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Chemoreflex control of breathing during wakefulness in healthy men and women

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Chemoreflex control of breathing during wakefulness in healthy men and women. J Appl Physiol 98: 822–828, 2005. First published November 19, 2004; doi:10.1152/japplphysiol.01208.2003. —This study used a modified CO2 rebreathing procedure to examine the effect of gender on the chemoreflex control of breathing during wakefulness in healthy men (n = 14) and women (n = 14). Women were tested in the follicular phase of the menstrual cycle. During rebreathing trials, subjects hyperventilated to reduce the partial pressure of end-tidal CO2 (PETCO2) below 25 Torr and were then switched to a rebreathing bag containing a normocapnic hypoxic or hyperoxic gas mixture. During the trial, PETCO2 increased, while O2 was maintained at a constant level. The point at which ventilation began to rise as PETCO2 increased was identified as the ventilatory recruitment threshold (VRT). Ventilation below the VRT was measured, and the slope of the ventilatory response above the VRT was determined. Gender had no effect on the hyperoxic or hypoxic VRT for CO2. Central chemoreflex sensitivity was significantly greater in men than women but not after correction for forced vital capacity. Measures of peripheral chemoreflex sensitivity were similar between genders. However, the slope of the tidal volume (VT) response to hypoxic and hyperoxic CO2 rebreathing (corrected and uncorrected) was greater in men than women, respectively. We conclude that central chemoreflex sensitivity is greater in men compared with women as reflected by differences in ventilatory (uncorrected) and VT (corrected and uncorrected) responses to CO2. However, gender has no significant effect on the central chemoreflex VRT for CO2. The peripheral chemoreflex control of breathing during wakefulness is similar between men and women.

Hypothesis: The ventilatory response to CO2 below and above the VRT would differ between men and women.

Methods

Subjects. Healthy, nonsmoking, normally active men (n = 14) and women (n = 14), aged 20–35 yr, were recruited at Queen’s University through posted announcements and flyers. Subjects had no history of cardiorespiratory disease, nor were they born at or recently returned from high altitude. None was taking medications that affect ventilatory control. Female subjects had regular menstrual cycles, as verified by questionnaire, and had not used oral contraceptives for 6 mo before experimental testing.

Before study entry, subjects attended an information session to familiarize them with the laboratory and study procedures. All subjects completed the revised Physical Activity Readiness Questionnaire (available online at www.csep.ca/forms.asp) to ensure that there were no contraindications to participation. Demographic information, including age, occupation, and occupational and recreational physical activity levels, were assessed by questionnaire. To evaluate menstrual activity levels, were assessed by questionnaire. To evaluate menstrual

Ovarian and testicular hormones influence the control of breathing via their effects on both central and peripheral chemoreflexes (2, 33). Progesterone increases central and peripheral chemosensitivity to hypercapnia and hypoxia (2, 33). This effect is potentiated by estrogen (2, 33). In addition, testosterone increases peripheral chemoreflex sensitivity to hypoxia (2, 17, 33), but its effect on central chemosensitivity remains unclear (2, 33). Given the effects of reproductive hormones on ventilatory control as well as gender differences in endocrine status, it is reasonable to expect that central and/or peripheral chemoreflex control characteristics may differ between healthy men and women during wakefulness.

Current evidence suggests that the apneic threshold and sensitivity of the ventilatory response to CO2, mediated by the central chemoreflex, is greater in men than women (20, 26, 35, 37), regardless of menstrual cycle status (20, 33, 35). Some studies have shown that the ventilatory response to hypoxia, mediated by the peripheral chemoreflex, is greater in men than women (20, 32, 33), whereas others have reported the opposite result (1). However, others have found that gender has no effect on either central (1, 13, 18, 26, 30, 34) or peripheral (13, 26, 27, 32, 34) chemoreflex sensitivity.

Divergent results from previous investigations may be due to different experimental designs. Some studies did not control for menstrual cycle status of female subjects (20, 21, 28, 30, 32, 34), whereas others made comparisons between men and women in the luteal phase (37) and follicular phase (FP) (1, 13, 26, 27) of the menstrual cycle. Also, some studies have not considered oral contraceptive use among female volunteers (21, 28, 30) or gender differences in body size and/or lung functional indexes (20, 26, 28).

Recent epidemiological studies (4, 36) have demonstrated that the prevalence of sleep-disordered breathing (SDB), a condition of repeated apneas, hypopneas, and breathing oscillations, is two to three times greater in men than women (4, 36). Differences in the central and/or peripheral chemoreflex control of breathing during wakefulness may reflect a similar difference during sleep that could contribute to the high male prevalence of SDB (12, 37).

This study investigated the effect of gender on the ventilatory recruitment threshold (VRT) for CO2 and the ventilatory response below (i.e., behavioral drives) and above (i.e., chemoreflex sensitivity) this threshold while maintaining constant backgrounds of hyperoxia and hypoxia, respectively. We hypothesized that neither the hyperoxic nor hypoxic VRT for CO2 would differ between men and women. In addition, we hypothesized that the ventilatory response to CO2 below and above this threshold would be greater in men than women, independent of the level of O2.

Methods

Subjects. Healthy, nonsmoking, normally active men (n = 14) and women (n = 14), aged 20–35 yr, were recruited at Queen’s University through posted announcements and flyers. Subjects had no history of cardiorespiratory disease, nor were they born at or recently returned from high altitude. None was taking medications that affect ventilatory control. Female subjects had regular menstrual cycles, as verified by questionnaire, and had not used oral contraceptives for 6 mo before experimental testing.

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cycle status, female subjects identified the first day of their last menstrual cycle and the average length of their cycle. This approach has been validated in our laboratory by using plasma progesterone measurements (23). 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.

Basic physical measurements included height and body mass, forced vital capacity (FVC), forced expired volume in 1 s (FEV1), and peak expiratory flow (model S-301, Pneumoscan). Body mass index (BMI) was calculated as body mass/height² (kg/m²). Body surface area (BSA; m²) was calculated from height and body mass measurements. All female subjects were tested in the FP (range: 2–13 days from the first day of menstruation) of the menstrual cycle when circulating estrogen and progesterone levels are low and gender differences in endocrine status are minimized.

Modified rebreathing protocol. Volunteers abstained from aerobic and muscular conditioning exercise as well as caffeine and alcohol on the day of testing. Two rebreathing experiments, each separated by at least 45 min, were completed in the morning (AM) and evening (PM) of the same day. AM and PM trials were separated by ~12 h.

A modified version of Read’s (24) rebreathing procedure that includes prior hyperventilation and maintenance of a constant (i.e., isooxia) end-tidal O₂ tension (PETO₂) was used to evaluate central and peripheral chemoreflex control characteristics (9). During each trial, subjects breathed room air for 5 min, using a slow and deliberate breathing pattern to avoid the short-term potentiation effect described by Folgering and Durlinger (10). Prior hyperventilation lowered the body stores of CO₂ below 25 Torr, thereby allowing the VRT to be identified as the partial pressure of end-tidal CO₂ (PETCO₂) increased from hypo- to hypercapnia. Furthermore, prior hyperventilation permits measurement of the ventilatory response below this threshold, independent of the ventilatory chemoreflexes (i.e., basal), estimating behavioral drives (29). After hyperventilation, subjects were switched from room air to a rebreathing bag containing a normocapnic (~42 Torr) hypoxic or hyperoxic gas mixture. Rebreathing began with three deep breaths, producing rapid equilibration of the PCO₂ in the bag, lungs, and arterial blood to that of the mixed venous blood. The equilibration was verified by a plateau in PETCO₂ and was a prerequisite for continuing the test. On equilibration, subjects were asked to breathe as they felt the need.

During rebreathing, PETCO₂ increased while isooxia was maintained, under computer control, at a constant hyperoxic (150 Torr) or hypoxic (50 Torr) level. In its hyperoxic form, the modified rebreathing procedure measures central chemoreflex sensitivity equivalent to that measured using Read’s original technique (19). Continuous measures of arterial blood O₂ saturation (SaO₂) (model OXI, Radiometer, Copenhagen, Denmark) and heart rate (HR) (Max-1 electrocardiograph, Marquette) were obtained throughout each test. Rebreathing was terminated if ventilation exceeded 100 l/min, PETCO₂ exceeded 60 Torr, SaO₂ fell below 70%, and/or subject discomfort.

Rebreathing apparatus. During rebreathing, subjects wore nose clips and breathed through a mouthpiece connected to one side of a three-way T-shaped manual directional valve (11.9-ml dead space; model 2100a, Hans Rudolph) that permitted switching from room air to the rebreathing bag. Subjects rebreathed from a 10-liter plastic bag connected to a volume turbine (model VMM-1100, Alpha Technologies). The volume turbine was coupled to the expiratory end of the T valve and monitored breath-by-breath changes in minute ventilation (VE), tidal volume (VT) and respiratory rate (f). A sampling tube connected to the mouthpiece side of the T valve permitted continuous analysis of PETCO₂ and PETO₂ by using a respiratory mass spectrometer (model MGA 1100, Perkin-Elmer) at a sample flow rate of 64 ml/min. Isoxia was maintained by a flow of 100% O₂ to the bag side of the T valve. The testing system was calibrated with gases of known concentrations and a standard 3,004-liter volume syringe (model 1922, CS-3000 AM Systems) before each rebreathing test.

A 12-bit analog-to-digital converter (DAQCard-6062E, National Instruments) digitized the continuous analog output signals from all monitoring devices for computer analysis using custom-written software (LabVIEW, National Instruments). The data-acquisition software calculated breath-by-breath measures of VE, f, VT, PETCO₂, PETO₂, SaO₂, and HR.

Data analyses. Data from rebreathing experiments were imported to an analysis program (LabVIEW, National Instruments) designed specifically for this purpose (J. Duffin, personal communication). Barometric pressure and room temperature were entered, and measured volumes were corrected to body temperature and pressure, saturated (bTPS). Data from the first equilibration at the start of rebreathing and any aberrant points detected by the data-acquisition software during the experiments were excluded from further analysis. Subsequently, breath-by-breath PETCO₂ was plotted against time and fitted with a least squares regression line, whose slope depends on the metabolic rate of CO₂ production (VCO₂). The equation for this line provided a predicted value of PETCO₂ vs. time, thereby minimizing interbreath variability due to measurement. Thereafter, VE, VT, and f were plotted against the predicted PETCO₂.

Each of these plots was fitted with a model made up of the sum of two segments separated by one breakpoint. 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) using commercial software (SigmaPlot 7.0, SPSS). Figure 1 is an example of the lines fitted to the VE response of a representative subject during an isooxic hyperoxic CO₂ rebreathing procedure. The first segment of the response was an exponential decline to a final value (i.e., “basal”). This value was taken as a measure of VE, VT, and f below the VRT for CO₂, respectively. Rarely, a short-term potentiation of breathing was observed after hyperventilation, and its waning was modeled as an exponential decay.

The point at which VE, VT, and f began to rise in a linear fashion in conjunction with a rise in PETCO₂ was taken as the VRT for CO₂ (Fig. 1). On the basis of the modeling of Duffin et al. (9), we assumed that the VRT measured under hyperoxic conditions originated from the central chemoreflex alone, because hyperoxia abolishes virtually
all peripheral chemoreflex activity (7). Moreover, it was assumed that the VRT measured under hypoxic conditions derived from the sum of the central and peripheral chemoreflexes.

The slope of the line fitted to V̇E, V̇T, and f responses above the VRT was taken as a measure of chemoreflex sensitivity to increases in P̂ETCO2 (Fig. 1). We assumed that the slope of the V̇E, V̇T, and f response measured during hyperoxic trials represented central chemoreflex sensitivity, whereas the slope recorded from hypoxic trials represented the additive effects of central and peripheral chemoreflex stimulation (9). Thus the slope of the hyperoxic response was subtracted from the slope of the hypoxic response for each subject to isolate peripheral chemoreflex sensitivity. Because lung volumes vary in proportion to body size and are usually higher in men than women, basal and sensitivity measures of V̇E and V̇T were corrected for body height, body mass, BSA, BMI, FEV1, and FVC, respectively. Comparisons of f responses (i.e., basal, VRT, and sensitivity) were made between nine women and six men.

Minimum sample size estimate. Sensitivity and VRT measures from hyperoxic and hypoxic rebreathing conditions were selected as important outcome data for between-gender effects. Minimum sample size was calculated on the basis of 80% power and a confidence interval of 0.05 using an unpaired subject formula for the comparison of means (22). Sample sizes capable of detecting between-gender differences of 1.5 l·min⁻¹·Torr⁻¹ and 2 Torr were estimated for sensitivity and VRT parameters, respectively, using standard deviations from Mateika and Elythy (16). The resulting critical sample sizes were estimated to be 6 and 9 for hyperoxic and hypoxic chemoreflex sensitivity parameters and 10 and 9 for hyperoxic and hypoxic VRT parameters, respectively. Therefore, a sample size of 14 subjects per group was adequate for this study.

Statistical analyses. Student’s t-statistics for independent samples were used for simple between-gender comparisons of physical characteristics. A three-way ANOVA was used to detect main effects for gender, isoaxia, and time of day among the calculated parameters. Time of day had no effect on the mean data, independent of isoaxia. Furthermore, significant AM-PM correlations (Pearson r) were observed for all hyperoxic (range: r = 0.45–0.75; P < 0.01) and hypoxic (range: r = 0.67–0.78; P < 0.01) rebreathing responses. Therefore, to reduce random intrasubject variability, data collected during AM and PM trials were averaged for each subject. A two-way ANOVA was used to detect differences between genders (men vs. women) and levels of O2 (hyperoxia vs. hypoxia) on the mean data. The post hoc test of Tukey (honestly significant difference) was used to identify between-gender differences under each experimental condition.

Unpaired Student’s t-statistics were used to identify significant between-group differences in peripheral chemoreflex sensitivity. Pearson product-moment correlation coefficients (Pearson r) were calculated between hyperoxic and hypoxic chemoreflex parameters and subject physical characteristics, respectively. Results for all statistical tests were considered significant if P < 0.05. Values are presented as means ± SE.

RESULTS

Physical characteristics. Mean age and BMI were similar between men and women (Table 1). As expected, body height, body mass, peak flow, FEV1, FVC, and BSA were significantly greater in men than women. FEV1/FVC was significantly greater in men than women. All women were tested within the FP of the menstrual cycle (8.4 ± 1.0 days from the first day of menstruation).

Effect of gender on chemoreflex control characteristics. Significant correlations were observed between body height, FVC, FEV1, BSA, and both hyperoxic and hypoxic chemoreflex characteristics (Table 2).

### Table 1. Physical characteristics of subjects

<table>
<thead>
<tr>
<th>Variable</th>
<th>Men (n = 14)</th>
<th>Women (n = 14)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, yr</td>
<td>25.4 ± 1.12</td>
<td>22.9 ± 0.66</td>
</tr>
<tr>
<td>Body height, cm</td>
<td>181 ± 1.8</td>
<td>164 ± 1.9*</td>
</tr>
<tr>
<td>Body mass, kg</td>
<td>83.4 ± 3.29</td>
<td>65 ± 3.00*</td>
</tr>
<tr>
<td>BML kg/m²</td>
<td>26.1 ± 0.9</td>
<td>24.1 ± 0.9</td>
</tr>
<tr>
<td>Peak flow, l/s</td>
<td>7.43 ± 0.18</td>
<td>4.98 ± 0.27*</td>
</tr>
<tr>
<td>FVC, liters</td>
<td>5.02 ± 0.14</td>
<td>3.29 ± 0.14*</td>
</tr>
<tr>
<td>FEV1, liters</td>
<td>3.79 ± 0.12</td>
<td>2.79 ± 0.11*</td>
</tr>
<tr>
<td>FEV1/FVC, %</td>
<td>75.7 ± 2.26</td>
<td>85.5 ± 2.58*</td>
</tr>
<tr>
<td>BSA, m²</td>
<td>2.07 ± 0.05</td>
<td>1.72 ± 0.04*</td>
</tr>
</tbody>
</table>

*Significant between-gender difference; P < 0.05.

Basal V̇E and V̇T were significantly greater in men than women under both hyperoxic and hypoxic conditions (Table 3). However, these differences were abolished after the data were normalized for FVC (P = 0.50, P = 0.48). There was no main effect of gender on the basal f response (F = 0.82, P = 0.37) during hyperoxic and hypoxic rebreathing.

No main effect of gender on the V̇E (F = 2.39, P = 0.13) and V̇T (F = 2.58, P = 0.11) recruitment threshold for CO2 was observed, independent of the level of isoaxia (Table 3). In contrast, the CO2 recruitment threshold for f was significantly greater in men than women under both isoaxic rebreathing conditions.

V̇E and V̇T chemoreflex sensitivities to CO2 were significantly greater in men than women under both hyperoxic and hypoxic conditions (Table 3). When comparisons were made between genders after normalization for FVC, mean V̇E chemoreflex sensitivity was not significantly different between genders (F = 0.10, P = 0.76), independent of the level of O2. In contrast, gender differences in the sensitivity of the V̇T response to CO2 during hyperoxic and hypoxic trials persisted after normalization for FVC. In this regard, elimination of the gender difference in the V̇E response to CO2 at high and low levels of O2 could be explained by the effect FVC has on f because gender differences in the V̇T response to CO2 remain after correcting for FVC.

Gender had no effect on the peripheral chemoreflex contribution to the increase in V̇E (corrected and uncorrected), V̇T (corrected and uncorrected), or f during isoaxic hypoxic CO2 rebreathing (Table 4). The observed difference in the uncorrected V̇E response to hypoxic-hypercapnia may be attributed to an effect of gender on central chemoreflex sensitivity.

Effects of isoaxic P̂ETCO2 (150 vs. 50 Torr) on chemoreflex control characteristics. There was no main effect of isoaxia on basal V̇E (F = 0.32, P = 0.57), V̇T (F = 0.44, P = 0.51), or f (F = 0.01, P = 0.93), irrespective of gender (Table 3). The CO2 recruitment threshold for V̇E and V̇T was lower, whereas chemoreflex responsiveness was higher, during hypoxic vs. hyperoxic rebreathing trials, independent of gender. In contrast, the f recruitment threshold for CO2 was significantly greater, whereas f chemoreflex responsiveness was identical (F = 0.81, P = 0.38), during hyperoxic and hypoxic rebreathing trials, independent of gender.

Correlations between chemoreflex characteristics. Basal V̇E, V̇T, and V̇E chemosensitivity estimates obtained from all subjects were pooled and grouped according to the isoaxic rebreathing condition (hyperoxic or hypoxic). Correlations
among these characteristics were tested by using a Pearson product-moment correlation analysis. Neither basal \( V_{\text{E}} \) and VRT (\( r = 0.34, P = 0.08 \)) or basal \( V_{\text{E}} \) and \( V_{\text{E}} \) chemosensitivity (\( r = 0.36, P = 0.06 \)) were significantly correlated for hyperoxic tests. Similarly, basal \( V_{\text{E}} \) and VRT were not significantly correlated (\( r = 0.07, P = 0.72 \)) for hypoxic tests. However, a significant correlation between basal \( V_{\text{E}} \) and \( V_{\text{E}} \) chemosensitivity (\( r = 0.48, P = 0.01 \)) was identified for hypoxic trials.

**DISCUSSION**

The primary findings of this study suggest that central chemoreflex sensitivity is greater in men than women as reflected by differences in \( V_{\text{E}} \) (uncorrected) and Vr (both corrected and uncorrected) responses to hyperoxic and hypoxic CO\(_2\) rebreathing. However, the central chemoreflex VRT for CO\(_2\) is similar in men compared with women. In addition, gender has no significant effect on the peripheral chemoreflex control of breathing during wakefulness when women are tested in the FP of their menstrual cycle.

**Critique of methods.** The benefits of the modified rebreathing procedure have been described previously (18, 19). Despite its advantages, the modified rebreathing technique may initiate responses, such as induction of short-term potentiation, respi-
of the apneic threshold, then the VRT for CO2 measured using threshold (7). If the extrapolated threshold were a true estimate consequently the ventilatory response to CO2. Although basal, have increased our subjects’ state of arousal and/or anxiety and alternative or traditional methods.

### Table 4. Effect of gender on the central and peripheral chemoreflex contribution to the ventilatory, tidal volume, and breathing frequency response to CO2

<table>
<thead>
<tr>
<th>Sensitivities to CO2</th>
<th>Central Chemoreflex</th>
<th>Peripheral Chemoreflex</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Women</td>
<td>Men</td>
</tr>
<tr>
<td>Vt, l/min · mmHg⁻¹</td>
<td>2.83 ± 0.27</td>
<td>4.35 ± 0.39*</td>
</tr>
<tr>
<td>Vt/FVC, l/min · mmHg⁻¹l⁻¹</td>
<td>0.90 ± 0.10</td>
<td>0.89 ± 0.08</td>
</tr>
<tr>
<td>Vt, ml/min · mmHg⁻¹</td>
<td>102.58 ± 8.80</td>
<td>220.09 ± 20.51*</td>
</tr>
<tr>
<td>Vt/FVC, ml/min · mmHg⁻¹l⁻¹</td>
<td>31.56 ± 2.39</td>
<td>43.71 ± 3.72*</td>
</tr>
<tr>
<td>f, breaths/min · mmHg⁻¹</td>
<td>1.50 ± 0.25</td>
<td>0.95 ± 0.15</td>
</tr>
</tbody>
</table>

Values are means ± SE. *Significant between-gender difference, P < 0.05.

(3). Subjects also rested for at least 45 min between trials to avoid respiratory muscle fatigue.

Alterations in CBF due to hypoxia and hypocapnia could affect the sensitivity and/or VRT for CO2 by modifying the arteriovenous difference and thus the relationship between PETCO2 and brain tissue PCO2 (PiCO2). Although this is a concern when steady-state methods are used (18), it is of lesser importance when the modified rebreathing procedure is employed (18, 24). Thus the initial equilibration of CO2 at the start of rebreathing ensures that changes in CBF do not affect the arteriovenous difference via washout of PiCO2, and consequently the slope of the ventilatory response to CO2 (25). In addition, because the subject and bag are a closed system, both blood circulation and pulmonary ventilation act as mixing addition, because the subject and bag are a closed system, both blood circulation and pulmonary ventilation act as mixing.

The modified rebreathing procedure permits direct measurement of the VRT for CO2 mediated by the central and peripheral chemoreflex. In contrast, other methods identify the chemoreflex threshold by extrapolation of the linear relation between Ve and PETCO2 to the x-axis (7). Consequently, the threshold is taken as the PETCO2 axis intercept and/or apneic threshold (7). If the extrapolated threshold were a true estimate of the apneic threshold, then the VRT for CO2 measured using the modified rebreathing procedure would depend on basal Ve, independent of the level of O2. However, a significant correlation was not observed in this study or previous studies (14, 16). In addition, exercise-induced increases in basal Ve have no effect on the VRT for CO2 mediated by either the central (5) or peripheral chemoreflex (8).

An additional limitation of the extrapolation method is that subjects with lower chemoreflex sensitivities will inevitably have much lower extrapolated thresholds than those with higher chemoreflex sensitivities. Findings from our study and past investigations (15, 16) have shown that no significant correlation exists between threshold and sensitivity parameters. Therefore, the modified rebreathing procedure provides a more accurate and representative estimate of the VRT for CO2 than alternative or traditional methods.

Performing a novel task (i.e., voluntary hyperventilation, breathing through a mouthpiece) without familiarization may have increased our subjects’ state of arousal and/or anxiety and consequently the ventilatory response to CO2. Although basal, VRT, and sensitivity responses were higher than those reported by Duffin et al. (9), they are consistent with ongoing studies in Duffin’s laboratory (J. Duffin, personal communication) as well as our own (D. Jensen and L. A. Wolfe, unpublished observations). If a lack of subject familiarity had had an effect on our results, differences between AM and PM trials would have been expected. This was not the case. Furthermore, environmental factors known to influence the state of arousal (i.e., lights, noise) and thus Ve (29) were controlled and maintained for each subject and trial, respectively.

It could be argued that random intrasubject variability due to testing at different times within the same day may have masked systematic differences between genders. However, we observed significant AM-PM correlation’s for all hyperoxic and hypocoxic responses, which demonstrates sufficient reproducibility within the pooled data. In addition, AM and PM responses were averaged for each subject to further reduce both biological and methodological variability, thereby increasing statistical power and the probability of finding between-gender differences. Furthermore, minimum sample size calculations were made before the study by using standard deviations from a recent report using similar subjects and identical methods (16) that verified the adequacy of statistical power in the present study. Therefore, it is unlikely that random intrasubject variability, subject familiarization, and/or other methodological factors influenced the interpretation of our results.

**Ventilatory response to hypocapnia in men and women.** To our knowledge, we are the first to report that the ventilatory response to hypocapnia (i.e., basal) is greater in men than women during wakefulness. This effect was attributed to a higher basal VT in male volunteers. However, when basal Ve and VT were corrected for FVC, the mean responses were identical. Therefore, the higher FVCs in men compared with women accounted for the difference in the uncorrected data.

The ventilatory response to hypoxia in the presence of hypocapnia has been studied with conflicting results. Findings from our study and previous investigations (6, 9, 19) have shown that Ve is similar between hyperoxic and hypocoxic trials when PETCO2 is below the recruitment threshold. Duffin et al. (9) recently demonstrated that the ventilatory responses to hypocapnia in the presence of isoxic PETCO2 values of 150, 100, 80, 60, and 40 Torr were not significantly different. Thus current evidence suggests that hypoxia has no independent effect on Ve when PETCO2 is below the VRT. These data support the hypothesis that ventilation is influenced by a wakefulness drive to breathe (29), independent of the ventilatory chemoreflexes.

**Effect of gender on the central chemoreflex.** The existence of gender differences in the central chemoreflex control of breathing is controversial. Regensteiner et al. (26) have reported that gender has no effect on absolute measures of central chemoreflex sensitivity, whereas other studies (1, 21, 28, 30, 34, 35, 37) have reported that the sensitivity of the central chemoreflex is greater in men than women, regardless of menstrual cycle.
status (20, 35, 37). In keeping with these latter results, we have shown that both $V_t$ (uncorrected) and $V_T$ (corrected and uncorrected) responses to isoxic hyperoxic $CO_2$ rebreathing are greater in men than women. Thus our data support an effect of gender on central chemoreflex sensitivity that may be attributed to increased levels of circulating testosterone in men relative to women. In this regard, laboratory animals treated with testosterone demonstrate significant increases in central chemoreflex responsiveness (33).

Only a few studies have examined the effect of gender on the central chemoreflex threshold for $CO_2$. Most studies have shown that chemoresponsiveness, and consequently the apneic threshold for $CO_2$, is greater in men than women (21, 35, 37), whereas Regensteiner et al. (26) reported that no gender difference exists. Furthermore, Morelli and coworkers (20) recently showed that gender has no effect on the VRT for $CO_2$ estimated from isoxic hyperoxic $CO_2$ rebreathing trials. Our data confirm these latter results as estimates of the central chemoreflex VRT for $CO_2$ did not differ between genders.

**Effect of gender on the peripheral chemoreflex.** Several studies have used the progressive isocapnic hypoxia procedure to examine the effect of gender on peripheral chemoreflex sensitivity (1, 13, 26, 34, 35). However, this procedure is problematic because neither the sensitivity nor threshold of the interaction between hypoxia and $CO_2$ is apparent from the curvilinear relation between $V_E$ and PETCO2. Moreover, the PETCO2 chosen to represent isocapnia may be above or below the VRT for $CO_2$, resulting in an over- or underestimation of peripheral chemoreflex sensitivity, respectively. For these reasons, we employed the modified rebreathing procedure that, in its hypoxic form, permits measurement of the threshold and sensitivity of the ventilatory response to $CO_2$, mediated by the sum of both central and peripheral chemoreflexes.

The sensitivity of the $V_E$ response to $CO_2$ during hypoxic trials was significantly greater in men than women but not after correction for FVC. However, gender differences in the slope of the $V_T$ response persisted even after correction for FVC. Because the peripheral chemoreflex contribution to the $V_E$ (corrected and uncorrected), $V_T$ (corrected and uncorrected), and $f$ responses were not significantly different between genders, we concluded that only central chemoreflex sensitivity is different between men and women. White et al. (35) reported that peripheral chemoreflex sensitivity (even after correction for BSA) was significantly greater in men compared with women. Similarly, Morelli et al. (20) recently demonstrated that the ventilatory response to isoxic hypoxic $CO_2$ rebreathing is significantly greater in men than women. However, it is unclear whether gender had an effect on peripheral chemoreflex sensitivity, as Morelli et al. failed to determine the peripheral chemoreflex contribution to the hypoxic-hypercapnic ventilatory response. Nevertheless, our findings are consistent with the majority of previous investigations showing that gender has no significant effect on peripheral chemoreflex sensitivity (1, 13, 26, 27, 32, 34). Moreover, findings from our study and a past investigation (20) demonstrate that the peripheral chemoreflex VRT for $CO_2$ is not significantly different between genders. Taken together, we conclude that gender has no significant effect on the peripheral chemoreflex control of breathing.

**Effect of gender on the ventilatory patternning response to $CO_2$.** Inconsistencies in the literature regarding the effect of gender on the chemoreflex control of breathing may exist because $V_T$ and $f$ were not considered. As previously discussed, the slope of the $V_T$ response to $CO_2$ (both corrected and uncorrected) at high and low levels of $O_2$ were significantly greater in men than women. In addition, the $f$ recruitment threshold for $CO_2$ was significantly lower in women, independent of the level of $O_2$, even though the slope of the $f$ response was similar. However, gender had no effect on the peripheral chemoreflex contribution to the $V_E$ (corrected and uncorrected), $V_T$ (corrected and uncorrected), and $f$ response during hypoxic $CO_2$ rebreathing. These findings are consistent with Sajkov et al. (27) and suggest that the ventilatory response to $CO_2$, mediated by the central chemoreflex, is more critically dependent on an increase in $f$ than $V_T$ in women than men. This may be due, at least in part, to an effect of gender and/or sex hormones on central neuromodulatory mechanisms that influence the regulation of respiratory rhythm (2). Future studies are needed to confirm this hypothesis.

**Implications for SDB.** Recent epidemiological studies (4, 36) have identified male gender as a predisposing risk factor for the development of SDB. Differences in airway morphology, fat distribution, endocrine status, and/or the chemoreflex control of breathing may explain the greater incidence of SDB in men compared with women (12).

Chemoreflex drives are the sole support of rhythmic breathing during sleep, as behavioral drives present during wakefulness are withdrawn (31). Zhou et al. (37) recently demonstrated that both chemosensitivity and the apneic threshold for $CO_2$ are greater in men than women. These differences, in combination with the observation that men require a significantly smaller reduction in PETCO2 to induce central apnea and/or hypopnea than women (37), may explain the high male prevalence of SDB.

However, we have shown, in accordance with previous investigations (15, 16, 20), that behavioral drives to breathe have no effect on either the central or peripheral VRT for $CO_2$. Thus our data suggest that gender has no effect on the central and peripheral VRT for $CO_2$, proceeding withdrawal of behavioral stimuli during sleep. Therefore, gender-related differences in the central and peripheral VRT for $CO_2$ may not contribute to the greater incidence of SDB in men relative to women.

We have demonstrated that central chemoreflex sensitivity is greater in men than women during wakefulness. Therefore, men may be more susceptible to ventilatory overshoot and a more pronounced hypopcapnia (i.e., decrease in central respiratory drive, initiation and prolongation of recurrent apneic events) on apnea termination than women, despite similar central and peripheral VRTs for $CO_2$. However, body size and therefore $V_{CO_2}$ were significantly greater in men compared with women. Thus the degree of ventilatory overshoot required to reduce PETCO2 below the central and peripheral VRT for $CO_2$ during sleep is also greater in men than women because of the increasing hyperbolic relation between alveolar $CO_2$ and alveolar ventilation at a higher $V_{CO_2}$ (7). Taken together, gender-related differences in the central and peripheral chemoreflex control of breathing may not solely explain the pathogenesis and high male prevalence of SDB (12, 36).
Summary. The present study supports the hypothesis that central chemoreflex sensitivity is greater in men than women. However, gender has no effect on the central chemoreflex VRT for CO₂. There is no significant effect of gender on the peripheral chemoreflex control of breathing during wakefulness when women are tested in the FP of the menstrual cycle. Further study is recommended to confirm and clarify the effect of gender and sex hormones on the chemoreflex and neural control of breathing during wakefulness and sleep.

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REFERENCES