Effect of menopause on the chemical control of breathing and its relationship with
acid-base status

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Running Title: Menopause and ventilatory control
ABSTRACT

This study examined the role of alterations in the chemoreflex control of breathing, acid-base balance and their interaction in postmenopausal ventilatory adaptations. A modified iso-oxic hyperoxic- and hypoxic-CO$_2$ rebreathing procedure was employed to evaluate central and peripheral chemoreflex drives to breathe, respectively, in 15 healthy postmenopausal (POST) and 20 premenopausal (PRE) women of similar age. Arterialized venous blood samples were collected at rest for the estimation of arterial PCO$_2$ (PaCO$_2$) and [H$^+$]; plasma strong ion difference ([SID]) and total weak acid ([A$_{TOT}$]) concentrations; and serum progesterone ([P$_4$]) and 17$\beta$-estradiol ([E$_2$]) concentrations. In POST vs. PRE: PaCO$_2$, [SID] and the central chemoreflex ventilatory recruitment threshold for PCO$_2$ (VRTCO$_2$) were higher, while [P$_4$] and [E$_2$] were lower (all p<0.05) with no significant change in central or peripheral chemoreflex sensitivity, peripheral chemoreflex VRTCO$_2$ and [A$_{TOT}$]. The acidifying effect of an increased PaCO$_2$ was offset by the alkalizing effect of an increased [SID], such that [H$^+$] was preserved in POST vs. PRE. PaCO$_2$ correlated positively with the central chemoreflex VRTCO$_2$ ($r$=0.67, p<0.01), which in turn correlated positively with [SID] ($r$=0.53, p<0.01) within the pooled data. In conclusion, the relative alveolar hypoventilation and attendant arterial hypercapnia in healthy post- compared with premenopausal women could be explained, in part, by the interaction of (1) reduced central, but not peripheral, chemoreflex VRTCO$_2$, (2) increased [SID] and (3) reduced circulating female sex steroid hormone concentrations.

Key Words: Chemoreflex, strong ion difference, ageing, progesterone
Introduction

The increasing menopausal demographic in North America raises several important questions regarding the underlying physiological status of this population of women, including the impact of menopause on ventilatory control, acid-base balance and their complex interaction. Increases in circulating concentrations of progesterone ([P₄]) and estrogen ([E₂]), such as occurs in human pregnancy and across the menstrual cycle, are characterized by consistent increases in resting ventilation (\(\dot{V}_E\)) with attendant reductions in arterial PCO₂ (PaCO₂) (25; 28; 40; 48; 51; 54). Hormone replacement therapy studies reported that medroxyprogesterone acetate (a synthetic progestin) administered alone, and in combination with estrogen, consistently reduced resting PaCO₂ by ~6 mmHg in postmenopausal women (39; 43-46). Withdrawal of these hormones would therefore be expected to decrease resting \(\dot{V}_E\), thereby increasing PaCO₂ in otherwise healthy postmenopausal women. Although the underlying mechanisms of these ventilatory adaptations remain speculative, the combined facilitatory effects of progesterone and estrogen on central and/or peripheral chemoreflex drives to breathe may be involved (3; 4; 11; 26; 28; 29; 31; 48).

Classical concepts of hydrogen ion (H⁺) homeostasis and their link to the chemoreflex control of breathing have evolved from strict Henderson-Hasselbach interpretations to embrace the physicochemical approach described by Dr. Peter A. Stewart (52; 53). Stewart’s approach identifies PaCO₂, total weak acid ([A_TOT]) and the strong ion difference ([SID], i.e., the concentration difference of strongly dissociated positive and negative ions in solution) as the three independent variables involved in the regulation of [H⁺] in
biological fluids (23; 24; 33). In keeping with the experimental results of Jennings (23; 24) and Oren et al. (37; 38), Duffin (12) recently demonstrated that the alkalizing effects of an increased plasma and cerebrospinal fluid (CSF) [SID] alters the relationship between the measured (PCO₂) and presumptive ([H⁺]) stimulus to the peripheral and central chemoreceptors, such that arterial and CSF [H⁺] are reduced at any given PaCO₂ and CSF PCO₂, respectively (refer to Figures 7 and 14 of Stewart, (52)). Consequently, the chemoreflex ventilatory recruitment threshold for PCO₂ (VRTCO₂) is increased, which in turn decreases resting \( \dot{V}_E \) with attendant increases in PaCO₂. Although evidence suggests that plasma [SID] may be higher in post- compared with premenopausal women (18-21), this is the first study to examine the role of acid-base balance in the chemoreflex control of breathing in healthy postmenopausal women.

In the present study, we postulated that reductions in circulating female sex hormone concentrations, alone or in combination with the alkalizing effect of an increased plasma [SID], contribute significantly to the relative hypoventilation and attendant arterial hypercapnia observed following the onset of menopause. We hypothesized that these outcomes would be mechanistically linked to reductions in central and peripheral chemoreflex drives to breathe. Our primary objective, therefore, was to determine if resting PaCO₂ was increased in healthy post- compared to premenopausal women of similar age, and to identify whether or not this difference was associated with reductions in \([P_4]\), \([E_2]\), and central and/or peripheral chemoreflex drives to breathe. Our second objective was to determine if increases in plasma [SID] contribute to a higher resting PaCO₂ in postmenopausal women by increasing the chemoreflex VRTCO₂.
METHODS

Subjects
Subjects included healthy, non-smoking, physically active pre- (PRE, n=20) and postmenopausal (POST, n=15) women between 42 and 54 years of age. Subjects were excluded if they were perimenopausal (i.e. sporadic and irregular menstrual cycles); taking any form of medication, including oral contraceptives and hormone replacement therapy, within six months of participation; had a history of cardiorespiratory, metabolic, haematologic and/or eating disorder(s); were born at and/or had recently returned from a trip at high altitude; or if menopause was surgically-induced. Detailed overnight polysomnography was not performed to rule out the presence of sleep-disordered breathing. Nevertheless, no individual in either group presented with daytime somnolence or a clinical diagnosis of sleep-disordered breathing; and available evidence suggests that obstructive sleep apnea has no independent effect on the central and peripheral chemoreflex response to hypercapnia and hypoxia, respectively (8; 50). Prior to participation, subjects completed the revised Physical Activity Readiness Questionnaire (www.csep.ca/forms.asp) and obtained medical clearance from their primary healthcare provider. PRE women were eumenorrheic and reported no menstrual cycle irregularities or disturbances prior to and throughout study participation. POST status was defined as the absence of menses for ≥1 year prior to study participation (56).

Study Design
This was a controlled, cross-sectional study in which written informed consent was obtained from all subjects. The study protocol and consent form were approved by the
Queen’s University and Affiliated Teaching Hospitals Health Sciences Human Research Ethics Board in accordance with the Declaration of Helsinki. Subjects were tested on two occasions, separated by ≥3 days; and abstained from caffeine, alcohol and strenuous exercise on each test day. No restrictions were placed on menstrual cycle phase for the first laboratory visit. During the second laboratory visit, however, PRE women were tested in the follicular phase of their menstrual cycle (confirmed by serum [P₄] and [E₂] measurements), which was determined using the first day of their last menstrual cycle and the average length of at least three previous menstrual cycles (9).

During the first laboratory visit, basic physical characteristics, including body height and mass, blood pressure and routine spirometry, were recorded; and each subject performed a modified iso-oxic hyperoxic CO₂ rebreathing procedure (see below) for familiarization purposes. During the second laboratory visit, subjects completed both a modified iso-oxic hyperoxic- and hypoxic-CO₂ rebreathing procedure (see below). These tests were separated by ≥45 min, and the order was randomized between subjects. Following a rest period of ≥90 min, breath-by-breath measurements of \( \dot{V}_E \), tidal volume (\( V_T \)), breathing frequency (\( f_R \)), estimated alveolar ventilation (\( \dot{V}_{A,est} \)), oxygen consumption (\( \dot{V}O₂ \)) and carbon dioxide production (\( \dot{V}CO₂ \)) were collected during 10 min of quiet resting breathing in accordance with previously published methods (17). During the 6\(^{th}\) min of this rest period, arterialized venous blood samples for the estimation of arterial blood gas and acid-base status; serum [P₄] and [E₂]; and plasma osmolality concentrations were collected and analyzed in accordance with previously published methods from our laboratory (25; 34; 51).
**Modified Rebreathing Procedure**

A modified version of Read’s (1967) rebreathing procedure that included 5-min of prior hyperventilation and maintenance of a constant (iso-oxic) hyperoxic and hypoxic end-tidal PO2 ($P_{ETO2}$) was employed to evaluate the effect(s) of menopause on central and peripheral chemoreflex drives to breathe, respectively (13; 27). A critique of the rebreathing procedure and its model assumptions have been described previously in detail (13; 27; 30).

Before rebreathing, subjects voluntarily hyperventilated room air for 5 min and were coached to maintain an end-tidal PCO2 ($P_{ETCO2}$) between 19 and 23 mmHg using a deep and deliberate breathing pattern to avoid induction of short-term potentiation (STP) (15). Prior hyperventilation permits (1) measurement of sub-VRTCO2 ventilation, estimating non-chemoreflex (‘wakefulness’) drives to breathe (14; 49) and (2) measurement, rather than extrapolation, of the chemoreflex VRTCO2.

Following hyperventilation, subjects expired to residual volume and were then switched from breathing room air to a rebreathing bag containing either a hyperoxic-hypercapnic (24% O2, 6% CO2, N2 balanced) or hypoxic-hypercapnic (4.5% O2, 6% CO2, N2 balanced) gas mixture. Rebreathing began with 3-5 deep breaths causing rapid equilibration of the PCO2 in the rebreathing bag, lungs, and arterial blood with that of the mixed-venous blood, thereby minimizing the central (or brain tissue)-to-arterial PCO2 difference. This equilibration ensures that (1) changes in $P_{ETCO2}$ accurately reflect changes in arterial and central PCO2/$[H^+]$ and (2) hypercapnia- and hypoxia-induced increases in cerebral blood flow have little or no effect on central PCO2/$[H^+]$ and therefore determination of the slope (sensitivity) of the ventilatory response to CO2. Equilibration was verified by
the observance of a plateau in $P_{ETCO_2}$ and was a prerequisite for continuing the test. Following equilibration, subjects were instructed to relax and breathe as they felt the need.

During rebreathing, $P_{ETCO_2}$ increased progressively from hypo- to hypercapnia while iso-oxia was maintained at a hyperoxic (150 mmHg) or hypoxic (50 mmHg) $P_{ETO_2}$ by providing a computer-controlled flow of 100% O$_2$ to the rebreathing bag. Arterial O$_2$ saturation and heart rate were monitored continuously with an ear oximeter (OXI; Radiometer Copenhagen, Copenhagen, Denmark). Rebreathing was terminated when $V_E$ exceeded 100 l min$^{-1}$, $P_{ETCO_2}$ exceeded 60 mmHg and/or subject discomfort.

During rebreathing, subjects were comfortably seated, wore nose clips and breathed through a mouthpiece connected to a 3-way T-shaped wide-bore manual directional valve (Model 2100a; Hans Rudolph, Inc., Kansas City, MO) that permitted switching from room air to the rebreathing bag. Subjects rebreathed from a 10 L plastic bag connected to a low resistance bi-directional volume turbine (VMM-2A; Alpha Technologies, Laguna Niguel, CA). End-tidal P$_{CO_2}$ and P$_{O_2}$ were measured at the mouth using a respiratory mass spectrometer (Perkin Elmer MGA 1100) at a sampling flow rate of 64 ml min$^{-1}$. The rebreathing system was calibrated with precision analyzed gases of known concentrations and a 3 L volume syringe prior to each test. A 12-bit analog-to-digital converter (DAQCard-6062E; National Instruments, Austin, TX) digitized the analog output signals from all monitoring devices for computer analysis using custom written software (Labview 6.1, National Instruments, Austin, TX). The data acquisition software calculated $V_E$, $V_T$, $f_R$, $P_{ETCO_2}$ and $P_{ETO_2}$ on a breath-by-breath basis.
Data from rebreathing experiments were imported into an analysis program designed specifically for this purpose (Labview 6.1, National Instruments, Austin, TX). Measured volumes were corrected to body temperature and pressure, saturated with water vapour. Data from the first equilibration at the start of rebreathing and any aberrant points (e.g. sighs, swallows) were excluded from further analysis. Breath-by-breath \( P_{ETCO_2} \) was then plotted against time and fitted with a least-squares regression line whose slope depends on \( \dot{V}CO_2 \). The equation for this line provided a predicted value of \( P_{ETCO_2} \) vs. time, thereby minimizing inter-breath variability. Thereafter, \( \dot{V}_E \) was plotted against the predicted \( P_{ETCO_2} \) and fitted with a model made up of the sum of two segments separated by a single breakpoint (13). All segments were fitted through an iterative process whereby the breakpoint and other parameters were varied to obtain an optimal fit to the observed data by minimizing the sum of squares (Levenberg-Marquardt algorithm). The ventilatory response to hyperoxic- and hypoxic-hypocapnia below the VRTCO2, respectively, was modeled as an exponential decay to a final value (13). This sub-VRTCO2 ventilation value (\( \dot{V}E_B \)) was taken as an estimate of non-chemoreflex drives to breathe (14; 49). The \( P_{ETCO_2} \) at which \( \dot{V}_E \) increased with progressive increases in \( P_{ETCO_2} \) was taken as the VRTCO2. Finally, the slope of the linear relation between \( \dot{V}_E \) and \( P_{ETCO_2} \) above the VRTCO2 was taken as an estimate of chemoreflex sensitivity (\( \dot{V}ES \)). We assumed that the VRTCO2 and \( \dot{V}ES \) measured under iso-oxic hyperoxic conditions originated from the central chemoreflex alone, as hyperoxia attenuates the peripheral chemoreflex response to CO2 (10), while the
same measurements recorded under iso-oxic hypoxic conditions represented the sum of both central and peripheral chemoreflex stimulation.

**Statistical Analysis**

A conventional power calculation formula for the comparison of two independent populations of unequal sizes was used to estimate the minimum sample size assuming 80% power and \( \alpha = 0.05 \). The outcome variables considered most important were PaCO\(_2\), [SID], hyperoxic and hypoxic VRTCO\(_2\) and \( \dot{V}E_S \), respectively. Standard deviations from Slatkovska et al. (51) were used to calculate sample sizes capable of detecting between-group differences of 2 mmHg and 2.5 mEq l\(^{-1}\) for PaCO\(_2\) and [SID], respectively. Standard deviations from Jensen et al. (27) were used to calculate sample sizes capable of detecting between-group differences of 2.0 l min\(^{-1}\) mmHg\(^{-1}\) and 2.5 mmHg for hyperoxic and hypoxic \( \dot{V}E_S \) and VRTCO\(_2\), respectively. The resulting estimates were 11, 14, 15 and 14 subjects per group for PaCO\(_2\), [SID], hyperoxic/hypoxic \( \dot{V}E_S \) and VRTCO\(_2\), respectively.

Independent t-tests were used to identify between-group differences for the general physical characteristics, resting cardiorespiratory variables and blood biochemistry parameters. A general linear model for repeated measures was used to identify significant group (PRE vs. POST), condition (hyperoxia vs. hypoxia) and group*condition interactions for each of the rebreathing parameters. Pearson product-moment correlation coefficients were calculated using pooled data from both groups to identify significant associations between PaCO\(_2\) and each of \( \dot{V}E_B \), \( \dot{V}E_S \), VRTCO\(_2\) and [SID]. Serum [P\(_4\)] and [E\(_2\)] were not used in the correlative analysis due to the inherent between-group differences in their
circulating concentrations. A p<0.05 level of statistical significance was used for all analyses. Data are presented as means ± SD. Statistical analyses were performed using SPSS 14.0 software (SPSS Inc., Chicago, Illinois).

RESULTS

The mean age of the PRE and POST groups differed significantly by ~7 yr (Table 1). Group vs. age comparisons demonstrated that menopausal status served as a better predictor of the between-group differences in measured variables (i.e. age did not improve the predictability of any of the variables after accounting for menopausal status), which suggests that the between-group differences presented herein reflect the effect(s) of menopausal status rather than age.

Subject characteristics are presented in Table 1. PRE and POST groups were well matched for important physical characteristics other than age. POST women had not experienced a menstrual period for at least one year (1.8 ± 1.2 yr; range: 1-5 yrs) prior to testing. Menopause-related changes in resting cardiorespiratory parameters are shown in Table 2: \( \dot{V}_E \), \( \dot{V}_O_2 \) and \( \dot{V}CO_2 \) were not significantly different between-groups; however, \( \dot{V}_{A,\text{est}} \) and \( V_T \) were consistently lower, while \( f_R \) was consistently higher in POST compared with PRE.

Blood Biochemistry

Blood biochemistry measurements were not available for one of the PRE subjects. Resting PaCO₂, [SID], [HCO₃⁻] and plasma [osmolality] were consistently higher, while [E₂] and
[P₄] were significantly lower in POST compared with PRE (Table 2). Increases in PaCO₂ and [SID], in the setting of an unchanged [A_TOT], offset each other such that [H⁺] did not differ between-groups (Table 2).

**Hyperoxic and Hypoxic CO₂ Rebreathing Responses**

Despite prior familiarization, 3 PRE women and 1 POST woman were unwilling to complete both rebreathing trials due to the discomfort/anxiety associated with performing this test. Therefore, complete rebreathing data were available from 17 PRE and 14 POST women. PRE and POST groups remained well matched for important physical characteristics, but still differed with respect to age. Menopause-related changes in the ventilatory response to iso-oxic hyperoxic- and hypoxic-CO₂ rebreathing are presented in Figure 1 and Table 3. Sub-VRTCO₂ ventilation was not significantly different between-groups. Hyperoxic and hypoxic VRTCO₂ values were consistently higher (by 3.5 and 2.9 mmHg, respectively) in POST compared with PRE (Table 3). The magnitudes of these differences were quantitatively similar, indicating that the central, but not peripheral, chemoreflex VRTCO₂ increased from PRE to POST. Hyperoxic and hypoxic \( \dot{V}_{E_S} \) values tended to be reduced in POST compared with PRE; however, these differences did not reach statistical significance (Table 3). The magnitude of these differences, although not statistically significantly, were quantitatively similar in POST vs. PRE (1.1 vs. 1.0 l min⁻¹ mmHg⁻¹, respectively), suggesting that central, but not peripheral, chemoreflex sensitivity tended to be reduced in the former. \( \dot{V}_{E_B} \) and \( \dot{V}_{E_S} \) were higher, and the VRTCO₂ was lower under hypoxic vs. hyperoxic rebreathing conditions, independent of menopausal status.
**Correlations**

Significant relationships were observed between resting PaCO$_2$ and each of the following independent variables within the pooled data: hyperoxic ($r=-0.36$, $p<0.05$) and hypoxic ($r=-0.36$, $p<0.05$) $\dot{V}_{E_b}$; hyperoxic ($r=0.67$, $p<0.01$ (Figure 2)) and hypoxic ($r=0.54$, $p<0.01$) VRTCO$_2$. Plasma [SID] correlated positively with both the hyperoxic ($r=0.53$, $p<0.01$ (Figure 2)) and hypoxic ($r=0.46$, $p<0.01$) VRTCO$_2$ within the pooled data.

**DISCUSSION**

The results of our study provide several novel observations regarding the control of ventilation and its relationship with acid-base balance in healthy post- compared to premenopausal women of similar age. These include (1) postmenopausal women possessed a reduced central, but not peripheral, chemoreflex drive to breathe, as evidenced by an increased VRTCO$_2$ and modestly reduced $\dot{V}_{E_S}$ response to iso-oxic hyperoxic-hypercapnia; (2) the reduced central chemoreflex drive to breathe was associated with decreased circulating female sex steroid hormone concentrations, and resulted in a modest alveolar hypoventilation with attendant arterial hypercapnia; and (3) postmenopausal women experienced acid-base adaptations characterized by increased plasma [SID] that compensated for the arterial hypercapnia and maintained $[H^+]$ unchanged in healthy postmenopausal compared to premenopausal women.

**Effect of Menopause on the Chemoreflex Control of Breathing**

Resting PaCO$_2$ was consistently higher by an average of 3.5 mmHg in POST women. This difference could be explained, in large part, by a 3.5 mmHg increase in the central
Chemoreflex VRTCO\textsubscript{2} in POST vs. PRE (Figures 1 and 2). These observations suggest that reductions in central chemoreflex drives to breathe are responsible for the increased PaCO\textsubscript{2} in healthy POST women.

**Mechanisms of the Increased Resting PaCO\textsubscript{2} in Postmenopausal Women**

Several hormone replacement therapy studies have demonstrated that medroxyprogesterone acetate administered alone, and in combination with estrogen, consistently reduce resting PaCO\textsubscript{2} in (1) POST women with (45; 46) and without respiratory insufficiency (39) and (2) healthy men (6; 22; 36; 47; 58) and ovariohysterectomized women (7; 28; 41; 48). At present, it is unclear whether female sex hormones alter resting PaCO\textsubscript{2} by acting directly on central chemoreceptor cells and/or indirectly on neuromodulatory structures involved in the processing, integration, and translation of central chemoreceptor activity into pulmonary ventilation (1-4; 11; 26; 42). Regardless of the cellular mechanism(s) involved, our data support the view that reductions in circulating [P\textsubscript{4}] and [E\textsubscript{2}] are responsible, at least in part, for the increased resting PaCO\textsubscript{2} in healthy POST women via their direct/indirect effects on central chemoreflex drives to breathe.

Duffin (12) recently described the role of acid-base balance in the chemoreflex control of breathing, using Stewart’s physicochemical principles (52; 53). He concluded that increases in [SID] would decrease [H\textsuperscript{+}] (the presumptive stimulus to the chemoreceptors) at any given PCO\textsubscript{2} (the measured stimulus to the chemoreceptors), thereby increasing the chemoreflex VRTCO\textsubscript{2}, which in turn increases resting PaCO\textsubscript{2}. Our findings in POST women strongly support this hypothesis since we found: (1) the acidifying effects of an increased PaCO\textsubscript{2} were effectively offset by the alkalizing effects of an increased
[SID], such that arterial [H+] was unchanged compared to PRE; (2) the central chemoreflex VRTCO2 was consistently higher in POST compared with PRE; and (3) PaCO2 correlated positively with the central chemoreflex VRTCO2 (Figure 2), which in turn correlated positively with plasma [SID] (Figure 2). Collectively, these findings support the novel hypothesis that increases in plasma [SID] contributed to the increased PaCO2 in healthy POST compared with PRE women, by increasing the central chemoreflex VRTCO2, secondary to its effects on the PCO2-[H+] relationship.

The cross-sectional design of our study does not permit detailed examination of the time-course of ventilatory, renal and humoral adaptations during the pre- to peri- to postmenopausal transition. Therefore, at the present time we do not know whether or not (1) the acidifying effect of progressive increases in resting PaCO2 (secondary to the abovementioned direct/indirect effects of reduced [P4] and [E2] on central chemoreflex drives to breathe) initiate a compensatory (renal) increase in plasma [SID] so as to maintain arterial [H+] constant or (2) the alkalizing effect of progressive increases in [SID] (secondary to the direct effects of reduced [P4] and [E2] on the activity of the renin-angiotensin aldosterone system (35)) initiate a compensatory decrease in alveolar ventilation via reductions in central chemoreceptor efferent activity, which in turn increases PaCO2 so as to regulate arterial [H+] at a constant level.

**Methodological Considerations**

A constraint of our study is the cross-sectional design, in addition to the modest, but anticipated, between-group difference in age. Menopause is an age-related biological phenomenon and therefore we chose not to match our groups for age since this would
introduce a bias due to the pre-selection of women who were PRE or POST beyond and before the age normally reported, respectively. We attempted to ameliorate the effects of our cross-sectional design by studying well-matched groups of healthy PRE and POST women that were ‘relatively’ similar in age. Furthermore, we recruited a sample size capable of detecting physiologically meaningful between-group differences in our primary outcome variables based on a priori power calculations. Finally, we utilized statistical methods to ensure that that the between-group differences reported herein reflected primarily differences in menopausal status as opposed to age. Therefore, the results of our study suggest that controlled, longitudinal studies of the ventilatory, hormonal and acid-base adaptations during the pre- to peri- to postmenopausal transition would provide significant insight into the underlying causes of age-related ventilatory adaptations.

**Perspectives and Significance**

To the best of our knowledge, this is the first study to systematically examine the role of central and peripheral chemoreflex drives to breathe, acid-base balance and their interaction in the ventilatory adaptations associated with menopause. The novel results of this study indicated that central, but not peripheral, chemoreflex drives to breathe were attenuated in postmenopausal women (as evidenced by a reduced hyperoxic VRTCO₂), and that this change was associated with decreased concentrations of female sex steroid hormones and complex acid-base interactions resulting in an increased resting PaCO₂. A methodological strength of our study was the comparison to premenopausal women of similar age.

Research examining the impact of menopause on ventilatory control is timely given the rapidly growing postmenopausal demographic in North America and the increased need
to understand the physiological milieu upon which age- and sex-related respiratory disorders are imposed. The results of the present study, therefore, have potentially important clinical and physiological implications with respect to our understanding of (1) the increased prevalence of sleep-disordered breathing following the onset menopause (5; 57) and (2) the effects of sex (27; 32; 55) and hormone replacement therapy (39; 43; 45) on ventilatory control and acid-base balance at rest and during exercise in health and disease.
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Figure Legends

**Figure 1.** Central ventilatory chemoreflex response to hyperoxic-hypercapnia in a representative pre- (●) and postmenopausal (o) subject.

**Figure 2.** Relationship between the central chemoreflex ventilatory recruitment threshold for CO₂ (VRTCO₂) and each of [SID] and PaCO₂. [SID], plasma strong ion difference concentration; PaCO₂, arterialized venous partial pressure of carbon dioxide. Closed and open diamonds represent pre- and postmenopausal women, respectively. R values were derived from pooled data.
## Table 1. Subject characteristics

<table>
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<tr>
<th>Variable</th>
<th>Premenopausal (n=20)</th>
<th>Postmenopausal (n=15)</th>
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<tbody>
<tr>
<td>Age, years</td>
<td>45.3 ± 3.2</td>
<td>52.1 ± 1.8**</td>
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<tr>
<td>Height, cm</td>
<td>165.4 ± 5.9</td>
<td>162.2 ± 8.0</td>
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<tr>
<td>Weight, kg</td>
<td>68.2 ± 14.3</td>
<td>64.3 ± 8.5</td>
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<td>BMI, kg m⁻²</td>
<td>24.8 ± 4.6</td>
<td>24.5 ± 2.9</td>
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<tr>
<td>PEFR, l sec⁻¹ (% predicted)</td>
<td>5.4 ± 0.8 (86 ± 12)</td>
<td>5.4 ± 0.7 (91 ± 15)</td>
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<tr>
<td>FVC, l (% predicted)</td>
<td>3.2 ± 0.4 (90 ± 10)</td>
<td>3.1 ± 0.3 (98 ± 15)</td>
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<tr>
<td>FEV₁, l (% predicted)</td>
<td>2.6 ± 0.3 (97 ± 11)</td>
<td>2.5 ± 0.3 (103 ± 16)</td>
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<td>FEV₁/FVC, % (% predicted)</td>
<td>83.0 ± 7.1 (108 ± 9)</td>
<td>79.6 ± 9.6 (106 ± 12)</td>
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<tr>
<td>SBP, mmHg</td>
<td>118.5 ± 10.6</td>
<td>118.3 ± 8.0</td>
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<tr>
<td>DBP, mmHg</td>
<td>72.1 ± 8.0</td>
<td>75.3 ± 6.6</td>
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Values are means ± SD. BMI, body mass index; PEFR, peak expiratory flow rate; FVC, forced vital capacity; FEV₁, forced expired volume in one second; SBP, systolic blood pressure; DBP, diastolic blood pressure. **significantly different from the premenopausal group (p<0.01).
Table 2. Resting cardiorespiratory and blood biochemistry

<table>
<thead>
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<th>Variable</th>
<th>Premenopausal (n=20)</th>
<th>Postmenopausal (n=15)</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \dot{V}_E ), l min(^{-1} )</td>
<td>8.0 ± 1.7</td>
<td>8.5 ± 4.5</td>
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<tr>
<td>( V_T ), ml</td>
<td>638.9 ± 228.0</td>
<td>498.7 ± 105.8*</td>
</tr>
<tr>
<td>( f_R ), breaths min(^{-1} )</td>
<td>13.5 ± 3.2</td>
<td>17.0 ± 4.9*</td>
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<td>( \dot{V}_{A,est} ), l min(^{-1} )</td>
<td>6.2 ± 1.4</td>
<td>5.3 ± 0.7*</td>
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<tr>
<td>( \dot{V}_O_2 ), ml min(^{-1} )</td>
<td>295.1 ± 39.7</td>
<td>298.1 ± 71.6</td>
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<tr>
<td>( \dot{V}_C O_2 ), ml min(^{-1} )</td>
<td>253.0 ± 31.7</td>
<td>262.6 ± 56.3</td>
</tr>
<tr>
<td>( \dot{V}_E/\dot{V}_O_2 )</td>
<td>28.5 ± 8.3</td>
<td>25.7 ± 3.9</td>
</tr>
<tr>
<td>( \dot{V}_E/\dot{V}_C O_2 )</td>
<td>32.4 ± 6.2</td>
<td>29.6 ± 6.1</td>
</tr>
<tr>
<td>([H^+]), nEq l(^{-1} )</td>
<td>38.4 ± 2.0</td>
<td>38.7 ± 2.6</td>
</tr>
<tr>
<td>( PaCO_2 ), mmHg</td>
<td>37.7 ± 3.9</td>
<td>41.2 ± 4.3*</td>
</tr>
<tr>
<td>([SID]), mEq l(^{-1} )</td>
<td>37.0 ± 1.4</td>
<td>38.7 ± 1.9**</td>
</tr>
<tr>
<td>([HCO_3^-]), mmol l(^{-1} )</td>
<td>23.7 ± 1.4</td>
<td>25.5 ± 1.5**</td>
</tr>
<tr>
<td>([ATOT]), mEq</td>
<td>16.4 ± 0.6</td>
<td>16.5 ± 0.7</td>
</tr>
<tr>
<td>Osmolality, mosmol kg(^{-1})H(_2)O(^{-1} )</td>
<td>280.2 ± 3.6</td>
<td>283.9 ± 3.2**</td>
</tr>
<tr>
<td>([P_4]), nmol l(^{-1} )</td>
<td>6.7 ± 9.7</td>
<td>1.1 ± 0.8 *</td>
</tr>
<tr>
<td>([E_2]), pmol l(^{-1} )</td>
<td>306.1 ± 255.6</td>
<td>67.3 ± 49.1**</td>
</tr>
</tbody>
</table>

Values are means ± SD. \( \dot{V}_E \), minute ventilation; \( V_T \), tidal volume; \( f_R \), breathing frequency; \( \dot{V}_{A,est} \), estimated alveolar ventilation; \( \dot{V}_O_2 \), metabolic rate of oxygen consumption; \( \dot{V}_C O_2 \), metabolic rate of carbon dioxide production; \( \dot{V}_E/\dot{V}_O_2 \), ventilatory equivalent for oxygen; \( \dot{V}_E/\dot{V}_C O_2 \), ventilatory
equivalent for carbon dioxide. $[\text{H}^+]$, arterialized venous hydrogen ion concentration; $\text{PaCO}_2$, arterialized venous partial pressure of carbon dioxide; $[\text{SID}]$, strong ion difference concentration; $[\text{A}_{\text{TOT}}]$, total weak acid concentration; $[\text{HCO}_3^-]$, bicarbonate concentration; $[\text{P}_4]$, serum progesterone concentration; $[\text{E}_2]$, 17β-estradiol concentration. *significantly different from the premenopausal group ($p<0.05$), **significantly different from the premenopausal group ($p<0.01$).
Table 3. Ventilatory responses to carbon dioxide under hyperoxic and hypoxic rebreathing conditions.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Hyperoxic</th>
<th>Hypoxic</th>
<th>P for Group</th>
<th>P for Condition</th>
<th>P Group*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Premenopausal</td>
<td>Postmenopausal</td>
<td>Premenopausal</td>
<td>Postmenopausal</td>
<td></td>
</tr>
<tr>
<td>( \dot{V}_E), l min(^{-1})</td>
<td>10.6 ± 5.2</td>
<td>10.2 ± 5.5</td>
<td>14.1 ± 5.4</td>
<td>11.4 ± 4.5</td>
<td>0.483</td>
</tr>
<tr>
<td>VRTCO(_2), mmHg</td>
<td>45.7 ± 3.4</td>
<td>49.2 ± 3.0**</td>
<td>42.2 ± 3.1</td>
<td>45.1 ± 3.2*</td>
<td>0.005</td>
</tr>
<tr>
<td>( \dot{V}_E), l min(^{-1}) mmHg(^{-1})</td>
<td>3.3 ± 1.6</td>
<td>2.2 ± 0.8</td>
<td>3.9 ± 2.1</td>
<td>2.9 ± 0.9</td>
<td>0.061</td>
</tr>
</tbody>
</table>

Values are means ± SD. \( \dot{V}_E\), sub-VRTCO\(_2\) (wakefulness) ventilation; VRTCO\(_2\), the ventilatory recruitment threshold for CO\(_2\); \( \dot{V}_E\), ventilatory chemoreflex sensitivity to CO\(_2\). Condition refers to the rebreathing trial (hyperoxic or hypoxic). *significantly different from the premenopausal group within condition (p<0.05). **significantly different from the premenopausal group within condition (p<0.01).
Figure 1
Figure 2