Chronic intermittent hypoxia increases the CO2 reserve in sleeping dogs

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Katayama K, Smith CA, Henderson KS, Dempsey JA. Chronic intermittent hypoxia increases the CO2 reserve in sleeping dogs. J Appl Physiol 103: 1942–1949, 2007. First published October 11, 2007; doi:10.1152/japplphysiol.00735.2007.—We hypothesized that chronic intermittent hypoxia (CIH) would induce a predisposition to apnea in response to induced hypocapnia. To test this, we used pressure support ventilation to quantify the difference in end-tidal partial pressure of CO2 (PETCO2) between eupnea and the apneic threshold (“CO2 reserve”) as an index of the propensity for apnea and unstable breathing during sleep, both before and following up to 3-wk exposure to chronic intermittent hypoxia in dogs. CIH consisted of 25 s of PETO2 = 35–40 Torr followed by 35 s of normoxia, and this pattern was repeated 60 times/h, 7–8 h/day for 3 wk. The CO2 reserve was determined during non-rapid eye movement sleep in normoxia 14–16 h after the most recent hypoxic exposure. Contrary to our hypothesis, the slope of the ventilatory response to CO2 below eupnea progressively decreased during CIH (control, 1.36 ± 0.18; week 2, 0.94 ± 0.12; week 3, 0.73 ± 0.05 1-min−1·Torr−1, P < 0.05). This resulted in a significant increase in the CO2 reserve relative to control (P < 0.05) following both 2 and 3 wk of CIH (control, 2.6 ± 0.6; week 2, 3.7 ± 0.8; week 3, 4.5 ± 0.9 Torr). CIH also 1) caused no change in eupneic, air breathing PaCO2; 2) increased the slope of the ventilatory response to hypercapnia after 2 wk but not after 3 wk compared with control; and 3) had no effect on the ventilatory response to hypocapnia. We conclude that 3-wk CIH reduced the sensitivity of the ventilatory response to transient hypocapnia and thereby increased the CO2 reserve, i.e., the propensity for apnea was reduced.

Patients with severe obstructive sleep apnea (OSA) have been shown to be predisposed to instability in central ventilatory control during sleep. That is, when upper airway obstruction was relieved via tracheostomy (21), continuous positive airway pressure (CPAP; Ref. 29), or proportional assist ventilation (32), the patients with the most severe OSA still tended to show unstable breathing patterns. This tendency could result from an inherent enhancement of the slope of the ventilatory response to CO2 above and/or below eupnea in these patients. Alternatively, the chronic intermittent hypoxia (CIH) experienced by these patients during OSA repeated over many nights might also influence the slope of the ventilatory response to CO2 above and/or below eupnea, thereby exacerbating ventilatory instability. For example, acute hypoxia in sleeping dogs or healthy humans has been shown to increase the ventilatory response slope to transient reductions in PETCO2 below eupnea (hypocapnia), thereby increasing the predisposition for apnea and instability by decreasing the difference between eupneic end-tidal partial pressure of CO2 (PETCO2) and the PETCO2 at the apneic threshold (CO2 reserve; Refs. 18, 31). In addition, both acute and short-term hypoxemia are known to increase the ventilatory responsiveness to hypercapnia (5, 12, 19), which would tend to enhance the propensity for transient ventilatory “overshoots,” hypocapnia, and unstable breathing. Accordingly, in the present study our primary hypothesis was that CIH would increase the slope of the ventilatory response to hypocapnia, thereby decreasing the CO2 reserve. In addition, we also hypothesized that CIH would increase the slope of the ventilatory response to hypercapnia and hypoxia, as these responses may be determinants of the magnitude of the ventilatory overshoot. We exposed healthy dogs to 3 wk of intermittent hypoxia (7–8 h daily), which mimicked an apnea index of 60 events per hour and then determined the apneic threshold and CO2 reserve by means of a pressure support ventilation technique.

Methods

Four unanesthetized female mixed-breed dogs weighing between 16 and 18 kg were studied. The dogs were trained to lie quietly in an air conditioned (19–21°C), sound-attenuated chamber. The surgical and experimental protocols of this study were approved by the Animal Care and Use Committee of the University of Wisconsin-Madison.

Chronic Instrumentation

One surgical procedure was performed under general anesthesia with strict sterile surgical techniques and appropriate postoperative analgesics and antibiotics (3, 18). A chronic tracheostomy was created, and a five-lead electroencephalogram (EEG)/electrooculogram montage was installed. Electrode wires were tunneled subcutaneously to the cephalad portion of the dog’s back where they were exteriorized. To remove the influence of cyclic changes in ovarian hormones, ovariectomy/hysterectomy was also performed. In addition, a chronic femoral arterial catheter was installed in three of the four dogs.

Experimental Setup—CIH

Dogs were intubated via their chronic tracheostomies and placed in a cage 71 cm in width, 104 cm in length, 79 cm in height cage with a soft, padded floor. The dogs had enough room to stand and turn around and were free to choose their body position. A length of lightweight tubing connected the dogs to the gas mixing system via a swivel. Intermittent hypoxia was provided by a computer-controlled gas mixing system such that PETO2 of 35–40 Torr was achieved for 25 s followed by 35 s of normoxia, and this pattern was repeated 60 times an hour, 7–8 h/day (1000 to 1700–1800) for 3 wk. During the daily hypoxic sessions, the dogs were disconnected from the system every 2 h and removed from the cage for 10 min, during which time they had free access to food and water and could also defecate and urinate if desired. No attempt was made to assess sleep/wakefulness when the dogs were in the cage but most chose to lie prone or in lateral recumbency and appeared to sleep/doze most of the time. At the end of the 7–8 h-exposure, the dogs were returned to normal housing.

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Experimental Setup—Determination of the CO₂ Reserve and Ventilatory Response to Hypercapnia

For determination of the CO₂ reserve and other ventilatory variables, the dogs were placed on a soft bed in our canine sleep laboratory 14–16 h after the most recent hypoxic exposure. All measurements were performed in a normoxic background. Standard canine criteria were applied to identify the sleep stages (25). Non-rapid eye movement (NREM) sleep was defined as a synchronized low-frequency (<10 Hz) EEG associated with an absence of rapid eye movement. EEG arousal was defined as a desynchronization and speeding (>10 Hz) of the EEG for >3 s. Ventilatory and cardiovascular parameters were recorded for at least 10 min during NREM sleep and wakefulness. The dogs breathed via a cuffed endotracheal tube (10.0 mm outer diameter; Shirley, Irvine, CA), which was inserted into the chronic tracheostomy. Airflow was measured via a heated pneumotachograph system (model 3700; Hans Rudolph, Kansas City, MO, and model MP-45-14-871; Validyne, Northridge, CA) connected to the endotracheal tube. The pneumotachograph was calibrated before each study with four known flows. Tracheal pressure was measured with a pressure transducer (model MP-45–871; Validyne) connected to a port in the endotracheal tube by means of a 1.7 mm inner diameter high durometer polyvinyl chloride tubing (Abbott Laboratories, North Chicago, IL). The pressure transducer was calibrated before each study by applying four known pressures. Airway fractions of CO₂ and O₂ were monitored by means of an infrared CO₂ analyzer (Sable Systems, Las Vegas, NV) and/or a mass spectrometer (model MGA-1100, Perkin-Elmer, Norwalk, CT) depending on experimental requirements. Arterial blood pressure was continuously monitored with a pressure transducer (Statham) connected to the femoral catheter. The blood pressure transducer was calibrated against five known pressures before each measurement. Mean arterial blood pressure and heart rate were calculated beat by beat from blood pressure wave forms obtained from the blood pressure transducer. All signals were digitized (128-Hz sampling frequency) and stored in the hard disk of a personal computer for subsequent analysis. Key signals were also recorded continuously on a polygraph (AstroMed K2G). All ventilatory and blood pressure data were analyzed with software developed in our laboratory. Arterial blood samples were obtained from the femoral catheter and analyzed for partial pressure of CO₂ and O₂ at the end of each period for at least 10 min during NREM sleep and wakefulness. The dogs breathed via a cuffed endotracheal tube (10.0 mm outer diameter; Shirley, Irvine, CA), which was inserted into the chronic tracheostomy. Airflow was measured via a heated pneumotachograph system (model 3700; Hans Rudolph, Kansas City, MO, and model MP-45-14-871; Validyne, Northridge, CA) connected to the endotracheal tube. The pneumotachograph was calibrated before each study with four known flows. Tracheal pressure was measured with a pressure transducer (model MP-45–871; Validyne) connected to a port in the endotracheal tube by means of a 1.7 mm inner diameter high durometer polyvinyl chloride tubing (Abbott Laboratories, North Chicago, IL). The pressure transducer was calibrated before each study by applying four known pressures. Airway fractions of CO₂ and O₂ were monitored by means of an infrared CO₂ analyzer (Sable Systems, Las Vegas, NV) and/or a mass spectrometer (model MGA-1100, Perkin-Elmer, Norwalk, CT) depending on experimental requirements. Arterial blood pressure was continuously monitored with a pressure transducer (Statham) connected to the femoral catheter. The blood pressure transducer was calibrated against five known pressures before each measurement. Mean arterial blood pressure and heart rate were calculated beat by beat from blood pressure wave forms obtained from the blood pressure transducer. All signals were digitized (128-Hz sampling frequency) and stored in the hard disk of a personal computer for subsequent analysis. Key signals were also recorded continuously on a polygraph (AstroMed K2G). All ventilatory and blood pressure data were analyzed with software developed in our laboratory. Arterial blood samples were obtained from the femoral catheter and analyzed for partial pressure of CO₂ and O₂ (Paco₂ and PacO₂) and pH with a blood-gas analyzer (model ABL-505, Radiometer, Copenhagen, Denmark). The blood gas analyzer was calibrated daily with dog blood tonometered with three different combinations of PO₂ and PCO₂ covering the range encountered in the experiments. Samples were corrected for both body temperature and systematic errors revealed by tonometry. This is the same system that we have used in several previous studies (3, 4, 17, 18).

Measurements

Baseline parameters. Baseline ventilatory and cardiovascular variables were measured during both NREM sleep and wakefulness. Three 1-ml arterial blood samples were obtained within a 2-min sampling period, and data from two such periods were obtained in each condition. Ventilatory and cardiovascular variables from the two periods in each condition were averaged.

Apneic threshold. CO₂ reserve determination was performed during NREM sleep. All trials that had arousals and/or sleep state change during the control or experimental periods were excluded from further analysis. The inspiratory and expiratory tubes of the ventilator were connected to the pneumotachograph using a Y-connector such that the dog could breathe spontaneously from room air. Data from the two trials were combined.

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Ventilatory and blood pressure responses to hypoxia. Ventilatory and arterial blood pressure (BP) responses to isocapnic hypoxia were measured using a steady-state method. Dogs breathed air and three levels of increased CO₂ fraction (3, 4, and 5% CO₂ in air) with a normoxic background and each level of CO₂ was presented for 3 min. Inspired minute ventilation (V̇I) and PETCO₂ from the last minute of each 3-min period were averaged. The same procedure was repeated five to six times with a 5-min interval between trials. Data from all trials were combined, and the slope of hypercapnic ventilatory response was determined by linear regression (ΔV̇I/ΔPETCO₂, 1/min<sup>−1</sup>-torr<sup>−1</sup>; Ref. 28).

Interpreting the CO₂ reserve. The CO₂ reserve as defined in the previous paragraph is an index of the propensity for apnea at the prevailing background ventilatory drive. It is the result of two factors, namely the gain of the ventilatory response to CO₂ below eupnea (“controller gain”; the slope determined by a line connecting the eupneic point with the apneic point, i.e., ΔV̇I/ΔPETCO₂) and the “plant gain” (ΔPETCO₂/ΔV̇A; where V̇A is alveolar ventilation) as determined under the prevailing eupneic conditions (i.e., by the point of intersection of PETCO₂ with V̇A along a given isometabolic line defined by the equation: PacO₂ = [V̇CO₂/V̇A]k, where V̇CO₂ is CO₂ production and k is a constant (3, 4)).

Ventilatory response to hypercapnia (gain above eupnea). The measurement of hypercapnic ventilatory response was performed during wakefulness. The steady-state method was used to determine the response, i.e., dogs breathed air and three levels of increased CO₂ fraction (3, 4, and 5% CO₂ in air) with a normoxic background and each level of CO₂ was presented for 3 min. Inspired minute ventilation (V̇I) and PETCO₂ from the last minute of each 3-min period were averaged. The same procedure was repeated five to six times with a 5-min interval between trials. Data from all trials were combined, and the slope of hypercapnic ventilatory response was determined by linear regression (ΔV̇I/ΔPETCO₂, 1/min<sup>−1</sup>-torr<sup>−1</sup>; Ref. 28).

Experimental Protocol

Measurements were performed over the course of 2–10 days before (control) and during 2 wk and 3 wk of CIH. All experimental trials were conducted from 0700 to 1000. More than one laboratory session was required to obtain a sufficient number of acceptable pressure support ventilation trials during NREM sleep.
Accordingly, in each experimental week, Monday through Thursday was devoted to determination of the CO₂ reserve. On Tuesdays, in addition to CO₂ reserve determinations, more extensive baseline ventilatory and blood gas determinations were performed. Friday was devoted to determination of the ventilatory response to CO₂, and Saturday was used for the determination of the ventilatory response to hypoxia. On Tuesday, in addition to CO₂ reserve determinations, baseline eupneic blood gases, ventilatory, and cardiovascular measurements were obtained.

Statistical Analysis

Values are expressed as means ± SE. The changes in all parameters during the experimental period periods were analyzed using one-way ANOVA with repeated measurements and the Bonferroni correction was used post hoc to test for significance between time periods (control, week 2, week 3). The StatView statistical package (SAS Institute, Tokyo, Japan) was used for these analyses. A P ≤ 0.05 was considered to be significant.

RESULTS

Baseline Parameters

Table 1 shows that there were no significant changes in any of the eupneic blood gas, ventilatory, and cardiovascular variables following 2 and 3 wk of CIH in normoxia during NREM sleep. It was clear from visual inspection that there were no signs of unstable/periodic breathing during spontaneous eupneic breathing in the sleeping animal in control conditions or following the intermittent hypoxic exposures. Similarly, during wakefulness, there were no significant changes in any of the variables (data not shown).

CO₂ Reserve

Figure 2 is a compilation of polygraph recordings of representative trials using PSV to determine the apneic threshold and CO₂ reserve during NREM sleep before and following 2 and 3 wk of CIH (PSV performed in normoxia 14–16 h after most recent hypoxic exposure). In this dog before CIH (control), PB occurred when CO₂ reserve was 2.7 Torr; decreases in PETCO₂ < 2.7 Torr did not produce apnea. Following 2 wk of CIH, the CO₂ reserve increased to 4.9 Torr. Following 3 wk of CIH, the CO₂ reserve increased further to 6.4 Torr. Figure 3 shows the changes in the CO₂ reserve in each dog throughout the experimental periods. The mean CO₂ reserve was widened significantly following both 2 and 3 wk of CIH compared with control (control, 2.6 ± 0.6; week 2, 3.7 ± 0.8; week 3, 4.5 ± 0.9 Torr; P < 0.05). The increase in the CO₂ reserve between week 3 and week 2 was just short of statistical significance (P = 0.0522).
Ventilatory Response to CO2 Above Eupnea and NREM sleep in normoxia

Table 1. Ventilatory and cardiovascular parameters during NREM sleep in normoxia

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Week 2</th>
<th>Week 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ti, s</td>
<td>2.15±0.77</td>
<td>1.69±0.28</td>
<td>1.95±0.40</td>
</tr>
<tr>
<td>Te, s</td>
<td>4.08±1.89</td>
<td>3.25±0.42</td>
<td>3.67±1.21</td>
</tr>
<tr>
<td>Vt, ml/min</td>
<td>331.4±93.4</td>
<td>291.8±91.2</td>
<td>292.4±51.8</td>
</tr>
<tr>
<td>fb, breaths/min</td>
<td>11.5±3.7</td>
<td>12.7±19</td>
<td>11.5±3.9</td>
</tr>
<tr>
<td>VI, l/min</td>
<td>3.40±0.52</td>
<td>3.68±0.53</td>
<td>3.26±0.51</td>
</tr>
<tr>
<td>PETCO2, Torr</td>
<td>40.1±1.6</td>
<td>39.6±1.7</td>
<td>39.0±2.7</td>
</tr>
<tr>
<td>PETO2, Torr</td>
<td>108.9±4.1</td>
<td>111.2±1.7</td>
<td>109.6±1.2</td>
</tr>
<tr>
<td>Paco2, Torr</td>
<td>3.71±0.3</td>
<td>36.8±3.45</td>
<td>35.3±2.45</td>
</tr>
<tr>
<td>Pao2, Torr</td>
<td>95.4±3.8</td>
<td>97.8±9.6</td>
<td>100.7±11.0</td>
</tr>
<tr>
<td>PHa</td>
<td>7.35±0.03</td>
<td>7.35±0.02</td>
<td>7.37±0.03</td>
</tr>
<tr>
<td>[HCO3]a, mEq/l</td>
<td>20.0±1.6</td>
<td>19.6±1.1</td>
<td>19.8±2.3</td>
</tr>
<tr>
<td>HR, beats/min</td>
<td>80.9±6.8</td>
<td>74.1±4.2</td>
<td>70.2±11.2</td>
</tr>
<tr>
<td>MBP, mmHg</td>
<td>88.4±9.4</td>
<td>85.6±7.7</td>
<td>86.8±7.4</td>
</tr>
</tbody>
</table>

Values are means ± SE. Ti, inspiratory time; Te, expiratory time; Vt, tidal volume; fb, breathing frequency; VI, inspired minute ventilation; PETCO2, end-tidal partial pressure of CO2; PETO2, end-tidal partial pressure of O2; Paco2, partial pressure of arterial CO2; Pao2, partial pressure of arterial O2; [HCO3]a, arterial bicarbonate concentration; HR, heart rate; MBP, mean arterial blood pressure. (n = 4 for Ti, Te, Vt, fb, VI, and PETCO2; n = 3 for PETO2, Paco2, Pao2, PHa, [HCO3]a, HR, and MBP).

In general, the greater the CO2 reserve, the greater was the pressure support required to achieve it. The mean amount of pressure support required to produce apnea and define the CO2 reserve increased from control (9.4 ± 0.4 cmH2O) to 11.8 ± 1.7 cmH2O in week 2 (increased in 3 of 4 dogs; not significant) and 13.0 ± 4 cmH2O in week 3 (increased in 4 of 4 dogs; P < 0.05). These increases in pressure support required to produce apnea are consistent with the greater reduction in PETCO2 required to cause apnea as a result of intermittent hypoxic exposure.

Ventilatory Response to CO2 Below Eupnea

Figure 4 shows the change in the slope of the ventilatory response to CO2 below eupnea, i.e., the hypocapnic ventilatory response, before and following CIH. The slope of the hypocapnic ventilatory response indicated a progressive decrease during CIH; the slopes at 2 and 3 wk were significantly (P < 0.05) lower than control (−29.4 ± 7.5% and −44.0 ± 7.0%).

Ventilatory Response to CO2 Above Eupnea

The change in the slope of the ventilatory response to hypercapnia before and following CIH is shown in Figure 5. The slope of the hypocapnic ventilatory response following 2 wk of CIH was significantly (P < 0.05) higher (52.0 ± 19.6%) than control. However, the slope of the ventilatory response to CO2 above eupnea after 3 wk was not significantly different than control, although it was increased in three of the four dogs (26.6 ± 19.2%).

Ventilatory and BP Responses to Isocapnic Hypoxia

Figure 6 shows the lack of changes in the ventilatory and mean BP responses to isocapnic hypoxia before and after CIH.

DISCUSSION

The major finding of the present study was that CIH induced a progressive decrease in the slope of the CO2 response below eupnea. This resulted in a significant increase in the CO2 reserve over the course of 3 wk of daily hypoxic exposures. This increased CO2 reserve was achieved despite no change in the eupneic PaCO2, i.e., all of the increase in the CO2 reserve was accounted for by the decrease in the CO2 response slope below eupnea. These data do not support our hypothesis that long-term exposure to CIH that mimicked the severity, duration, and number of the hypoxic episodes experienced in severe sleep apnea would sensitize the apneic threshold and decrease the CO2 reserve, thereby increasing the propensity for apnea following a ventilatory overshoot. Indeed, the data suggest that the opposite was true, i.e., CIH stabilized ventilation in the face of transient hypocapnia. The reasons for these findings are unclear but several possibilities exist.

Sex

We studied female dogs only. There appear to be sex-related quantitative differences in ventilatory control, at least in humans and rats (9, 16). However, all dogs in the present study underwent ovariectomy and hysterectomy, so ventilatory effects of ovarian hormones (1, 30, 34) were not present. Thus our female dogs would have an endocrinological profile that mimicked that of post-menopausal women whose incidence of sleep-disordered breathing is similar to that of men (33).

CO2 Reserve Determination

Our method of determining the CO2 reserve was intended to find the smallest decrease in PETCO2 that resulted in apnea and periodic breathing, i.e., the “apneic threshold.” Accordingly, multiple trials covering a 7- to 8-Torr range of decreases in PETCO2, below eupnea were employed to identify this point for a given week of the study (control, week 2, week 3; see METHODS). While quite conservative, our approach could be criticized for its dependence on a single point. An error in the determination of this point could cause us to over- or underestimate the CO2 reserve and affect our ability to accurately detect time-dependent changes in the CO2 reserve. However, we do not think this occurred for the following reasons. 1) The large number of trials employed per animal (16–33) each week assured that the pressure support ventilation-induced decreases in PETCO2, were closely spaced over the range of hypocapnia studied. Thus there were no large gaps in the independent variable that would have contributed to uncertainty in the determination of the CO2 reserve. 2) All four dogs exhibited a similar pattern of response, i.e., a progressive increase in the CO2 reserve and a progressive increase in the level of pressure support ventilation required to cause apnea over the duration of intermittent hypoxic exposure. This supports our contention that we detected real increases in the CO2 reserve. 3) When two of the four dogs in the present study were restudied several weeks after CIH exposures to assess the effects of chronic hypoxia (see discussion below), their control CO2 reserve values had returned to their prechronic intermittent hypoxia control levels, again supporting our ability to detect real changes in the CO2 reserve. 4) Previous studies from our laboratory using this technique in control conditions (3, 18) demonstrated across-dogs standard deviations (n = 6) in the CO2 reserve of between 0.6 and 0.8 Torr. In these studies, we were capable of detecting significant differences in the CO2 reserve between control and experimental conditions of <1 Torr.
Why did CIH Fail to Decrease the CO2 Reserve?

In the present study, the severity of hypoxia and pattern of normoxic/hypoxic cycles mimicked that experienced by human sleep apnea sufferers with an apnea hypopnea index of 60, which should have been adequate to sensitize the apneic threshold chronically (i.e., more than a few hours after the last hypoxic period) if CIH, per se, does indeed cause such a sensitization. Our experiments were limited to 3-wk duration so it is possible that much longer exposures to CIH are required to elicit this effect. However, studies in rats and humans have shown that relatively short durations of daily exposure to CIH in the unanesthetized state can elicit changes in ventilatory responses to hypoxia and hypercapnia that persist for many hours after normoxic conditions are restored. For example, Peng and Prabhakar (23) exposed rats to cycles of 15 s of hypoxia followed by 5 min of normoxia nine times per hour, 8 h/day for 10 days. The rats were studied under anesthesia for carotid sinus nerve recording or were killed for ex vivo recording of the carotid sinus nerve in a superfused carotid body preparation 16 h after the last hypoxic exposure. The authors observed that the carotid sinus nerve discharge in response to hypoxia was enhanced both in vivo and ex vivo. Interestingly, there was no change in the responsiveness of the carotid sinus nerve discharge to hypercapnia. Ling et al. (13) made similar observations. They exposed rats to alternating episodes of 5 min of hypoxia, 5 min of normoxia 12 h/day for 7 days. The rats were studied under anesthesia 4–8 h after the last intermittent hypoxic exposure. They observed that the phrenic discharge response to acute hypoxia was increased and the magnitude of long-term facilitation up to 60 min following the acute hypoxic exposure was enhanced. In the human, Garcia et al. (7) observed that 2 h of hypoxia per day for 12 days increased the hypoxic ventilatory response (determined 22 h after the last hypoxic exposure) progressively with the maximum effect on day 5 followed by a decline to near control values by day 12. Also in humans, Serebrovskaya et al. (27) found that three daily episodes of FIO2 0.07– 0.08 for 5– 6 min each for 14 days was sufficient to increase the ventilatory response to moderate isocapnic hypoxia. Mahamad and Duffin (14) exposed humans to 20 min of isocapnic hypoxia per day for 14 days and observed that the ventilatory threshold for CO2 response in a background of moderate hypoxia (via an isoxic rebreathe test) decreased progressively over the course of the experiment, indicating a sensitization of ventilatory responsiveness to CO2. Katayama et al. exposed humans to 3 h of hypoxia per day for up to 2 wk (10) or 1 h of hypoxia per day for 1 wk (11). They found that the ventilatory response to hypoxia was enhanced with either paradigm of intermittent hypoxia when measured about 24 h after the last hypoxic exposure but the ventilatory response to hypercapnia was
increased only after 2-wk exposure to 3 h of hypoxia per day.  
Despite these changes in sensitivity of the ventilatory  
responses to hypoxia or hypercapnia, no changes in the eupneic,  
air breathing PETCO₂ were observed.

In summary, while it remains controversial whether CIH can  
affect the ventilatory response to CO₂ and whether the en-
hanced hypoxic response is mediated centrally and/or by the  
carotid bodies, it seems clear that, despite varying paradigms of  
intermittent hypoxic exposure (15 s to 3 h; 1–72 episodes per  
day), as little as 7–14 days of intermittent hypoxic exposure  
can elicit changes in chemoreceptor responsiveness. At least in  
the human studies (where PETCO₂ data were available) this  
ocurred despite no change in the eupneic PETCO₂ over the  
course of CIH (8, 10, 14, 27).

Fig. 3. Changes in the CO₂ reserve during NREM sleep  
before and during CIH (2 and 3 wk) in each dog. Top of bar,  
eupneic PETCO₂; bottom of bar, apneic threshold PETCO₂. The  
number in the boxes preceded by Δ are the CO₂ reserves in  
Torr for each condition.

Fig. 4. Changes in the slope of the ventilatory response to CO₂ below eupnea  
(hypocapnic ventilatory response) before (control, 1.36 ± 0.18 l·min⁻¹·Torr⁻¹)  
and during CIH (week 2, 0.94 ± 0.12 l·min⁻¹·Torr⁻¹; week 3, 0.73 ± 0.05,  
l·min⁻¹·Torr⁻¹). A: mean slopes are superimposed on an assumed theoretical  
isometabolic line for purposes of illustration (PaCO₂ = VCO₂/Vₐ·k. Vₐ was  
calculated assuming a reasonable VCO₂ for a 20-kg dog of 150 ml/min and k =  
0.863). B: mean values ± SE. *Significantly different than control (P < 0.05).

Fig. 5. Changes in the CO₂ slope above eupnea (hypercapnic ventilatory  
response) before (control) and during chronic intermittent hypoxia (week 2 and  
week 3) A: mean slopes are superimposed on an assumed theoretical isometabolic  
line for purposes of illustration (see Fig. 4 for description). Note that this  
is the same line plotted in Fig. 4 but on a slightly different scale to better  
illustrate slope changes. B: mean values ± SE. *Significantly different than  
control (P < 0.05).
Why did CIH Increase the CO2 Reserve?

It is well established that acclimatization to chronic hypoxia (as opposed to acute hypoxia) lessens the severity of periodic breathing in sleeping humans while in the hypoxic environment (26). Acute hypoxic exposure promotes apnea and periodic breathing in sleep due to the increased controller gain (Gc) caused by the concomitant hyperventilation (decreased eupneic PaCO2). Chronic hypoxic exposure, however, results in ventilatory acclimatization that is characterized by a further decrease in eupneic PaCO2 and decrease in Gp that would tend to increase the CO2 reserve despite the increased Gc. However, in the present study, ventilatory acclimatization cannot explain the decreased ventilatory response to CO2 below eupnea because the eupneic PaCO2, (i.e., as determined 14–16 h posthypoxia) did not change over the 3-wk course of the experiment. This finding is not unprecedented, as others have shown no change in eupneic PaCO2, (~24 h after the last hypoxic exposure) in response to CIH even in the face of increased ventilatory responsiveness to acute hypoxia (10, 11).

Our observations, obtained in normoxia 14–16 h after the most recent hypoxic exposure, would also appear to rule out a contribution from long-term facilitation in the widening of the CO2 reserve. If long-term facilitation were active as a result of CIH, one would predict continued hyperventilation (2, 20, 24), but we observed no hyperventilation after 14–16 h of normoxia in the present study. Although the mechanisms remain unknown, it is clear that CIH decreases the sensitivity of the ventilatory response to hypcapnia and increases the CO2 reserve in the absence of changes in eupneic ventilation. In two of the dogs in the present study we confirmed that intermittent hypoxia did have different effects on eupneic ventilation and the CO2 reserve relative to continuous hypoxia. When we exposed these dogs to 8 h of continuous hypoxia daily for 7 days, with the same PETO2 used in the CIH study (35–40 Torr), we observed that the CO2 reserve determined 14–16 h posthypoxia increased as it did following CIH but there was also persistent hyperventilation (PaCO2 remained 2.2–2.5 Torr below the eupneic value in normoxia), indicating that ventilatory acclimatization had occurred (data not shown). As we followed the change in CO2 reserve over time in the normoxic recovery period in these two dogs (up to 64 h), we found progressive increases in eupneic PaCO2 back toward normoxic control levels that correlated well with a return (decrease) in the CO2 reserve to control. Thus, in the acclimatized dogs exposed to chronic hypoxia, Ga did not increase in the face of a decreased Gp resulting in an increased CO2 reserve whereas dogs exposed to CIH had no change in Gp so the increased CO2 reserve was due solely to decreased Gc.

Gains of the Ventilatory Responses to CO2 Above and Below Eupnea

It is noteworthy that CIH decreased the gain of the ventilatory response to hypcapnia while at the same time the gain of the ventilatory response to hypercapnia was either increased (week 2) or unchanged (week 3). These findings are consistent with the observations of Mateika and Ellythy (15) in awake human OSA patients. These authors used a rebreathing technique to demonstrate that the ventilatory recruitment threshold for CO2 was increased in awake OSA patients relative to healthy control subjects (i.e., a reduced ventilatory gain to CO2 below eupnea) but the ventilatory response to elevated CO2 above the "recruitment threshold" was the same for both groups.

Gains of the Ventilatory Responses to Hypoxia

Despite observed increases in the ventilatory response to hypcapnia and the trend toward increased ventilatory response to hypercapnia, the ventilatory response to hypoxia was unchanged during 3 wk of CIH. The reasons for this lack of association are unclear, but the findings clearly suggest that CIH-induced changes in ventilatory response gain to CO2 are independent of changes in hypoxic ventilatory response gain. On the one hand, this is surprising in light of observations in rats showing an enhanced sensitivity of ex vivo carotid bodies to hypoxia following whole animal CIH (22, 23). We speculate that these differences are due to species differences and/or differences in the duration, timing, and severity of the CIH paradigm. On the other hand, lack of change of the sensitivity of the ventilatory response to CO2 in the face of increased hypoxic ventilatory sensitivity during chronic hypoxic exposure is not unprecedented (e.g., Refs. 6, 12), so this dissociation appears not to be unique to CIH.

In summary, 3 wk of daily CIH resulted in changes in ventilatory control that caused an increase in the CO2 reserve thereby promoting ventilatory stability during sleep in nor-
moxia. This enhanced stability was not the result of normal ventilatory acclimatization to hypoxia but apparently was unique to CIH.

**Implications for Sleep Apnea**

The present findings demonstrate that 3 wk of CIH in the dog did not increase the propensity for hypocapnia-induced apnea during sleep. Rather, the CIH promoted stability of breathing. If these findings can be extrapolated to human sleep apnea patients there are several implications. 1) CIH per se probably does not exacerbate central sleep apnea and, in fact, should act to minimize it. 2) It follows that ventilatory instability or increased loop gain (Gp + Gc) probably is not acquired in OSA patients (i.e., secondary to OSA-induced CIH) but may depend on preexisting deficits in the ventilatory control system that are manifested when the system is stressed by such factors as increased upper airway resistance, unstable sleep state, or alcohol consumption.

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**REFERENCES**