Chemoreflex control of breathing during wakefulness in healthy men and women

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Jensen, Dennis, Larry A. Wolfe, Denis E. O'Donnell, and Gregory A. L. Davies. 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 hyperoxic and hypoxic 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.

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

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cycle menstrual 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 CO₂ 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 hypoxic 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 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 hyperxia abolishes virtually

![Fig. 1. An example of the ventilatory response to progressive hypercapnia while O₂ was maintained at 150 Torr in a representative subject. Note the measurement of basal ventilation, the ventilatory recruitment threshold, and chemoreflex sensitivity. PETCO₂, end-tidal PCO₂. See text for details.](http://jap.physiology.org/)
all peripheral chemoreflex activity (7). Moreover, it was assumed that
the V̇E measured under hypoxic conditions derived from the sum of
the central and peripheral chemoreflexes.

The slope of the line fitted to V̇E, VT, and f responses above the
V̇E was taken as a measure of chemoreflex sensitivity to increases in
P(t)CO₂ (Fig. 1). We assumed that the slope of the V̇E, VT, and f
response measured during hyperoxic trials represented central che-
moreflex 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 sub-
tracted 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 VT were corrected for body
height, body mass, BSA, BMI, FEV₁, and FVC, respectively. Com-
parisons of f responses (i.e., basal, V̇E, and sensitivity) were made
between nine women and six men.

Minimum sample size estimate. Sensitivity and V̇E measures from
hyperoxic and hypoxic rebreathing conditions were selected as im-
portant 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·Torr⁻¹ and 2 Torr were estimated for
sensitivity and V̇E parameters, respectively, using standard devia-
tions from Mateika and Ellythy (16). The resulting critical sample
sizes were estimated to be 6 and 9 for hyperoxic and hypoxic
sensitivity parameters and 10 and 9 for hyperoxic and hypoxic V̇E
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 char-
acteristics. A three-way ANOVA was used to detect main effects for
gender, isooxia, and time of day among the calculated parameters.
Time of day had no effect on the mean data, independent of isooxia.
Furthermore, significant AM-PM correlations (Pearson r) were ob-
served 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. Pear-
son product-moment correlation coefficients (Pearson r) were calcu-
lated 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 women than men. 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 chemore-
flex characteristics (Table 2).

| Table 1. Physical characteristics of subjects |
|-----------------|-----------------|
| Variable        | Men (n = 14)    | Women (n = 14) |
| Age, yr         | 25 ± 1.12       | 22.9 ± 0.66    |
| Body height, cm | 181 ± 1.8       | 164 ± 1.9*     |
| Body mass, kg   | 83.4 ± 3.29     | 65.1 ± 3.00*   |
| BMI kg/m²       | 26.1 ± 0.9      | 24.1 ± 0.9     |
| Peak flow, l/s  | 7.43 ± 0.18     | 4.98 ± 0.27*   |
| FVC, liters     | 5.02 ± 0.14     | 3.29 ± 0.14*   |
| FEV₁, liters    | 3.79 ± 0.12     | 2.79 ± 0.11*   |
| FEV₁/FVC, %     | 75.7 ± 2.26     | 85.5 ± 2.58*   |
| BSA, m²         | 2.07 ± 0.05     | 1.72 ± 0.04*   |

Values are means ± SE; n, no. of subjects. BMI, body mass index; FVC, forced vital capacity; FEV₁, forced expired volume in 1 s; BSA, body surface area. *Significant between-gender difference; P < 0.05.

Basal V̇E and VT were significantly greater in men than women under both hypoxic and hypoxic conditions (Table 3). However, these differences were abolished after the data were normalized for FVC (Ḟ = 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 VT (F = 2.58, P = 0.11) recruitment threshold for CO₂ was observed, independent of the level of isooxia (Table 3). In contrast, the CO₂ recruitment threshold for f was significantly greater in men than women under both isooxic rebreathing conditions.

V̇E and VT chemoreflex sensitivities to CO₂ were signifi-
cantly greater in men than women under both hypoxic and
hypoxic rebreathing 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 VT response to CO₂ during hyperoxic and hypoxic trials
persisted after normalization for FVC. In this regard, elimina-
tion 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 VT response to CO₂
remain after correcting for FVC.

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

Effects of isooxic P(t)CO₂ (150 vs. 50 Torr) on chemoreflex
control characteristics. There was no main effect of isooxia on
basal V̇E (F = 0.32, P = 0.57), VT (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 VT was lower, whereas
chemoreflex responsiveness was higher, during hypoxic vs.
hyperoxic rebreathing trials, independent of gender. In con-
trast, 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 rebreath-
ing 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 isooxic
rebreathing condition (hyperoxic or hypoxic). Correlations

among these characteristics were tested by using a Pearson product-moment correlation analysis. Neither basal Ve and VRT (r = 0.34, P = 0.08) or basal Ve and Ve chemosensitivity (r = 0.36, P = 0.06) were significantly correlated for hyperoxic tests. Similarly, basal Ve and VRT were not significantly correlated (r = 0.07, P = 0.72) for hypoxic tests. However, a significant correlation between basal Ve and Ve 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 Ve (uncorrected) and VT (both corrected and uncorrected) responses to hyperoxic and hypoxic CO2 rebreathing. However, the central chemoreflex VRT for CO2 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, respiratory muscle fatigue, changes in cerebral blood flow (CBF), and state of arousal and/or anxiety, that could influence the ventilatory response after voluntary hyperventilation (14, 16, 18).

Short-term potentiation after hypocapnic hyperventilation is seldom observed during wakefulness in healthy subjects (14, 15, 19). When it is observed, the time constant is much less than the time required to reach the VRT for CO2 (14–16, 19). Furthermore, Morelli et al. (20) recently demonstrated that the time constant of the poststimulus potentiation induced by hypocapnic hyperventilation was not significantly different between men and women. Similarly, Jordan et al. (11) found no evidence of a gender or menstrual cycle phase effect on the time constant of the ventilatory decline after hypoxic and hypercapnic ventilatory stimulation, respectively. These findings suggest that short-term potentiation had no effect on the VRT calculated from hyperoxic and hypoxic rebreathing trials, independent of gender within the present study.

It is unlikely that the ventilatory response to hyperoxic and hypoxic CO2 rebreathing was influenced by respiratory muscle fatigue because subjects breathed through a low-resistance apparatus for a shorter period of time than that previously shown to cause diaphragmatic fatigue under loaded conditions.

### Table 2. Correlations between hyperoxic and hypoxic rebreathing responses (all subjects) and anthropometric and spirometric variables

<table>
<thead>
<tr>
<th>Basal (subthreshold)</th>
<th>Condition</th>
<th>Age, yr</th>
<th>Height, cm</th>
<th>Weight, kg</th>
<th>BMI, kg/m²</th>
<th>FVC, liters</th>
<th>FEV₁, liters</th>
<th>BSA, m²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ve, l/min</td>
<td>Hyperoxic</td>
<td>-0.32</td>
<td>0.44*</td>
<td>0.28</td>
<td>0.07</td>
<td>0.50*</td>
<td>0.44*</td>
<td>0.34</td>
</tr>
<tr>
<td></td>
<td>Hypoxic</td>
<td>0.22</td>
<td>0.36</td>
<td>0.34</td>
<td>0.22</td>
<td>0.47*</td>
<td>0.44*</td>
<td>0.37</td>
</tr>
<tr>
<td>Vt, ml (BTPS)</td>
<td>Hyperoxic</td>
<td>-0.19</td>
<td>0.44*</td>
<td>0.30</td>
<td>0.07</td>
<td>0.50*</td>
<td>0.48†</td>
<td>0.34</td>
</tr>
<tr>
<td></td>
<td>Hypoxic</td>
<td>0.02</td>
<td>0.41*</td>
<td>0.38*</td>
<td>0.20</td>
<td>0.57†</td>
<td>0.53†</td>
<td>0.40*</td>
</tr>
<tr>
<td>Sensitivities to CO₂</td>
<td>Ve, l/min⁻¹·Torr⁻¹</td>
<td>Hyperoxic</td>
<td>0.29</td>
<td>0.45*</td>
<td>0.36</td>
<td>0.15</td>
<td>0.44†</td>
<td>0.45*</td>
</tr>
<tr>
<td></td>
<td>Hypoxic</td>
<td>0.11</td>
<td>0.39*</td>
<td>0.20</td>
<td>-0.01</td>
<td>0.37</td>
<td>0.45†</td>
<td>0.27</td>
</tr>
<tr>
<td></td>
<td>Hyperoxic</td>
<td>0.15</td>
<td>0.74†</td>
<td>0.72†</td>
<td>0.37</td>
<td>0.75†</td>
<td>0.78†</td>
<td>0.77†</td>
</tr>
<tr>
<td></td>
<td>Hypoxic</td>
<td>0.26</td>
<td>0.69†</td>
<td>0.54†</td>
<td>0.17</td>
<td>0.64†</td>
<td>0.72†</td>
<td>0.61†</td>
</tr>
</tbody>
</table>

Ve, minute ventilation; Vt, tidal volume. *Significant correlation at the P < 0.05 level. †Significant correlation at the P < 0.01 level.

### Table 3. Effects of gender on the ventilation, tidal volume, and breathing frequency response to CO₂ under hyperoxic and hypoxic rebreathing conditions

<table>
<thead>
<tr>
<th>Basal (subthreshold)</th>
<th>Condition</th>
<th>Women</th>
<th>Men</th>
<th>Women</th>
<th>Men</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ve, l/min</td>
<td>Hyperoxic</td>
<td>9.9±1</td>
<td>16.3±2.1*</td>
<td>10.5±1.3</td>
<td>17.7±2.1*</td>
</tr>
<tr>
<td></td>
<td>Hypoxic</td>
<td>3.1±0.4</td>
<td>3.3±0.4</td>
<td>3.2±0.4</td>
<td>3.6±0.5</td>
</tr>
<tr>
<td>Vt/FVC, l/min⁻¹·l⁻¹</td>
<td>Hyperoxic</td>
<td>731±46</td>
<td>1,070±156*</td>
<td>784±59</td>
<td>1,159±126*</td>
</tr>
<tr>
<td></td>
<td>Hypoxic</td>
<td>229±20</td>
<td>209±28</td>
<td>242±18</td>
<td>229±22</td>
</tr>
<tr>
<td>Ve/FVC, ml/l</td>
<td>Hyperoxic</td>
<td>14.2±1.1</td>
<td>16.1±1.9</td>
<td>13.9±1.3</td>
<td>16.2±1.9</td>
</tr>
<tr>
<td></td>
<td>Hypoxic</td>
<td>48.8±1.0</td>
<td>50.6±0.5*</td>
<td>46.1±0.9</td>
<td>48.6±0.6*</td>
</tr>
<tr>
<td>Ventilatory recruitment threshold for CO₂</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ve, Torr</td>
<td>Hyperoxic</td>
<td>47.5±0.6</td>
<td>49.3±0.5</td>
<td>43.8±0.6</td>
<td>43.9±0.7</td>
</tr>
<tr>
<td></td>
<td>Hypoxic</td>
<td>46.8±0.8</td>
<td>48.4±0.6</td>
<td>43.3±0.6</td>
<td>43.8±0.5</td>
</tr>
<tr>
<td>Vt, Torr</td>
<td>Hyperoxic</td>
<td>48.8±1.0</td>
<td>50.6±0.5*</td>
<td>46.1±0.9</td>
<td>48.6±0.6*</td>
</tr>
<tr>
<td></td>
<td>Hypoxic</td>
<td>2.83±0.27</td>
<td>4.35±0.39*</td>
<td>4.16±0.50</td>
<td>5.98±0.69*</td>
</tr>
<tr>
<td>Sensitivities to CO₂</td>
<td>Ve, l/min⁻¹·mmHg⁻¹</td>
<td>Hyperoxic</td>
<td>0.90±0.10</td>
<td>0.89±0.08</td>
<td>1.27±0.14</td>
</tr>
<tr>
<td></td>
<td>Hypoxic</td>
<td>102.58±8.80</td>
<td>220.09±20.51*</td>
<td>157.01±15.68</td>
<td>335.72±43.30*</td>
</tr>
<tr>
<td></td>
<td>Hyperoxic</td>
<td>31.56±2.39</td>
<td>43.71±3.72*</td>
<td>47.89±4.35</td>
<td>66.99±8.25*</td>
</tr>
<tr>
<td></td>
<td>Hypoxic</td>
<td>1.50±0.25</td>
<td>0.95±0.15</td>
<td>1.45±0.28</td>
<td>1.50±0.26</td>
</tr>
</tbody>
</table>

Values are means ± SE. f, Respiratory rate. *Significant between-gender difference, P < 0.05.
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 CO\textsubscript{2} by modifying the arteriovenous difference and thus the relationship between PET\textsubscript{CO\textsubscript{2}} and brain tissue PCO\textsubscript{2} (PtiCO\textsubscript{2}). 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 CO\textsubscript{2} at the start of rebreathing ensures that changes in CBF do not affect the arteriovenous difference via washout of PtiCO\textsubscript{2}, and consequently the slope of the ventilatory response to CO\textsubscript{2} (25). In addition, because the subject and bag are a closed system, both blood circulation and pulmonary ventilation act as mixing forces to reduce any differences between PET\textsubscript{CO\textsubscript{2}} and PtiCO\textsubscript{2} induced by voluntary hyperventilation (18).

The modified rebreathing procedure permits direct measurement of the VRT for CO\textsubscript{2} mediated by the central and peripheral chemoreflex. In contrast, other methods identify the chemoreflex threshold by extrapolation of the linear relation between VE and PET\textsubscript{CO\textsubscript{2}} to the x-axis (7). Consequently, the threshold is taken as the PET\textsubscript{CO\textsubscript{2}} axis intercept and/or apneic threshold (7). If the extrapolated threshold were a true estimate of the apneic threshold, then the VRT for CO\textsubscript{2} measured using the modified rebreathing procedure would depend on basal VE, independent of the level of O\textsubscript{2}. 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 CO\textsubscript{2} 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 CO\textsubscript{2} 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 CO\textsubscript{2}. 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 hypoxic 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.

**Table 4. Effect of gender on the central and peripheral chemoreflex contribution to the ventilatory, tidal volume, and breathing frequency response to CO\textsubscript{2}**

<table>
<thead>
<tr>
<th>Sensitivities to CO\textsubscript{2}</th>
<th>Central Chemoreflex</th>
<th>Peripheral Chemoreflex</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Women</td>
<td>Men</td>
</tr>
<tr>
<td>V\textsubscript{E}, l/min\textsuperscript{-1}\textsuperscript{-1}mmHg\textsuperscript{-1}</td>
<td>2.83±0.27</td>
<td>4.35±0.39*</td>
</tr>
<tr>
<td>V\textsubscript{E}/FVC, l/min\textsuperscript{-1}mmHg\textsuperscript{-1}</td>
<td>0.90±0.10</td>
<td>0.89±0.08</td>
</tr>
<tr>
<td>V\textsubscript{T}, ml/min\textsuperscript{-1}mmHg\textsuperscript{-1}</td>
<td>102.58±8.80</td>
<td>220.09±20.51*</td>
</tr>
<tr>
<td>V\textsubscript{T}/FVC, ml/min\textsuperscript{-1}mmHg\textsuperscript{-1}</td>
<td>31.56±2.39</td>
<td>43.71±3.72*</td>
</tr>
<tr>
<td>f, breaths/min\textsuperscript{-1}mmHg\textsuperscript{-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.
Status (20, 35, 37). In keeping with these latter results, we have shown that both Ve (uncorrected) and Vt (corrected and uncorrected) responses to isooxic hyperoxic CO2 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 CO2. Most studies have shown that chemoresponsiveness, and consequently the apneic threshold for CO2, 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 CO2 estimated from isooxic hyperoxic CO2 rebreathing trials. Our data confirm these latter results as estimates of the central chemoreflex VRT for CO2 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 CO2 is apparent from the curvilinear relation between Ve and PETCO2. Moreover, the PETCO2 chosen to represent isocapnia may be above or below the VRT for CO2, 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 CO2, mediated by the sum of both central and peripheral chemoreflexes.

The sensitivity of the Ve response to CO2 during hypoxic trials was significantly greater in men than women but not after correction for FVC. However, gender differences in the slope of the Vt response persisted even after correction for FVC. Because the peripheral chemoreflex contribution to the Ve (corrected and uncorrected), Vt (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 isooxic hypoxic CO2 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 CO2 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 patterning response to CO2. Inconsistencies in the literature regarding the effect of gender on the chemoreflex control of breathing may exist because Vt and f were not considered. As previously discussed, the slope of the Vt response to CO2 (both corrected and uncorrected) at high and low levels of O2 were significantly greater in men than women. In addition, the f recruitment threshold for CO2 was significantly lower in women, independent of the level of O2, even though the slope of the f response was similar. However, gender had no effect on the peripheral chemoreflex contribution to the Ve (corrected and uncorrected), Vt (corrected and uncorrected), and f response during hypoxic CO2 rebreathing. These findings are consistent with Sajkov et al. (27) and suggest that the ventilatory response to CO2, mediated by the central chemoreflex, is more critically dependent on an increase in f than Vt 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 CO2 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 CO2. Thus our data suggest that gender has no effect on the central and peripheral VRT for CO2, proceeding withdrawal of behavioral stimuli during sleep. Therefore, gender-related differences in the central and peripheral VRT for CO2 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 hypopnea (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 CO2. However, body size and therefore VCO2 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 CO2 during sleep is also greater in men than women because of the increasing hyperbolic relation between alveolar CO2 and alveolar ventilation at a higher VCO2 (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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