108, 101–115.

- Moore, L.G., McCullough, R.E., Weil, J.V., 1987. Increased HVR in pregnancy: relationship to hormonal and metabolic changes. *J. Appl. Physiol.* 62, 158–163.
- Nielsen, M., Smith, H., 1952. Studies on the regulation of respiration to acute hypoxia. *Acta. Physiol. Scand.* 24, 293–313.
- Preston, R.J., Heenan, A.P., Wolfe, L.A., 2001. Physicochemical analysis of phasic menstrual cycle effects on acid-base balance. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 280, R481–R487.
- Rapanos, T., Duffin, J., 1997. The ventilatory response to hypoxia below the carbon dioxide threshold. *Can. J. Appl. Physiol.* 22, 23–36.
- Read, D.J.C., 1967. A clinical method for assessing the ventilatory response to CO₂. *Austr. Ann. Med.* 16, 20–32.
- Regensteiner, J.G., McCulloch, R.G., McCulloch, R.E., Pickett, C.K., Moore, L.G., 1990. Combined effects of female hormones and exercise on hypoxic ventilatory response. *Respir. Physiol.* 82, 107–114.
- Sahn, S.A., Zwillich, C.W., Dick, N., McCullough, R.E., Lakshminarayan, S., Weil, J.V., 1977. Variability of the ventilatory responses to hypoxia and hypercapnia. *J. Appl. Physiol.* 43, 1019–1025.
- Schoene, R.B., Robertson, H.T., Pierson, D.J., Peterson, A.P., 1981. Respiratory drives and exercise in menstrual cycles of athletic and non-athletic women. *J. Appl. Physiol.* 50, 1300–1305.
- Shea, S.A., 1996. Behavioural and arousal-related influences on breathing in humans. *Exp. Physiol.* 81, 1–26.
- Stephenson, R., Mohan, R.V., Duffin, J., Jarsky, T.M., 2000. Circadian rhythms in the chemoreflex control of breathing in humans. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 278, R282–R286.
- Takano, N., 1984. Reflex hypoxic drive to respiration during the menstrual cycle. *Respir. Physiol.* 56, 229–235.
- Takano, N., Sakai, A., Iida, Y., 1981. Analysis of alveolar P_{CO₂} control during the menstrual cycle. *Pflug. Arch.* 390, 56–62.
- Tatsumi, K., Moore, L.G., Hannhart, B., 1995. Influences of sex steroids on ventilation and ventilatory control. In: Dempsey, J.A., Pack, A.I. (Eds.), *Regulation of Breathing*. Marcel Dekker, New York, pp. 829–864.
- Weil, J.V., Byrne-Quinn, E., Sodal, L.E., Friesen, W.O., Underhill, B., Filley, G.F., Grover, R.F., 1970. Hypoxic ventilatory drive in normal man. *J. Clin. Invest.* 49, 1061–1072.
- Wolfe, L.A., Kemp, J.G., Heenan, A.P., Preston, R.J., Ohtake, P.J., 1998. Acid–base regulation and control of ventilation during human pregnancy. *Can. J. Physiol. Pharmacol.* 76, 815–827.
- Zhang, S., Robbins, P.A., 2000. Methodological and physiological variability within the ventilatory response to hypoxia in humans. *J. Appl. Physiol.* 88, 1924–1932.