Progesterone and estrogen influence baseline breathing parameters and chemoreflexes in menstruating women

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ABSTRACT

The hypoxic and hypercapnic ventilatory responses (HVR, HCVR, respectively) are the reflex increases in breathing in response to decreases in arterial oxygen or increases in arterial carbon dioxide partial pressures. These reflexes are highly variable both within individuals because of pathologies or environmental exposures, as well as across populations. However, the mechanisms underlying individual variation in these responses are still under investigation. Despite decades of research examining the effects of sex hormones progesterone and estrogen on ventilatory chemoreflexes, there remains no strong consensus and data are conflicting. Some studies have reported differences in the HVR in menstruating women compared to men and postmenopausal women, but few studies investigate this link further, and data within menstruating non-pregnant women are less conclusive. Understanding hormonal effects on ventilation could illuminate differences in risk factors and responses to control of breathing disorders. We directly measured plasma progesterone and estradiol levels and the HVR and HCVR using the Duffin modified rebreathing chemoreflex method, in 40 healthy, nonpregnant women. Our results indicate that higher progesterone levels were not associated with HVR, HCVR or the ventilatory recruitment threshold when measured in hyperoxic (inspired P_{O2} = 228 mmHg) or hypoxic (end-tidal P_{O2} = 50 mmHg) conditions, especially when adjusting for age as a covariate. Our results indicate a positive correlation with total ventilation and estradiol when adjusting for age as a covariate ($F(1, 77) = 16.9063$, $p = 9.739e\text{-}5$). Overall, these findings indicate that the impact of progesterone on the isocapnic HVR in menstruating women may be moderate at lower hormone levels, while estradiol seems to influence baseline ventilation. By analyzing these relationships, we can better understand the cause of sex-based differences in respiratory health.

KEYWORDS: Ventilatory chemoreflexes, menstruation, progesterone, estradiol

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INTRODUCTION

The hypoxic and hypercapnic ventilatory responses (HVR and HCVR, respectively) are the reflex increase in minute ventilation in response to hypoxic (low oxygen) or hypercapnic (high carbon dioxide) conditions. These ventilatory reflexes, as well as baseline respiratory drive, vary within and across individuals. For example, the HVR changes during acclimatization to sustained hypoxia (Pham et al., 2021) and in pregnancy (García-Rio et al., 1996). The molecular and neurophysiological mechanisms of this plasticity in chemoreflex responses is a large area of study. Understanding these variations can lead to a greater understanding of risk factors and variation in responses to control of breathing disorders. Some research suggests that sex may be one factor accounting for this variation. There seems to be significant differences between the ventilatory chemoreflexes of menstruating women and men of the same age group (D'Ambrosio et al., 2005). Interestingly, these differences are not significant in post-menopausal women when compared to men of the same age (Becklake & Kauffmann, 1999). These results indicate that menstrual hormones may impact ventilatory chemoreflexes.

There are three main phases of menstruation, each characterized by varying levels of menstrual hormones. In this study, we chose to focus on menstrual hormones estradiol 2 (also known as E2), the main form of estrogen found in menstruation women, and progesterone. During the menstrual cycle, plasma estradiol 2 levels peak during ovulation, then slightly decrease in the luteal phase, before reaching their lowest levels during the follicular phase (Anckaert et al., 2021). Plasma progesterone levels, however, greatly increase during the luteal phase before returning to relatively low levels for the follicular phase and ovulation (Anckaert et al., 2021). Existing data are conflicting on the influence of estrogen and progesterone on ventilatory chemoreflexes. Some studies have linked the increase in progesterone with the increase in ventilatory chemoreflexes during pregnancy (García-Rio et al., 1996). Other studies have suggested that progesterone and estrogen play a neuroprotective role against sleep-disordered breathing in post-menopausal women (D'Ambrosio et al., 2005). When considering progesterone's clinical use as a respiratory stimulant, this conclusion makes sense (Hall et al., 2016). However, there are studies which have found little to no link between estrogen or progesterone and ventilatory chemoreflexes (Citherlet et al., 2024).

Based on these data, we hypothesize that progesterone and estradiol 2 would both be associated with an increase in ventilatory chemoreflexes. To test our hypothesis, we used the Duffin rebreathing method to determine the ventilatory chemoreflex parameters of menstruating, nonpregnant women and directly measured their plasma estradiol 2 and progesterone levels.

METHODS

Ethical Approval

This study was approved by the UC Riverside Institutional Review Board (HS-20-128) and performed in accordance with the Declaration of Helsinki, except for registration in a database. Consent procedures were performed in the participant's native language with study personnel fluent in the language (English or Spanish). All participants received a copy of the consent form prior to their first appointment and were informed of the purpose of the study and expected risks and benefits of participating in the study. After all information was provided, both verbal and written consent were required to move forward with the study.

Participant Demographics and Inclusion Criteria

Between April 2022 and May 2023, 118 participants were recruited for a larger study examining the effects of COVID-19 on immune and respiratory health (Bergersen et al., 2023). Recruitment was conducted via word of mouth, social media, and flyers around the University of California, Riverside campus and the greater Riverside, California area. From the initial pool of 118 participants, 40 participants who self-identified as female were selected for this study (Table 1). When selecting participants for this study, female participants with high quality HVR data were prioritized, and those who ended their tests early or had irregular breathing patterns were excluded.

Participants self-reported their biological sex at birth, gender identity, age, and ethnicity. Inclusion criteria for participants included age ≥ 18 years and female sex and

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Variable	Follicular Phase	Ovulatory Phase	Luteal Phase	Total
Participants	12	24	4	40
Age	33 ± 14	27 ± 4	25 ± 8	28 ± 11
BMI	28 ± 5.7	21 ± 1.7	25 ± 5.5	25 ± 6

Table 1: Participant demographics grouped by menstrual phase.

Notes: Menstrual phase was approximated based on plasma progesterone using the hormone ranges measured in Anckeart et al., 2021. The plasma progesterone used for each phase was as follows: follicular at 0.159-0.616 nmol/L, ovulatory at 0.175-13.2 nmol/L, and luteal at 13.1-46.3 nmol/L (Anckaert et al., 2021). The distribution of participants in each phase, especially with so many participants in the ovulatory phase and so few in the luteal phase, is statistically unlikely. We hypothesis that this distribution could be due to a degradation of the progesterone when the plasma samples were sitting at room temperature, due to progesterone's short half-life (Kolatorova et al., 2022).

gender. Exclusion criteria included pregnancy, due to links between hypoxia exposure and development of preeclampsia (Tong & Giussani, 2019). Pregnancy was also excluded because this study focuses on menstrual hormone levels in currently menstruating women. Participants with current severe cardiac or pulmonary illness were excluded or participated in limited testing that was within safe limits for these participants. Finally, participants with confirmed or suspected active COVID-19 infection were also excluded.

For at least 12 hours prior to testing, participants were instructed to abstain from caffeine, anti-inflammatory medications, corticosteroids, and other medications that could interfere with control of breathing measures or inflammatory marker expression (Peña-Ortega, 2019). If participants were not able to stop taking the referenced medications, their data was excluded. Participants were not required to fast prior to their study appointment because, in a related study, the participants' blood samples were compared to ICU patient samples, for which it was not possible to require fasting (Bergersen et al., 2023).

Study Design

Prior to their study appointment, participants completed a screening questionnaire to confirm that they did not have a current COVID-19 infection or other illnesses. Upon arriving at their appointment, participants completed questionnaires reporting their past medical history, demographics, and long-COVID symptoms. Basic physiological measures were then collected. Blood pressure was collected with a stethoscope and manual sphygmomanometer, body temperature was collected with an infrared thermometer to the forehead, and height and weight were measured and recorded. Peripheral venous blood samples were then collected via standard venipuncture procedures by a licensed phlebotomist. Following blood sampling, a spirometry test was performed to measure baseline lung function. Finally, the participants' ventilatory chemoreflex measures were measured as described below. Each completed appointment took approximately two hours to complete.

Blood Sample Processing and Progesterone and Estradiol ELISAs

20 mL of blood was collected in two vacutainer tubes containing EDTA. Samples were kept at room temperature and processed within 1 hour. Tubes were centrifuged at 2500 x g for ten minutes. After separation, plasma used for inflammatory cytokine assays was stored at -20°C for short term storage and at -80°C for long term storage.

Prior to their use in this study, plasma samples underwent one freeze-thaw cycle. Plasma progesterone and estradiol were measured via ELISA (ab108670, abcam, Cambridge,

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UK; KAQ0622, invitrogen, ThermoFisher Scientific) following manufacturer protocols, and samples were tested in duplicates. Absorbance was quantified via spectrophotometer. Data was transferred to an Excel spreadsheet and the known standard curve was graphed. Using a line of best fit, the concentration of plasma estradiol 2 and progesterone were calculated from the absorbance measured.

Ventilatory Chemoreflex Testing and Data Analysis

Ventilatory chemoreflex measures were tested using the Duffin modified rebreathing method, as previously described in Frost et al (Duffin, 2007; Frost et al., 2024). Participants were seated in a semi recumbent position with legs uncrossed. They were fitted with a 3-lead electrocardiogram (ECG) placed under the right clavicle, under the left clavicle, and on the lower edge of the left ribcage. Participants were then fitted with a finger pulse oximeter and a vinyl mouthpiece (Vacumentrics, Ventura, CA, USA) attached to a rebreathing system. Downstream of the silicone mouthpiece was a disposable respiratory filter (MLA304, AD Instruments, Colorado Springs, CO, USA) and a flow meter (ML 1000, AD Instruments). After being fitted with a two-way valve allowing redirection of airflow from room air to a rebreathing bag, nose clips were utilized to ensure air was not being inhaled or exhaled through nasal pathways. The concentrations of O_2 and CO_2 were subsampled near the mouth at a rate of 200 mL/minute by an electromagnetic O_2 analyzer (VacuMed, model #17625) and an infrared CO_2 analyzer (VacuMed, model #17630, Ventura, CA). At the base of the rebreathing bag, a line for an O_2 concentrator (DeVilbiss) was attached to allow manual addition of O_2 throughout each test to maintain constant hyperoxic (elevated oxygen) or hypoxic (low oxygen) conditions.

Participants were first instructed to relax and breathe room air normally for five minutes. During this time and for the rest of the testing period, participants were instructed not to talk, move, or look at cell phones or other devices. After normally breathing room air, participants were instructed to voluntarily hyperventilate without panting by inhaling and exhaling slowly and deeply for approximately two minutes, or until their end-tidal P_{CO2} (ETP_{CO2}) reached 22 mmHg. The purpose of this phase was to reduce ETP_{CO2} below the ventilatory recruitment threshold (VRT) to ensure that the VRT is detected during the test. Additionally, this phase ensures that when the participant begins breathing from the rebreathing bag, their alveolar gas equilibrates with gas pressure in the bag while minimizing arteriovenous P_{CO2} differences to avoid cerebral blood flow changes. Equilibrium was detected by a plateau in ETP_{CO2} shortly after onset of rebreathing. Switching from breathing room air to breathing from the 6-L rebreathing bag, participants were instructed to take two large breaths, then relax. Participants remained on the bag for several minutes while ETP_{CO2} was allowed to slowly increase over time from their starting value to 60 mmHg. This process typically took 8-10 minutes, and the P_{O2} in the bag was maintained at a constant level by manual addition of O_2 from the oxygen concentrator. The test was terminated if the ETP_{CO2} reached 60 mmHg, the saturation of peripheral oxygen (SpO_2) approached 70%, the total ventilation reached 100 L/min, or if the participant voluntarily ended the test. This test was repeated twice. First, with a hyperoxic gas mixture maintaining an inspired oxygen concentration of 30%. This was followed by a rest period of 15 minutes, while breathing room air, to return to baseline. Second, the test was repeated with a hypoxic gas mixture to maintain ETP_{O2} levels at 50 mmHg (PIO_2 approximately 70 mmHg and allowed average desaturation to approximately 80-85%).

Data from all biomarker outputs was collected by a PowerLab data acquisition system (AD Instruments) and converted to a digital signal sent to a PC for collection in LabChart 8 software (AD Instruments). An integral flow channel was used to record inspiratory volumes, which were then converted to BTPS units. One minute of pre-test data at the end of the rest period was used to determine resting breathing parameters.

Data was pre-processed in LabChart 8 and analyzed in RStudio. The VRT in each test condition (hypoxia and hyperoxia) was determined using the Regression with Multiple Change Points (mcp) package in R (Lindeløv, 2020). This package uses a Bayesian inference to identify an ideal breakpoint in a two-slope line of best fit. The estimate of the mcp function plotter with raw ventilation data was used to visually validate the VRT estimate. The hypercapnic ventilatory reflex (HCVR) slope is seen as the slope of the second segment of the "hockey stick" shaped ventilatory

response curve. A VRT and HCVR value was calculated to each participant under two distinct oxygen tensions (hypoxia and hyperoxia), thus there were two VRT and HCVR values per participant.

The Duffin rebreathing technique allows the hypoxic ventilatory response (HVR) to be calculated at any P_{CO2} because it provides ventilation rates as a function of continuously increasing end-tidal PCO2 at two different PO2 levels. Thus, the HVR was calculated at four ETPCO2 levels: 45 mmHg, 50 mmHg, 55 mmHg, and 3 mmHg above the VRThyperoxia. To calculate the HVR at given ETPCO2 levels, three steps were performed. First, the SpO2 at the exact time when the target ETPCO2 was reached were recorded as SpO2hypoxia and SpO2hyperoxia. Second, the ventilation rate at each SpO2 level was determined using the equation for the linear HCVR response curve. These ventilatory rates were recorded as VEhypoxia and VEhyperoxia. Finally, the HVR at a given ETPCO2 was calculated as:

$$HVR = \frac{\dot{V}_{E_{hypoxia}} - \dot{V}_{E_{hyperoxia}}}{SpO_{2_{hyperoxia}} - SpO_{2_{hypoxia}}}$$

Table 2. Univariate Analysis of Hormones and Baseline Measures.

Baseline Measure	Shapiro-Wilks test	Progesterone (ng/mL)		Estradiol 2 (pg/mL)		Estradiol 2 to Progesterone ratio	
		r_s	p	r_s	p	r_s	p
V (L/min)	4.312e-5***	-0.15	0.17	0.075	0.51	0.21	0.063
V_T (L)	1.154e-5***	-0.24	0.035*	-0.075	0.51	0.27	0.018*
V_F (breaths/min)	7.83e-7***	0.11	0.31	0.023	0.84	-0.15	0.17
VRT	0.7569	-0.2	0.078	-0.069	0.56	0.14	0.24
HCVR	1.171e-5***	-0.063	0.58	0.052	0.65	0.13	0.25
HVR 45	1.118e-9***	0.29	0.12	0.079	0.68	-0.17	0.36
HVR 50	2.52e-11***	0.036	0.83	0.23	0.16	0.08	0.63
HVR 55	0.000187***	-0.43	0.03*	-0.11	0.59	0.47	0.017*
HVR vrt3	2.519e-7***	-0.12	0.51	0.19	0.3	0.21	0.25

Abbreviations: V = total ventilation, V_T = tidal volume, V_F = frequency, VRT = ventilatory recruitment threshold, HCVR = hypercapnic ventilatory response, HVR # = hypoxic ventilatory response where $ETPCO_2$ = # mmHg, HVR vrt3 = hypoxic ventilatory response where $ETPCO_2$ = 3 mmHg above $VRT_{hypoxia}$

*p<0.05, **p<0.01, ***p<0.001

Table 2: Univariate Analysis of Hormones and Baseline Measures.

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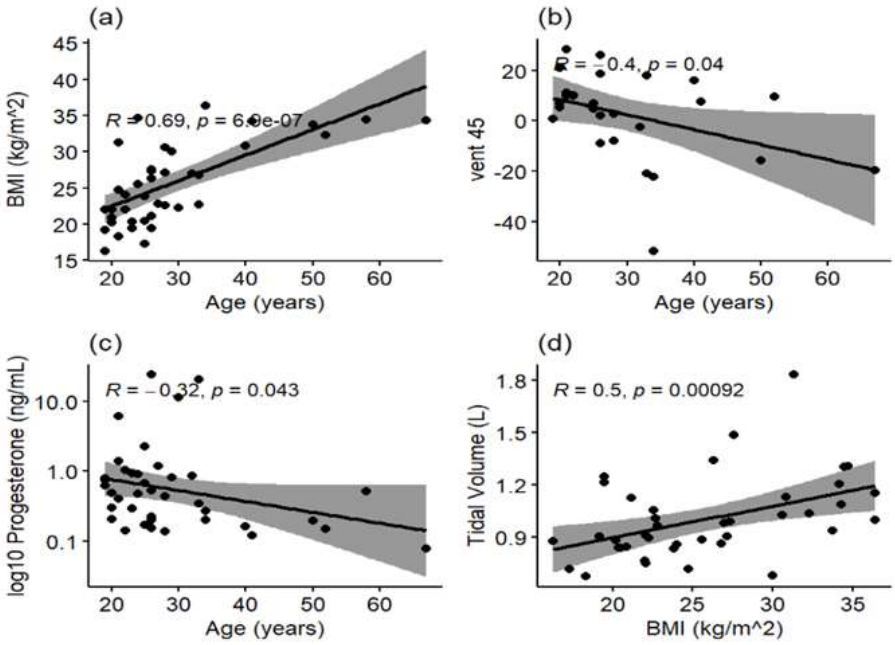

Figure 1: Univariate correlation scatter plots. BMI plotted as a function of age (a). Ventilation at ETPCO2 = 45 mmHg plotted as a function of age (b). Plasma progesterone plotted as a function of age (c). Tidal volume plotted as a function of BMI (d). Statistics represent Spearman Rho and p values for each univariate correlation.

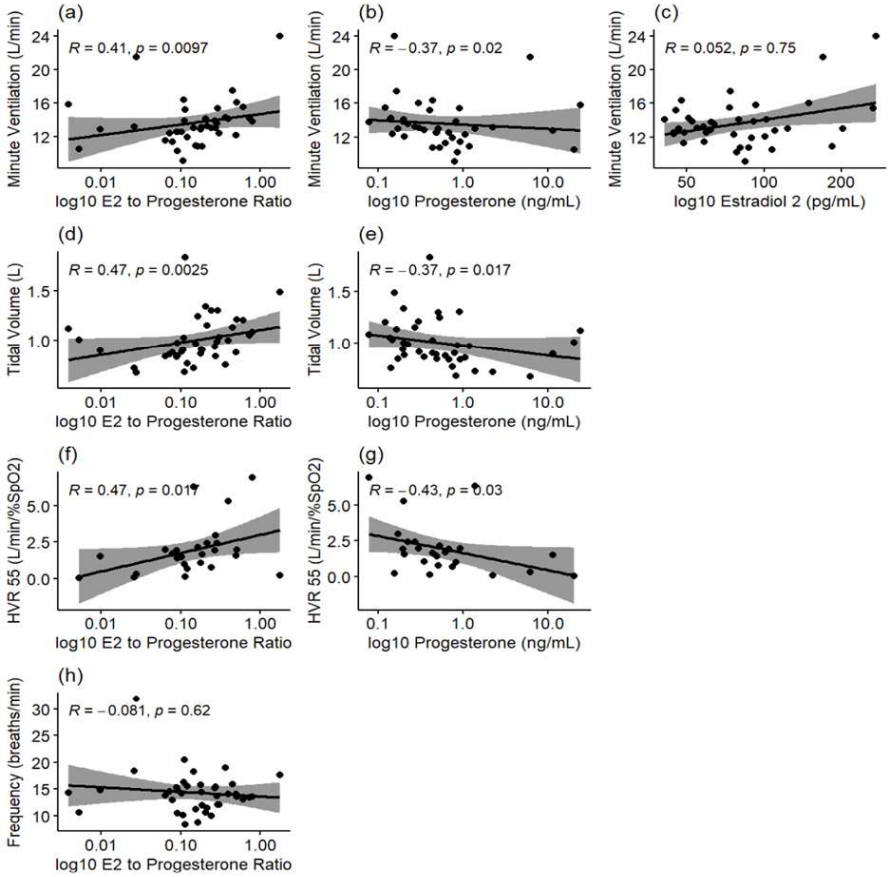

Figure 2: Univariate correlations of significance. Minute ventilation (a-c) plotted as a function of the E2 to progesterone ratio (a), progesterone (b), and E2 (c). Tidal volume (d-e) plotted as a function of the E2 to progesterone ratio (d) and progesterone (e). HVR at end-tidal PCO2 of 55 mmHg (f-g) plotted as a function of the E2 to progesterone ratio (f) and progesterone (g). Baseline breathing frequency plotted as a function of the E2 to progesterone ratio (h). Statistics represent Spearman Rho and p values for each univariate correlation.

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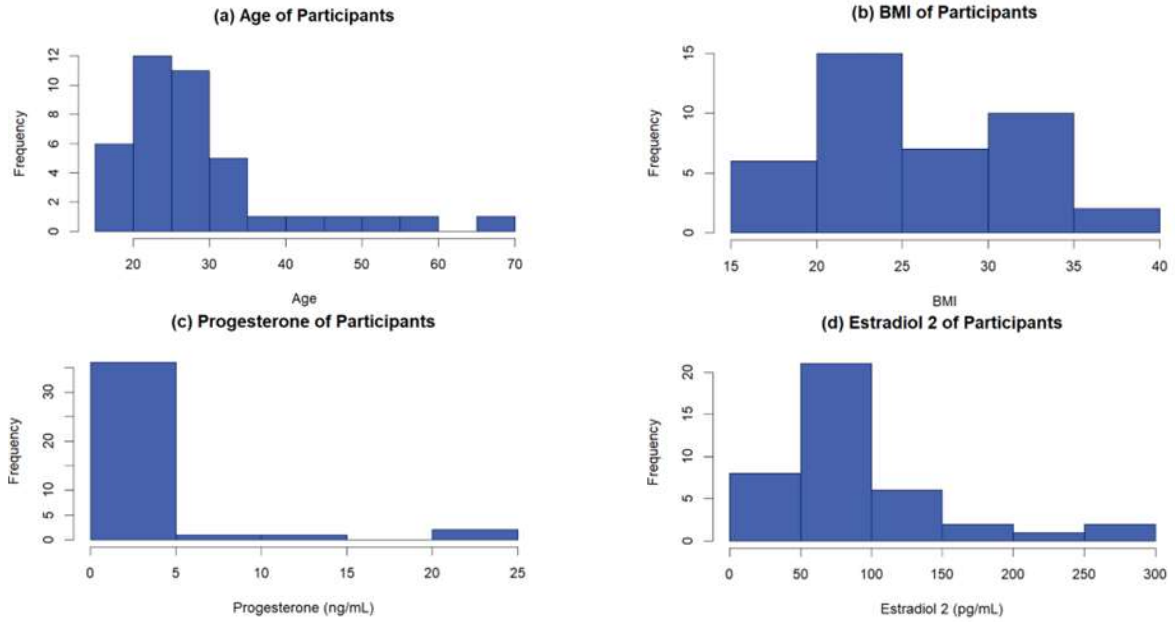

Figure 3: Distribution of participant demographics. The distribution of participants' age (a), BMI (b), plasma progesterone (c), and plasma estradiol 2 (d).

RESULTS

40 women were selected for this study. Their ages ranged from 19 to 67 years with an average of 29.175 years (**Figure 3a**). BMI ranged from 16.3 to 36.4 with an average BMI of 25.7 (**Figure 3b**). Of note, only four of the participants had a level of plasma progesterone above 4.1 ng/mL, which would be expected during the luteal phase, and 23 participants (more than half the total) had plasma progesterone which would be expected in the ovulatory phase (**Table 1; Figure 3c**). Furthermore, age and BMI were strongly positively correlated (rs = 0.69, p < 0.001) (**Figure 1a**).

To determine if progesterone influences variation in ventilatory measures, a univariate spearman correlation was performed with progesterone as the predictive variable and ventilatory measures as the outcome variables (**Table 2**). There was an unexpected negative association between progesterone and HVR at ETP_{CO2} = 55 mmHg (HVR 55) (r_s = -0.43, p = 0.03) (**Figure 2g**); however, this relationship appears to be impacted by the effect of age. When adjusted for age in a multivariate model, the effect of progesterone on HVR 55 was no longer significant (F(1, 23) = 2.08, p = 0.16), while age showed some significance (F(1, 23) = 3.6, p = 0.069). Similarly, a negative association between progesterone and the tidal volume (r_s = -0.24, p = 0.035) (**Figure 2e**) was found in the univariate model, but was not statistically significant when adjusting for age in a multivariate model (F(1, 77) = 0.49, p = 0.48). Interestingly, when adjusting for age and BMI in a multivariate model, the tidal volume was associated with BMI (F(1, 76) = 14, p = 0.00034) (**Figure 1d**).

To determine if estradiol 2 (E2) influences variation in ventilatory measures, a univariate spearman correlation was performed with E2 as the predictive variable and ventilatory measures as the outcome variables (**Table 2**). While none of the univariate comparisons with E2 showed significance, when adjusting for age in a multivariate model, E2 had a significant effect on resting minute ventilation (F(1, 77) = 16.9, p = 9.7e-5). When adjusting for both age and BMI in a separate multivariate model, the effect of E2 on minute ventilation remained significant (F(1, 76) = 15.06, p = 0.00022) (**Figure 2c**).

To determine if progesterone and E2 had interactive effects on ventilatory measures, the ratio between E2 and progesterone was calculated for each participant and

compared to ventilatory measures in a univariate model (**Table 2**). Similar to progesterone, there was a positive association between the E2 to progesterone ratio and tidal volume ($rs = 0.27$, $p = 0.018$) (**Figure 2d**), and the E2 to progesterone ratio and HVR 55 ($rs = 0.47$, $p = 0.017$) (**Figure 2f**). When adjusting for age in a multivariate model, the effect of the ratio on the tidal volume ($F(1, 77) = 0.74$, $p = 0.38$) and HVR 55 ($F(1, 23) = 0.11$, $p = 0.74$) were no longer significant. With this adjustment for age, however, the association of the E2 to progesterone ratio with total ventilation ($F(1, 77) = 15.9$, $p = 0.00014$) (**Figure 2a**) and frequency ($F(1, 77) = 5.92$, $p = 0.017$) (**Figure 2h**) were significant. When controlling for both age and BMI, the effects of the E2 to progesterone ratio on total ventilation ($F(1, 76) = 14.1$, $p = 0.00032$) and frequency ($F(1, 76) = 4.98$, $p = 0.028$) remained significant.

In the multivariate model adjusting for age in each of the hormone measures, age played a significant role in the ventilation at $ETP_{CO2} = 45$ mmHg (Vent 45) (**Figure 1b**). In the multivariate model with progesterone as the independent variable and age as the only covariate, the association of age with Vent 45 was $F(1, 51) = 8.70$, $p = 0.0047$. In the multivariate model with E2 as the independent variable and age as the only covariate, the association of age with Vent 45 was $F(1, 51) = 7.08$, $p = 0.010$. In the multivariate model with the E2 to progesterone ratio as the independent variable and age as the only covariate, the association of age with Vent 45 was $F(1, 51) = 11.7$, $p = 0.0012$. Interestingly, the effect of age on Vent 45 was no longer significant for the progesterone or E2 models when BMI was added as an additional covariate, while the effect of age stayed significant for the E2 to progesterone ratio model ($F(1, 50) = 4.11$, $p = 0.048$).

DISCUSSION

Current data regarding the effect of progesterone and E2 on breathing are conflicting (Brodeur et al., 1986; Marques et al., 2015). Our data adds rigor to these existing datasets because: we have quantified plasma hormone concentrations rather than using self-reported menstrual cycle data; we used a larger sample size than most similar studies; and we used more sophisticated measures of ventilatory reflexes, which include the ventilatory recruitment threshold. A limitation of our dataset is the lack of variation in serum progesterone across participants. In a normal menstrual cycle, women reach their highest progesterone concentration during the luteal phase (over 4.1 ng/mL), which is approximately one-half to one-third of their cycle length (Anckaert et al., 2021). Thus, we would expect roughly one-half to one-third of our participants to have high progesterone. However, only four of forty participants were determined to be in the luteal phase based on their progesterone (**Table 1**). We hypothesize that this was due to a degradation of progesterone when the plasma samples were left at room temperature for up to an hour during processing (Kolatorova et al., 2022). This would have led to some of the participants who were in the luteal phase appearing to be in the ovulatory phase, during which progesterone is at a lower concentration, and which we did observe (**Table 1**). Furthermore, all four participants in the luteal phase were younger than or closer to the average age of all participants (29 years) (**Figure 1c**). This limitation would explain why any significant effects of progesterone observed in the univariate model were no longer significant when adjusting for age.

Our data supports the hypothesis that menstrual hormones progesterone and E2 may not significantly influence ventilatory chemoreflex responses to hypoxia or hypercapnia among nonpregnant women in this age group (Citherlet et al., 2024; Marques et al., 2015). This is counterintuitive because the increased sensitivity to progesterone during pregnancy is thought to cause the increased chemosensitivity to carbon dioxide in the bloodstream. The mechanism by which this occurs is still under investigation (García-Rio et al., 1996). Additional evidence in women and rat pups suggests that estradiol amplifies progesterone's effects on ventilation by increasing the number of progesterone receptors, especially on the carotid bodies (Joseph et al., 2012; Regensteiner et al., 1989). Yet, we found no correlation between plasma progesterone and ventilatory drive. One theory for why we failed to find this connection is that physiologically normal fluctuations in progesterone concentrations during the menstrual cycle may not be enough to cause a significant effect on ventilatory drive. This could explain why there is significant evidence for progesterone as a ventilatory stimulant during pregnancy, when it increases to a much higher concentration than is physiologically normal during the menstrual cycle

(García-Rio et al., 1996). Further research on ventilatory chemoreflexes in pregnant women could help clarify the role of progesterone on ventilation.

In contrast to our findings regarding ventilatory chemoreflex sensitivity, we observed a positive association between E2 and resting minute ventilation. This result supports research which identifies E2's role in increasing resting ventilation and HVR in pregnant women (Regensteiner et al., 1989). However, much of the research concludes that E2 increases lung function by amplifying the effects of progesterone, rather than by having a direct effect (Regensteiner et al., 1989). While our results indicate an effect of the E2 to progesterone ratio on breathing frequency, which could be what drives the variation in total ventilation, we found no significant effect of progesterone on either frequency or total ventilation. Thus, our findings suggest there is an alternative mechanism by which E2 influences total ventilation.

Finally, we found an unexpected positive effect of BMI on tidal volume (**Figure 1d**). This is contrary to current literature, which is conflicting, but typically shows no correlation between BMI and tidal volume (Sadiqa & Munawar, 2019) or a negative correlation (Littleton, 2012). However, in one study on children with asthma, higher BMI was associated with higher tidal volume, which aligns with our findings (Afshar-Mohajer et al., 2022). The study suggests that the decreased tidal volume associated with increased BMI in adults could be due to restriction of pulmonary function because of multiple factors, including age, BMI, lifestyle, and smoke inhalation. However, this hypothesis does not account for why we observed a positive effect of BMI on tidal volume in our study on adults, especially when BMI was strongly positively correlated with age in our sample (**Figure 1a**).

In conclusion, our finding that neither progesterone nor E2 impact HVR is unexpected. However, the observed association between E2 and total ventilation indicates that E2 may have more of a role on baseline ventilatory function than on the hypoxic or hypercapnic ventilatory chemoreflexes. Furthermore, our findings support the hypothesis that progesterone concentrations within the normal physiological range during menstruation do not have a significant effect on ventilation. Focusing the scope of research towards pregnant women, who have much higher concentrations of both progesterone and E2, could determine if our findings are due to a dosage effect.

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REFERENCES

Afshar-Mohajer, N., Wu, T. D., Shade, R., Brigham, E., Woo, H., Wood, M., Koehl, R., Koehler, K., Kirkness, J., Hansel, N. N., Ramchandran, G., & McCormack, M. C. (2022). Obesity, tidal volume, and pulmonary deposition of fine particulate matter in children with asthma. *The European Respiratory Journal*, 59(3), 2100209. https://doi.org/10.1183/13993003.00209-2021

Anckaert, E., Jank, A., Petzold, J., Rohsmann, F., Paris, R., Renggli, M., Schönfeld, K., Schiettecatte, J., & Kriner, M. (2021). Extensive monitoring of the natural menstrual cycle using the serum biomarkers estradiol, luteinizing hormone and progesterone. *Practical Laboratory Medicine*, 25, e00211. https://doi.org/10.1016/j.plabm.2021.e00211

Becklake, M., & Kauffmann, F. (1999). Gender differences in airway behaviour over the human life span. *Thorax*, 54(12), 1119–1138.

Bergersen, K. V., Pham, K., Li, J., Ulrich, M. T., Merrill, P., He, Y., Alaama, S., Qiu, X., Harahap-Carrillo, I. S., Ichii, K., Frost, S., Kaul, M., Godzik, A., Heinrich, E. C., & Nair, M. G. (2023). Health disparities in COVID-19: Immune and vascular changes are linked to disease severity and persist in a high-risk population in Riverside County, California. *BMC Public Health*, 23(1), 1584. https://doi.org/10.1186/s12889-023-16462-5

Brodeur, P., Mockus, M., McCullough, R., & Moore, L. G. (1986). Progesterone receptors and ventilatory stimulation by progestin. *Journal of Applied Physiology*, 60(2), 590–595. https://doi.org/10.1152/jappl.1986.60.2.590

Citherlet, T., Raberin, A., Manferdelli, G., Pialoux, V., & Millet, G. P. (2024a). Menstrual cycle does not impact the hypoxic ventilatory response and acute mountain sickness prediction. *Scientific Reports*, 14(1), 26087. https://doi.org/10.1038/s41598-024-76404-y

Citherlet, T., Raberin, A., Manferdelli, G., Pialoux, V., & Millet, G. P. (2024b). Menstrual cycle does not impact the hypoxic ventilatory response and acute mountain sickness prediction. *Scientific Reports*, 14(1), 26087. https://doi.org/10.1038/s41598-024-76404-y

D'Ambrosio, C., Stachenfeld, N. S., Pisani, M., & Mohsenin, V. (2005). Sleep, breathing, and menopause: the effect of fluctuating estrogen and progesterone on sleep and breathing in women. *Gender Medicine*, 2(4), 238–245. https://doi.org/10.1016/S1550-8579(05)80053-1

Duffin, J. (2007). Measuring the ventilatory response to hypoxia. *Journal of Physiology,* 584(1), 286–293. https://doi.org/10.1113/jphysiol.2007.138883

Frost, S., Pham, K., Puvvula, N., Oeung, B., & Heinrich, E. C. (2024). Changes in hypoxic and hypercapnic ventilatory responses at high altitude measured using rebreathing methods. *Journal of Applied Physiology*, 137(2), 364–373. https://doi.org/10.1152/japplphysiol.00128.2024

García-Rio, F., Pino, J. M., Gómez, L., Alvarez-Sala, R., Villasante, C., & Villamor, J. (1996). Regulation of breathing and perception of dyspnea in healthy pregnant women. *Chest, 110(2)*, 446–453. https://doi.org/10.1378/chest.110.2.446

Hall, O. J., Limjunyawong, N., Vermillion, M. S., Robinson, D. P., Wohlgemuth, N., Pekosz, A., Mitzner, W., & Klein, S. L. (2016). Progesterone-based therapy protects against influenza by promoting lung repair and recovery in females. *PLOS Pathogens*, 12(9), e1005840. https://doi.org/10.1371/journal.ppat.1005840

Joseph, V., Niane, L. M., & Bairam, A. (2012). Antagonism of progesterone receptor suppresses carotid body responses to hypoxia and nicotine in rat pups. *Neuroscience*, 207, 103–109. https://doi.org/10.1016/j.neuroscience.2012.01.041

Kolatorova, L., Vitku, J., Suchopar, J., Hill, M., & Parizek, A. (2022). Progesterone: A steroid with wide range of effects in physiology as well as human medicine. *International Journal of Molecular Sciences*, 23(14), 7989. https://doi.org/10.3390/ijms23147989

Littleton, S. W. (2012). Impact of obesity on respiratory function. *Respirology*, 17(1), 43–49. https://doi.org/10.1111/j.1440-1843.2011.02096.x

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REFERENCES CONT.)

Marques, D. A., de Carvalho, D., da Silva, G. S. F., Szawka, R. E., Anselmo-Franci, J. A., Bícego, K. C., & Gargaglioni, L. H. (2015). Ventilatory, metabolic, and thermal responses to hypercapnia in female rats: Effects of estrous cycle, ovariectomy, and hormonal replacement. *Journal of Applied Physiology*, 119(1), 61–68. https://doi.org/10.1152/japplphysiol.00254.2015

Pamenter, M. E., & Powell, F. L. (2016). Time domains of the hypoxic ventilatory response and their molecular basis. *Comprehensive Physiology*, 6(3), 1345–1385. https://doi.org/10.1002/cphy.c150026

Peña-Ortega, F. (2019). Clinical and experimental aspects of breathing modulation by inflammation. *Autonomic Neuroscience: Basic and Clinical*, 216, 72–86. https://doi.org/10.1016/j.autneu.2018.11.002

Pham, K., Parikh, K., & Heinrich, E. C. (2021). Hypoxia and inflammation: Insights from high-altitude physiology. *Frontiers in Physiology*, 12, 676782. https://doi.org/10.3389/fphys.2021.676782

Regensteiner, J. G., Woodard, W. D., Hagerman, D. D., Weil, J. V., Pickett, C. K., Bender, P. R., & Moore, L. G. (1989). Combined effects of female hormones and metabolic rate on ventilatory drives in women. *Journal of Applied Physiology*, 66(2), 808–813. https://doi.org/10.1152/jappl.1989.66.2.808

Sadiqa, A., & Munawar, F. (2019). Correlation of BMI variation with tidal function in healthy young adults. *Journal of Islamic International Medical College*, 14(3), 136–141.

Tong, W., & Giussani, D. A. (2019). Preeclampsia link to gestational hypoxia. *Journal of Developmental Origins of Health and Disease*, 10(3), 322–333. https://doi.org/10.1017/S204017441900014X