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Genioglossal Muscle Response to CO₂ Stimulation During NREM Sleep

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Abstract

Study Objectives—The objective was to evaluate the responsiveness of upper airway muscles to hypercapnia with and without intrapharyngeal negative pressure during non-rapid eye movement (NREM) sleep and wakefulness.

Design—We assessed the genioglossal muscle response to CO₂ off and on continuous positive airway pressure (CPAP) (to attenuate negative pressure) during stable NREM sleep and wakefulness in the supine position.

Setting—Laboratory of the Sleep Medicine Division, Brigham and Women's Hospital.

Patients or Participants—Eleven normal healthy subjects.

Interventions—During wakefulness and NREM sleep, we measured genioglossal electromyography (EMG) on and off CPAP at the normal eupneic level and at levels 5 and 10 mm Hg above the awake eupneic level.

Measurements and Results—We observed that CO₂ could increase upper- airway muscle activity during NREM sleep and wakefulness in the supine position with and without intrapharyngeal negative pressure. The application of nasal CPAP significantly decreased genioglossal EMG at all 3 levels of PETCO₂ during NREM sleep ($13.0 \pm 4.9\%$ vs. $4.6 \pm 1.6\%$ of maximal EMG, $14.6 \pm 5.6\%$ vs. $7.1 \pm 2.3\%$ of maximal EMG, and $17.3 \pm 6.3\%$ vs. $10.2 \pm 3.1\%$ of maximal EMG, respectively). However, the absence of negative pressure in the upper airway did not significantly affect the slope of the pharyngeal airway dilator muscle response to hypercapnia during NREM sleep ($0.72 \pm 0.30\%$ vs. $0.79 \pm 0.27\%$ of maximal EMG per mm Hg PCO₂, respectively, off and on CPAP).

Conclusions—We conclude that both chemoreceptive and negative pressure reflex inputs to this upper airway dilator muscle are still active during stable NREM sleep.

Keywords

Genioglossus; hypercapnia; sleep; upper airway; pharynx; continuous positive airway pressure; respiration

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INTRODUCTION

Obstructive Sleep Apnea (OSA) is a common disorder characterized by recurrent collapse of the upper airway leading to repetitive episodes of hypoxemia, hypercapnia, and arousal from sleep.^{1,2} This disorder affects 2% to 4% of the middle-aged population³ and is associated with important adverse consequences for afflicted individuals.⁴⁻⁶ The pathogenesis of OSA is complicated and is likely due to the combination of an anatomically small pharyngeal airway⁷⁻⁹ and loss of reflex-driven neuromuscular compensation in pharyngeal dilator muscles during sleep.^{10,11} Therefore, it is important to understand the mechanisms controlling upper airway muscle activity during wakefulness and sleep.

Considerable effort has been exerted to define the stimuli modulating upper airway muscle activity. Identified variables influencing upper airway muscle activity include inputs from the chemoreceptors,^{12,13} the brainstem central respiratory pattern generator,¹⁴ vagal input from lung volume,^{15,16} a tonic wakefulness drive to the respiratory system,^{17,18} sex-specific hormones,¹⁹ and intrapharyngeal negative pressure.^{20,21} Evidence suggests that intrapharyngeal negative pressure acting via mechanoreceptors primarily in the larynx^{22,23} leads to substantial increases in upper airway muscle activity.^{11,24}

During wakefulness, chemoreceptor activation has been shown to increase upper airway muscle activity during normoxic and hyperoxic hypercapnia.^{12,25} In addition, studies of patients with laryngectomy or tracheotomy have demonstrated that hypercapnia can increase upper airway muscle activity without pressure changes in the upper airway.^{26,27} However, during non-rapid eye movement (NREM) sleep, it is unclear whether CO₂ can increase upper airway muscle activity and whether any such hypercapnic effect on upper airway muscle activity requires intrapharyngeal negative pressure. The available literature regarding the responsiveness of upper-airway muscles to hypercapnia during sleep is inconsistent. Pillar's and Stanchina's physiology studies have shown that the pharyngeal muscles have little responsiveness to CO₂ stimulation alone.^{2,28,29} However, Berry's and Worsnop's observations have shown that substantial increases in upper airway dilator muscle activity occur both in normal subjects between the onset of sleep and stable NREM sleep as PCO₂ rises and in patients with OSA across an apnea.^{30,31} One possible explanation for these conflicting data may be due to the fact that Pillar's and Stanchina's studies were completed with subjects in the lateral posture and Berry's and Worsnop's observations were completed with subjects in the supine posture. However, the actual stimulus to upper airway muscle activity during these studies was unclear because any increase in upper airway muscle activity with rising PCO₂ could be due to increased chemoreceptor activation or increasing negative pressure in the airway. Because nasal continuous positive airway pressure (CPAP) applied to the upper airway leads to a decrement in pharyngeal muscle activity,¹⁰ assumedly by attenuating the negative pressure reflex input, CPAP could be used to evaluate whether chemoreceptor or negative pressure activation is the primary stimulus to pharyngeal muscle activity as CO₂ is increased. Thus, the application of positive pressure can be used to dissociate chemoreceptor from negative pressure effects during NREM sleep.

In this study, we used the application of nasal CPAP and the administration of CO₂ to study the upper airway muscle responsiveness to hypercapnia with and without intrapharyngeal negative pressure during NREM sleep and wakefulness in normal healthy humans.

METHODS

Subjects

Eleven subjects were initially studied with monitoring of both genioglossus and tensor palatini muscle activity during sleep and wakefulness. Only 2 of the subjects could maintain adequate sleep to complete the protocol described below. For subject comfort, we subsequently discontinued the monitoring of tensor palatini muscle activity and focused the study on the genioglossus muscle. Eleven healthy subjects (6 men, 5 women) without sleep complaints finished this study. This sample size was based on preliminary data obtained in the first 3 subjects who completed this study and a prior study from our laboratory. We determined that 10 subjects would be required, during NREM sleep with CPAP in place, to detect a difference of 5% in genioglossal activity during hypercapnia (10 mm Hg above the awake eupneic level) with 80% power and at the 5% significance level. Thus, 11 subjects were study. Their mean age was 28.5 ± 2.7 (S.E.M.) years, and their mean body mass index was 23.9 ± 0.7 kg/m². Written informed consent was obtained from each subject, with the protocol having the prior approval of the Human Subjects Committee of the Brigham and Women's Hospital. Women were studied during the follicular phase (day 5–11) of their menstrual cycle, as determined by history.

Equipment and Techniques

Ventilation—Subjects wore a nasal mask (Respironics, Inc., Murraysville, PA) connected to a heated pneumotachometer (model 3700A, Hans Rudolph Inc., Kansas City, MO) and a differential pressure transducer (Validyne Corp., Northridge, CA), calibrated with a rotameter, for measurement of airflow. Inspiratory and expiratory times were determined from this signal and it was integrated for calculation of tidal volume and minute ventilation. The subjects were instructed to breathe exclusively through the nose. This was ensured by taping the mouth and using an infrared video camera to document that the mouth remained closed.

Muscle Activation—The genioglossal electromyogram (EMG) was measured with a pair of unipolar intramuscular electrodes referenced to a single ground, producing a bipolar recording. Two stainless-steel, Teflon-coated, 30-gauge wire electrodes were inserted approximately 15 mm into the body of the genioglossal muscle 3 mm lateral to the frenulum on each side, using a 25-gauge needle. The needles were removed immediately, leaving the wires in place. This technique has been used previously in our laboratory.²²

The raw genioglossal EMG was amplified (Grass Instrument, Quincy, MA), band-pass filtered (between 30 and 1000 Hz), rectified, and electronically integrated on a moving time average basis with a time constant of 100 milliseconds (CWE, Inc., Ardmore, PA). To define maximal EMG for the muscle, subjects were asked to perform 4 maneuvers. They were instructed to (1) swallow, (2) maximally protrude their tongue against the maxillary alveolar ridge, (3) inspire maximally against an occluded tube, and (4) suck and blow. Each maneuver was performed several times, and the maximal EMG recorded during this calibration was designated as 100%. Electrical zero was then determined, and the genioglossal EMG was quantified as a percentage of maximal activation for that subject.

Since the genioglossal muscle is an inspiratory phasic muscle, its activation was assessed at 2 points during the respiratory cycle. The tonic activation was defined as the lowest activation during expiration, the peak activation as the highest activation during inspiration (i.e., peak phasic), and the phasic activation as the difference between the peak and tonic activation.

Sleep-Wake State—Two channels of electroencephalography (C3-A2, O2-A1), left and right electrooculograms, and the submental EMG were recorded to document wakefulness or sleep and score the different sleep stages.³² Subjects were maintained in the supine position throughout this study, verified by infrared video camera.

Nasal CPAP Application—We sought to determine the CPAP level that yielded the lowest level of genioglossal EMG during wakefulness. To accomplish this, nasal CPAP was initially applied (BiPAP® S/T-D, Respironics, Inc.) at 4 cm H₂O and increased until the minimal level of genioglossal EMG was obtained or to a maximum of 8 cm H₂O. If no obvious reduction in genioglossal EMG was discernible, the subjects were studied on 6 cm H₂O. The level of CPAP (mean 6.7 ± 0.1 cm H₂O) producing this minimal genioglossal EMG was then used throughout the protocol (see below).

CO₂ Administration, PETCO₂, and SaO₂ Measurement—PETCO₂ was measured from expired air within the nostril using a calibrated infrared CO₂ analyzer (Capnograph/Oximeter Monitor, BCI, Waukesha, WI) while SaO₂ was measured using a pulse-oximeter probe attached to the index finger (Capnograph/Oximeter Monitor). To assess the hypercapnic response, the inspired fraction of CO₂ was increased using a calibrated gas source (25% CO₂-25% O₂-balance N₂) bled into the inspiratory line to obtain a PETCO₂ of 5 and 10 mm Hg above the eupneic level during wakefulness.

Protocol

Each subject reported to the laboratory at approximately 8:00 PM, having fasted for at least 4 hours. After informed consent was obtained, the sleep-staging electrodes and intramuscular EMG wires were placed. Subjects then assumed the supine posture in bed, and the nasal mask and pneumotachograph were attached. Subjects subsequently lay with their eyes open in this posture and were allowed to acclimatize to the equipment. During documented wakefulness, data were recorded at 3 PETCO₂ levels: the normal eupneic level and plus 5 and plus 10 mm Hg above the eupneic level. After all signals were recorded for 10 minutes of eupneic respiration, the PCO₂ was increased and stabilized for 3 minutes at each level, after which data were recorded for 3 minutes. These measurements, at all 3 levels, were collected both on and off nasal CPAP (order randomized). After data were recorded during wakefulness, subjects were allowed to fall asleep. Once the subjects were in stable NREM sleep (stage 2, 3, and 4), the response to rising CO₂ was again assessed. First, measurements at the eupneic PCO₂ level were recorded for 3 minutes. Then, inspired CO₂ was increased to elevate the PCO₂ to exactly the same levels as were studied during wakefulness (5 and 10 mm Hg above the awake eupneic level). As the PCO₂ rose during sleep, these levels were +2 to +3 and +7 to +8 mm Hg above the eupneic sleeping PCO₂. As during wakefulness, the subjects were held at the desired PCO₂ for a full 3 minutes before acquiring 3 minutes of data. The CO₂ response during NREM sleep was measured both on and off CPAP with the order (CPAP or no CPAP first) being randomized. If the subjects awoke during CO₂ stimulation, they were returned to room-air breathing, and the data were discarded. A data set (eupneic and 5 and 10 mm Hg above awake eupneic levels), both on and off CPAP during wakefulness and stable NREM sleep, were recorded 1 time for each subject. Of note, even in the “off-CPAP” condition, subjects wore a CPAP mask. Thus, temperature and flow through the mask were quite similar under the 2 conditions.

Data Recording and Analysis

All signals (Flow, VT, PETCO₂, SaO₂, electrooculogram, EMG, electrocardiogram, and raw and moving time-averaged genioglossal EMG) were simultaneously recorded on a 16-channel Grass model 78 polygraph (Grass Instrument, Astro-Medical Inc, West Warwick, RI) and a personal computer using an analog-to-digital converter (1401plus, Cambridge

Electronic Design, Ltd, Cambridge, UK) and data-acquisition software (Spike 2, version 5.03, Cambridge Electronic Design, Ltd). The sampling rate varied from 125 Hz for respiratory signals to 1000 Hz for the raw EMG. For each recording period (wakefulness and NREM sleep both on and off CPAP at each PCO₂ level), a mean value for ventilation and EMG was determined. These results were then compared statistically.

All statistical analyses were performed with commercially available software (Excel 2000, Microsoft; and SigmaStat + Sigmaplot, SPSS, Chicago, IL). All data are presented as means \pm S.E.M. unless otherwise stated. Repeated-measures analysis of variance with post hoc Student Newman-Keuls testing was used to assess the effect of CO₂ on both ventilation and genioglossal EMG during wakefulness and NREM sleep both on and off CPAP. Whenever data were not normally distributed, Friedman repeated-measures analysis of variance on ranks was used. Ventilation and genioglossal EMG during wakefulness were compared to values during sleep using a paired t test at each PCO₂ level both on and off CPAP. Standard linear regression was performed to investigate the relationships both between genioglossal EMG and PETCO₂ and between ventilation and PETCO₂, both on and off CPAP during wakefulness and NREM sleep. Regression slopes were compared between conditions. The Wilcoxon signed-rank test was used if data were not normally distributed. $P < .05$ was considered statistically significance.

RESULTS

Full data sets were acquired in 10 of 11 subjects who finished this study, with inspiratory phasic genioglossal activation being encountered at all CO₂ levels, on and off CPAP, awake and asleep. Figure 1 shows an example of raw data obtained in 1 individual during stable NREM sleep off and on CPAP at 3 PETCO₂ level: basal breathing and +5 and +10 mm Hg above the awake eupneic level. As can be seen, genioglossal EMG was increased during hypercapnia both off and on CPAP during stable NREM sleep. As can be seen in Tables 1 and 2, the PETCO₂ values at +5 mm Hg and +10 mm Hg were well matched during wakefulness and sleep. As expected, the baseline PETCO₂ level rose during sleep, as compared with wakefulness (41.5 ± 0.8 to 45.7 ± 0.7 mm Hg).

Genioglossal EMG Response to CO₂ On and Off CPAP

Peak, phasic, and tonic genioglossal EMG; ventilation; and SaO₂ at 3 different PETCO₂ levels on and off CPAP during stable NREM sleep and wakefulness are shown in Tables 1 and 2. During NREM sleep, peak and phasic genioglossal activity significantly increased with CO₂ stimulation both on and off CPAP, as is seen in Figure 2 and Table 1. Raising the PCO₂ augmented tonic genioglossal EMG during NREM sleep on but not off CPAP. The application of nasal CPAP significantly reduced peak, phasic, and tonic genioglossal EMG at all PETCO₂ levels during NREM sleep, with the exception of tonic genioglossal EMG at PETCO₂ 5 mm Hg above awake eupnea ($p = .056$). During wakefulness, hypercapnia significantly increased peak and phasic genioglossal activity both on and off CPAP (Figure 2 and Table 2). Tonic genioglossal EMG increased only on CPAP but not off CPAP under CO₂ stimulation. Comparing on CPAP with off CPAP during waking eupnea, peak, phasic, and tonic genioglossal EMG significantly decreased after CPAP application, but there were no differences in genioglossal activity on versus off CPAP at PETCO₂ levels 5 and 10 mm Hg above eupneic breathing.

The slopes of peak genioglossal EMG response to CO₂ stimulation during NREM sleep and wakefulness are shown in Figures 3A and 3B. The slopes of peak genioglossal EMG to CO₂ stimulation during NREM sleep were not different on and off CPAP ($0.79 \pm 0.27\%$ vs. $0.72 \pm 0.30\%$ of maximal EMG per mm Hg PCO₂). During wakefulness, the slopes of peak genioglossal EMG versus CO₂ off and on CPAP were not statistically different ($0.84 \pm$

0.35% vs. $0.59 \pm 0.18\%$ of maximal EMG per mm Hg PCO_2 , $p = .35$). Comparing the slopes of peak genioglossal EMG to CO_2 stimulation between wakefulness and NREM sleep, both on and off CPAP, the slopes were quite similar for each condition ($0.84 \pm 0.35\%$ vs. $0.72 \pm 0.30\%$ of maximal EMG per mm Hg PCO_2 , $p = \text{NS}$; $0.59 \pm 0.18\%$ vs. $0.79 \pm 0.27\%$ of maximal EMG per mm Hg PCO_2 , $p = \text{NS}$, respectively, off and on CPAP).

Ventilatory Response to CO_2 On and Off CPAP

During NREM sleep, both on and off CPAP, hypercapnic stimulation significantly increased minute ventilation via augmenting tidal volume and raising respiratory rate (with decreased expiratory time). Similarly, during wakefulness, both on and off CPAP, minute ventilation increased significantly via rising tidal volume and a higher respiratory rate under CO_2 stimulation.

The ventilatory response to CO_2 both on and off CPAP during NREM sleep and wakefulness are shown in Figure 4. NREM sleep significantly reduced the slope of the hypercapnic ventilatory response (HCVR) both off CPAP (1.52 ± 0.14 vs. 0.73 ± 0.08 L/min/mm Hg PCO_2 , $p = .001$) and on CPAP (1.37 ± 0.15 vs. 1.07 ± 0.07 L/min/mm Hg PCO_2 , $p = .036$). Application of CPAP during NREM sleep significantly increased the slope of the HCVR (1.07 ± 0.07 vs. 0.73 ± 0.08 L/min/mm Hg PCO_2 , $p = .002$), but CPAP had little effect on the HCVR during wakefulness.

DISCUSSION

The results of this study suggest that, in normal subjects, CO_2 can modulate upper airway muscle activity during NREM sleep and wakefulness in the supine position even in the absence of intrapharyngeal negative pressure. However, application of nasal CPAP significantly decreased dilator muscle activity at all levels of PETCO_2 during NREM sleep, indicating that the negative pressure reflex is still active. Thus both CO_2 via chemoreceptor stimuli and negative pressure reflex inputs to the upper airway dilator muscles remain active during stable NREM sleep. However, the absence of negative pressure in the upper airway did not affect the slope of the response of the pharyngeal airway dilator muscle to hypercapnia. In addition, this study not only confirms the previous finding that NREM sleep shifts the HCVR to the right and downward,³³ but also demonstrates that CPAP application, during NREM, shifts the HCVR to the left and upward, likely a result of decreasing upper airway resistance.

Previous work has suggested that chemoreceptor activation substantially modulates upper airway dilator muscle activity during sleep, while other studies have indicated no effect. For example, Badr and colleagues³⁴ reported that, in 7 normal subjects, hypercapnia increased genioglossal muscle activity during NREM sleep in the supine position. On the other hand, Pillar et al²⁸ investigated genioglossal muscle responsiveness to hypercapnia during NREM sleep and reported no significant increase in muscle activity. Similarly, Stanchina and coworkers²⁹ reported that neither inspiratory resistive loading nor normoxic hypercapnia alone could augment genioglossal muscle activity during NREM sleep. However, both of these 2 studies were conducted with subjects in the lateral decubitus position. These conflicting data may be attributable to not only different body positions, but also activating both chemoreceptors and mechanoreceptors simultaneously as more negative pressure is generated in the supine airway. Finally, Malhotra and colleagues³⁵ examined postural effects on genioglossal muscle responsiveness to mechanical stimuli (pulses of negative pressure) during wakefulness and NREM sleep and reported an increase in genioglossal muscle responsiveness to negative pressure stimuli during sleep, as compared with wakefulness, in the supine position but a decrease in the lateral one. Therefore, we speculated that the genioglossal muscle becomes more responsive to stimuli when the

pharyngeal airway is more vulnerable or when tongue protrusion is critical to prevent pharyngeal closure. One potential method of interpretation for the present study is that CPAP reduces the vulnerability of the pharyngeal airway to collapse, rather than simply removing the negative pressure stimulus. Thus, not only CO₂, but also pharyngeal airway mechanics (negative pressure, posture, or both) may influence the response of the genioglossal muscle to hypercapnic stimulation.

With the application of CPAP to minimize intrapharyngeal negative pressure, the genioglossus muscle could still respond to hypercapnia in the supine position during NREM sleep, suggesting that the muscle is controlled by respiratory neurons via chemoreceptor stimuli. We also observed that genioglossal activity decreased when CPAP was applied at all levels of PETCO₂ during NREM sleep. However, the slopes of the genioglossal EMG response to hypercapnia on and off CPAP during NREM sleep were parallel, indicating that CO₂ was the primary stimulus to muscle activation as PCO₂ levels rose. That being said, if one examines the effect of CPAP on the slope of the genioglossal EMG/CO₂ relationship during wakefulness (Figure 3a) versus sleep (Figure 3b), there are apparent differences. There is a greater drop (to a lower EMG) in the entire relationship during NREM sleep, as compared with wakefulness, that is probably a product of the elimination of higher intrapharyngeal negative pressures by CPAP during sleep, compared with wakefulness. On the other hand, during wakefulness, there is a trend (not significant) for the EMG/CO₂ slope to become steeper when CPAP is removed. This may indicate greater muscle responsiveness to intrapharyngeal negative pressure during wakefulness than during asleep.

The finding that sleep was associated with a decrease in the HCVR confirms previous observations.³³ The magnitude of the reduction was also similar. Application of CPAP did not change the slope of the HCVR during wakefulness, also confirming the previous work by Browne and coworkers.³⁶ During NREM sleep, CPAP did significantly increase the HCVR, probably as a result of reducing the elevated upper-airway resistance associated with sleep. However, even on CPAP, the HCVR was significantly decreased during sleep. Therefore, these results suggest that both the sleep-induced increment in upper airway resistance and loss of wakefulness itself impact the ventilatory response to CO₂ stimulation.³⁷

Despite its strengths, there are some potential limitations to this study. First, our ultimate goal is to provide insights into the pathogenesis of OSA. By studying only normal subjects, any conclusion regarding patients with OSA is speculative. However, patients with OSA are unlikely to achieve stable NREM sleep without CPAP, limiting the feasibility of the present study in people with this disease. In addition, an advanced understanding of normal upper airway dilator muscle control will ultimately improve our knowledge of the pathogenesis of OSA. Second, stable NREM sleep was selected for this study because hypercapnic stimulation cannot be meaningfully assessed during wake-sleep transitions due to their transient nature. Although assessment during rapid eye movement sleep is potentially achievable, it was not attempted in this study. Third, the application of nasal CPAP causes additional physiologic changes other than simply lowering upper airway resistance and eliminating intrapharyngeal negative pressure. These include an increase in resting lung volume, as well as a potential change in pharyngeal airway shape.^{38,39} Hemodynamics can be affected as well. Therefore, the effects of CPAP are probably more complicated than the simplified view presented here. Multiple studies, including several from our group, have shown that increasing lung volume decreases upper-airway resistance with a subsequent decrement in genioglossal EMG.⁴⁰ Thus, the decrease in genioglossal EMG with CPAP could have multiple sources (decreased intrapharyngeal negative pressure or increased lung volume). Moreover, pharyngeal mechanoreceptors may respond to pressure changes rather than absolute values of pressure, implying that negative pressure stimulation may still occur

while subjects are on CPAP. However, application of CPAP during NREM sleep substantially decreases muscle activity at all levels of PETCO₂. Thus, although pressure fluctuations above 0 cm H₂O may influence muscle activity, this in no way invalidates the observations of this study. Thus, we achieved our goal with CPAP, which was to dissociate mechanoreceptive and chemoreceptive mechanisms. Fourth, we have reported the responsiveness of only 1 upper airway dilator muscle, the genioglossus, in this study. We believe that there are substantial data supporting the importance of this muscle in the maintenance of upper airway patency. However, its behavior may be different from that of other upper airway muscles, such as the tensor palatini, a tonic upperairway muscle having less respiratory-related activity and less response to chemical and mechanical stimuli.⁴¹ Accordingly, our findings in this study may not reflect the activity of all pharyngeal airway muscles. Finally, we studied muscle activity at fixed levels of CO₂ rather than conducting a more-standard progressive response. We used this method because it allowed us to establish steady-state conditions of elevated CO₂, allowing careful wakesleep comparisons. Thus, we believe this to be a valid approach. Despite these potential limitations, we believe that our findings are credible and that this study advances our understanding of apnea pathophysiology.

In summary, we have observed that CO₂ modulates upper airway muscle activity during NREM sleep and wakefulness in the supine position in normal subjects even without intrapharyngeal negative pressure. Application of nasal CPAP significantly decreased dilator muscle activity at all levels of PETCO₂ during NREM sleep, indicating that this negative pressure reflex is active during NREM sleep. Thus, both chemoreceptor stimuli and negative pressure reflex inputs to this upper airway dilator muscle remain active during stable NREM sleep. As a result, this dilator muscle can respond to the challenges imposed by pharyngeal collapse (partial or complete) during NREM sleep. Thus, the ability of patients with OSA to maintain stable airway patency some of the time during sleep may reflect these muscle responses.

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REFERENCES

1. Remmers JE, deGroot WJ, Sauerland EK, Anch AM. Pathogenesis of upper airway occlusion during sleep. *J Appl Physiol.* 1978; 44:931–938. [PubMed: 670014]
2. Akaoshi T, White DP, Edwards JK, Beauregard J, Shea SA. Phasic mechanoreceptor stimuli can induce phasic activation of upper airway muscles in humans. *J Physiol.* 2001; 531:677–691. [PubMed: 11251050]
3. Young T, Palta M, Dempsey J, Skatrud J, Weber S, Badr S. The occurrence of sleep-disordered breathing among middle-aged adults. *N Engl J Med.* 1993; 328:1230–1235. [PubMed: 8464434]

4. Teran-Santos J, Jimenez-Gomez A, Cordero-Guevara J. The association between sleep apnea and the risk of traffic accidents. Cooperative Group Burgos-Santander. *N Engl J Med.* 1999; 340:847–851. [PubMed: 10080847]
5. Peppard PE, Young T, Palta M, Skatrud J. Prospective study of the association between sleep-disordered breathing and hypertension. *N Engl J Med.* 2000; 342:1378–1384. [PubMed: 10805822]
6. Shahar E, Whitney CW, Redline S, et al. Sleep-disordered breathing and cardiovascular disease: cross-sectional results of the Sleep Heart Health Study. *Am J Respir Crit Care Med.* 2001; 163:19–25. [PubMed: 11208620]
7. Haponik EF, Smith PL, Bohlman ME, Allen RP, Goldman SM, Bleecker ER. Computerized tomography in obstructive sleep apnea. Correlation of airway size with physiology during sleep and wakefulness. *Am Rev Respir Dis.* 1983; 127:221–226. [PubMed: 6830039]
8. Horner RL, Shea SA, McIvor J, Guz A. Pharyngeal size and shape during wakefulness and sleep in patients with obstructive sleep apnoea. *Q J Med.* 1989; 72:719–735. [PubMed: 2602554]
9. Schwab RJ, Gupta KB, Gefter WB, Metzger LJ, Hoffman EA, Pack AI. Upper airway and soft tissue anatomy in normal subjects and patients with sleep-disordered breathing. Significance of the lateral pharyngeal walls. *Am J Respir Crit Care Med.* 1995; 152:1673–1689. [PubMed: 7582313]
10. Mezzanotte WS, Tangel DJ, White DP. Waking genioglossal electromyogram in sleep apnea patients versus normal controls (a neuromuscular compensatory mechanism). *J Clin Invest.* 1992; 89:1571–1579. [PubMed: 1569196]
11. Fogel RB, Malhotra A, Pillar G, et al. Genioglossal activation in patients with obstructive sleep apnea versus control subjects. Mechanisms of muscle control. *Am J Respir Crit Care Med.* 2001; 164:2025–2030. [PubMed: 11739130]
12. Onal E, Lopata M, O'Connor TD. Diaphragmatic and genioglossal electromyogram responses to CO₂ rebreathing in humans. *J Appl Physiol.* 1981; 50:1052–1055. [PubMed: 6785263]
13. Onal E, Lopata M, O'Connor TD. Diaphragmatic and genioglossal electromyogram responses to isocapnic hypoxia in humans. *Am Rev Respir Dis.* 1981; 124:215–217. [PubMed: 6792954]
14. Bianchi AL, Denavit-Saubie M, Champagnat J. Central control of breathing in mammals: neuronal circuitry, membrane properties, and neurotransmitters. *Physiol Rev.* 1995; 75:1–45. [PubMed: 7831394]
15. Van de Graaff WB. Thoracic influence on upper airway patency. *J Appl Physiol.* 1988; 65:2124–2131. [PubMed: 3209556]
16. Bartlett D Jr, Knuth SL, Gdovin MJ. Influence of laryngeal CO₂ on respiratory activities of motor nerves to accessory muscles. *Respir Physiol.* 1992; 90:289–297. [PubMed: 1480840]
17. Wiegand L, Zwillich CW, Wiegand D, White DP. Changes in upper airway muscle activation and ventilation during phasic REM sleep in normal men. *J Appl Physiol.* 1991; 71:488–497. [PubMed: 1938720]
18. Orem J. The nature of the wakefulness stimulus for breathing. *Prog Clin Biol Res.* 1990; 345:23–30. discussion 31. [PubMed: 2198591]
19. Popovic RM, White DP. Influence of gender on waking genioglossal electromyogram and upper airway resistance. *Am J Respir Crit Care Med.* 1995; 152:725–731. [PubMed: 7633734]
20. Horner RL, Innes JA, Holden HB, Guz A. Afferent pathway(s) for pharyngeal dilator reflex to negative pressure in man: a study using upper airway anaesthesia. *J Physiol.* 1991; 436:31–44. [PubMed: 2061834]
21. Horner RL, Innes JA, Murphy K, Guz A. Evidence for reflex upper airway dilator muscle activation by sudden negative airway pressure in man. *J Physiol.* 1991; 436:15–29. [PubMed: 2061830]
22. Wheatley JR, Mezzanotte WS, Tangel DJ, White DP. Influence of sleep on genioglossus muscle activation by negative pressure in normal men. *Am Rev Respir Dis.* 1993; 148:597–605. [PubMed: 8368629]
23. Mezzanotte WS, Tangel DJ, White DP. Influence of sleep onset on upper-airway muscle activity in apnea patients versus normal controls. *Am J Respir Crit Care Med.* 1996; 153:1880–1887. [PubMed: 8665050]

24. Malhotra A, Fogel RB, Edwards JK, Shea SA, White DP. Local mechanisms drive genioglossus activation in obstructive sleep apnea. *Am J Respir Crit Care Med.* 2000; 161:1746–1749. [PubMed: 10806181]
25. Patrick GB, Strohl KP, Rubin SB, Altose MD. Upper airway and diaphragm muscle responses to chemical stimulation and loading. *J Appl Physiol.* 1982; 53:1133–1137. [PubMed: 7174406]
26. Redline S, Strohl KP. Influence of upper airway sensory receptors on respiratory muscle activation in humans. *J Appl Physiol.* 1987; 63:368–374. [PubMed: 3624139]
27. Innes JA, Morrell MJ, Kobayashi I, Hamilton RD, Guz A. Central and reflex neural control of genioglossus in subjects who underwent laryngectomy. *J Appl Physiol.* 1995; 78:2180–2186. [PubMed: 7665415]
28. Pillar G, Malhotra A, Fogel RB, et al. Upper airway muscle responsiveness to rising PCO₂ during NREM sleep. *J Appl Physiol.* 2000; 89:1275–1282. [PubMed: 11007559]
29. Stanchina ML, Malhotra A, Fogel RB, et al. Genioglossus muscle responsiveness to chemical and mechanical stimuli during non-rapid eye movement sleep. *Am J Respir Crit Care Med.* 2002; 165:945–949. [PubMed: 11934719]
30. Worsnop C, Kay A, Kim Y, Trinder J, Pierce R. Effect of age on sleep onset-related changes in respiratory pump and upper airway muscle function. *J Appl Physiol.* 2000; 88:1831–1839. [PubMed: 10797148]
31. Berry RB, McNellis MI, Kouchi K, Light RW. Upper airway anesthesia reduces phasic genioglossus activity during sleep apnea. *Am J Respir Crit Care Med.* 1997; 156:127–132. [PubMed: 9230736]
32. Rechtschaffen, A.; Kales, A. A manual of standardised terminology, techniques and scoring systems for sleep stages of human subjects. UCLA Los Angeles, USA: National Institute of health; 1968.
33. Douglas NJ, White DP, Weil JV, Pickett CK, Zwillich CW. Hypercapnic ventilatory response in sleeping adults. *Am Rev Respir Dis.* 1982; 126:758–762. [PubMed: 7149440]
34. Badr MS, Skatrud JB, Simon PM, Dempsey JA. Effect of hypercapnia on total pulmonary resistance during wakefulness and during NREM sleep. *Am Rev Respir Dis.* 1991; 144:406–414. [PubMed: 1859068]
35. Malhotra A, Trinder J, Fogel R, et al. Postural effects on pharyngeal protective reflex mechanisms. *Sleep.* 2004; 27:1105–1112. [PubMed: 15532204]
36. Browne HA, Adams L, Simonds AK, Morrell MJ. Ageing does not influence the sleep-related decrease in the hypercapnic ventilatory response. *Eur Respir J.* 2003; 21:523–529. [PubMed: 12662012]
37. Skatrud JB, Dempsey JA, Badr S, Begle RL. Effect of airway impedance on CO₂ retention and respiratory muscle activity during NREM sleep. *J Appl Physiol.* 1988; 65:1676–1685. [PubMed: 3141362]
38. Deegan PC, Nolan P, Carey M, McNicholas WT. Effects of positive airway pressure on upper airway dilator muscle activity and ventilatory timing. *J Appl Physiol.* 1996; 81:470–479. [PubMed: 8828699]
39. Alex CG, Aronson RM, Onal E, Lopata M. Effects of continuous positive airway pressure on upper airway and respiratory muscle activity. *J Appl Physiol.* 1987; 62:2026–2030. [PubMed: 3298198]
40. Stanchina ML, Malhotra A, Fogel RB, et al. The influence of lung volume on pharyngeal mechanics, collapsibility, and genioglossus muscle activation during sleep. *Sleep.* 2003; 26:851–856. [PubMed: 14655919]
41. Sauerland EK, Orr WC, Hairston LE. DMG patterns of oropharyngeal muscles during respiration in wakefulness and sleep. *Electromyogr Clin Neurophysiol.* 1981; 21:307–316. [PubMed: 7262010]

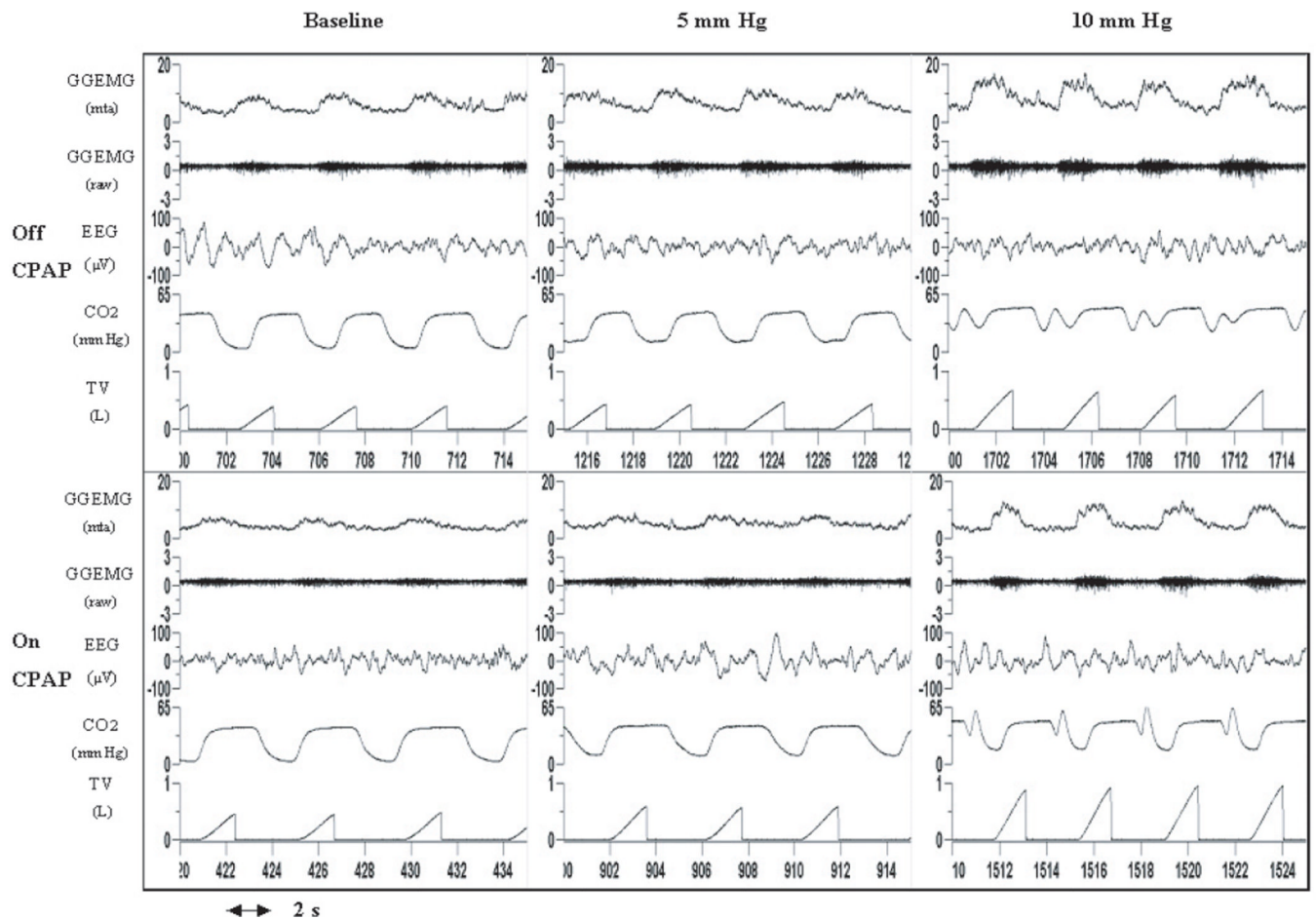


Figure 1.

Individual raw data traces from 1 subject at 3 end-tidal PCO₂ (PETCO₂) levels: basal breathing and +5 and +10 mm Hg above awake eupneic level during stable non-rapid eye movement (NREM) sleep both off and on continuous positive airway pressure (CPAP). Carbon dioxide (CO₂) can increase genioglossal muscle activity both off and on CPAP during stable NREM sleep. Note that the inspiratory increment in the PCO₂ trace at 10 mm Hg above eupnea both on and off CPAP indicates CO₂ accumulation in the inspiratory line during expiration. GGEMG refers to genioglossal electromyogram; EEG, electroencephalogram; TV, tidal volume.

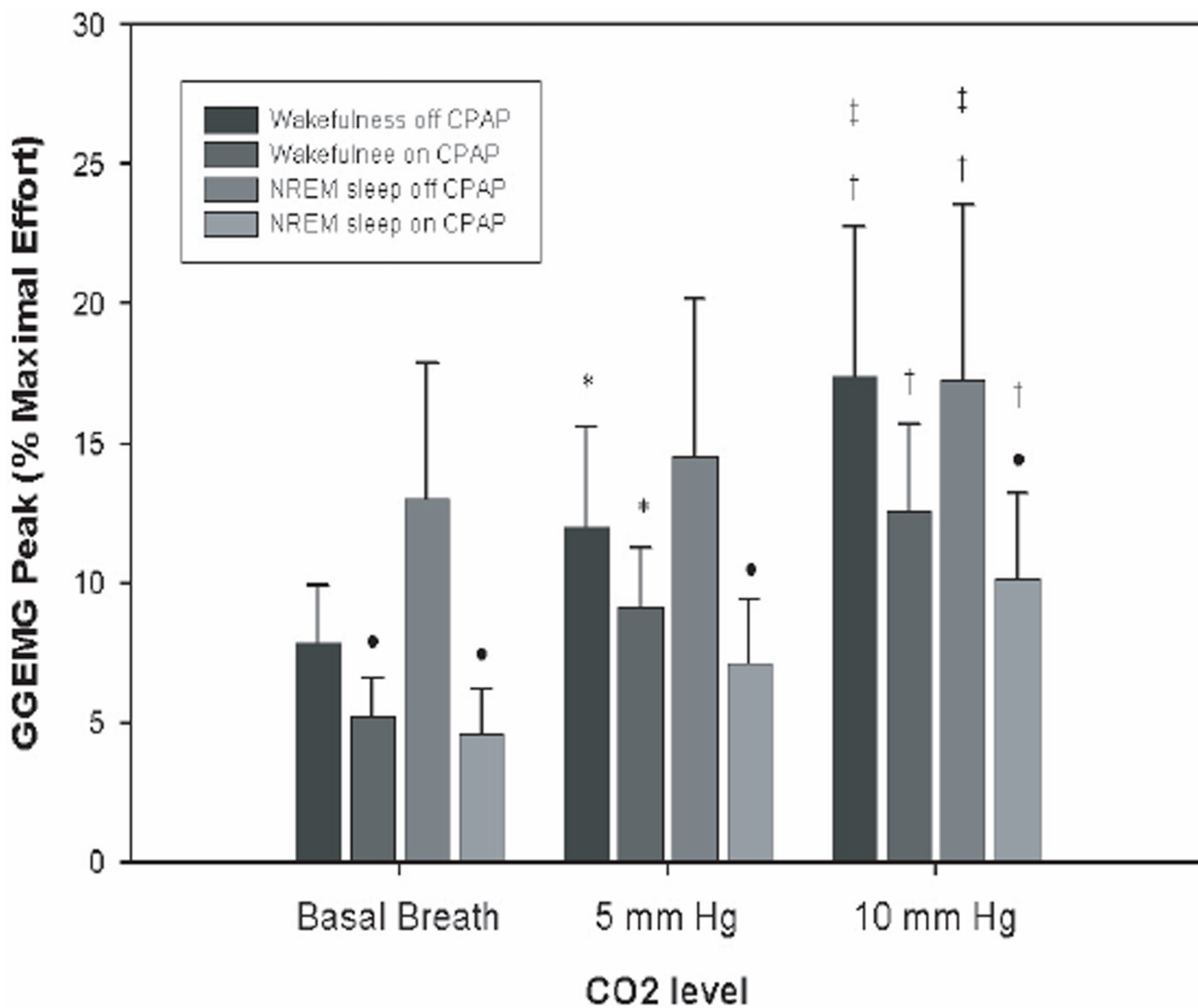


Figure 2.

Bar graphs show the mean (\pm S.E.M.) peak genioglossal electromyogram (GGEMG) response to CO₂ stimulation at 3 end-tidal PCO₂ (PETCO₂) levels during wakefulness and non-rapid eye movement (NREM) sleep both on and off continuous positive airway pressure (CPAP). CPAP significantly decreases GGEMG at all levels of PCO₂ during NREM sleep but only at the eupneic level during wakefulness. During wakefulness and NREM sleep, both on and off CPAP, the genioglossal muscle can significantly respond to CO₂ stimulation. •P < .05, off vs on CPAP; *P < .05, eupneic vs 5 mm Hg above eupneic levels; †P < .05, eupneic vs 10 mm Hg above eupneic levels; ‡P < .05, 5 mm Hg vs 10 mm Hg above eupneic levels.

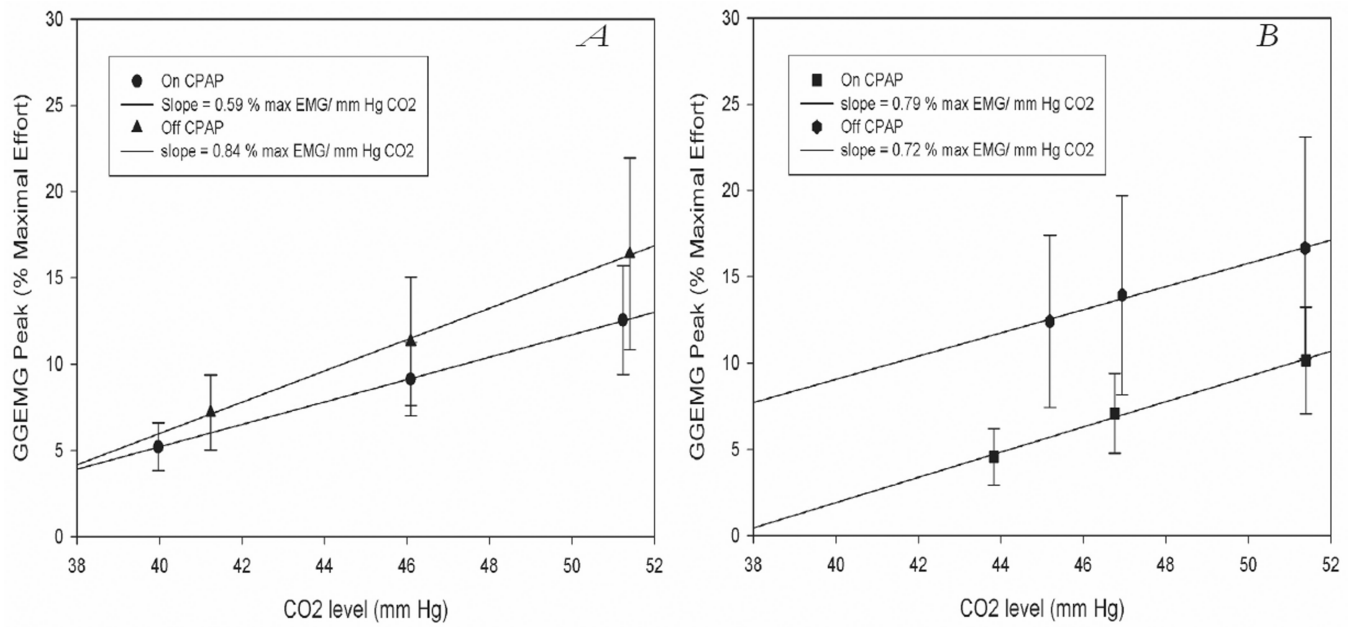


Figure 3.

During wakefulness, there is a trend (not significant) for continuous positive airway pressure (CPAP) to reduce the slope of peak genioglossal electromyogram (GGEMG) response to CO₂ stimulation (A). During non-rapid eye movement (NREM) sleep, on and off CPAP, the slopes of peak GGEMG response to CO₂ stimulation are parallel with CPAP shifting the curve to the left (B).

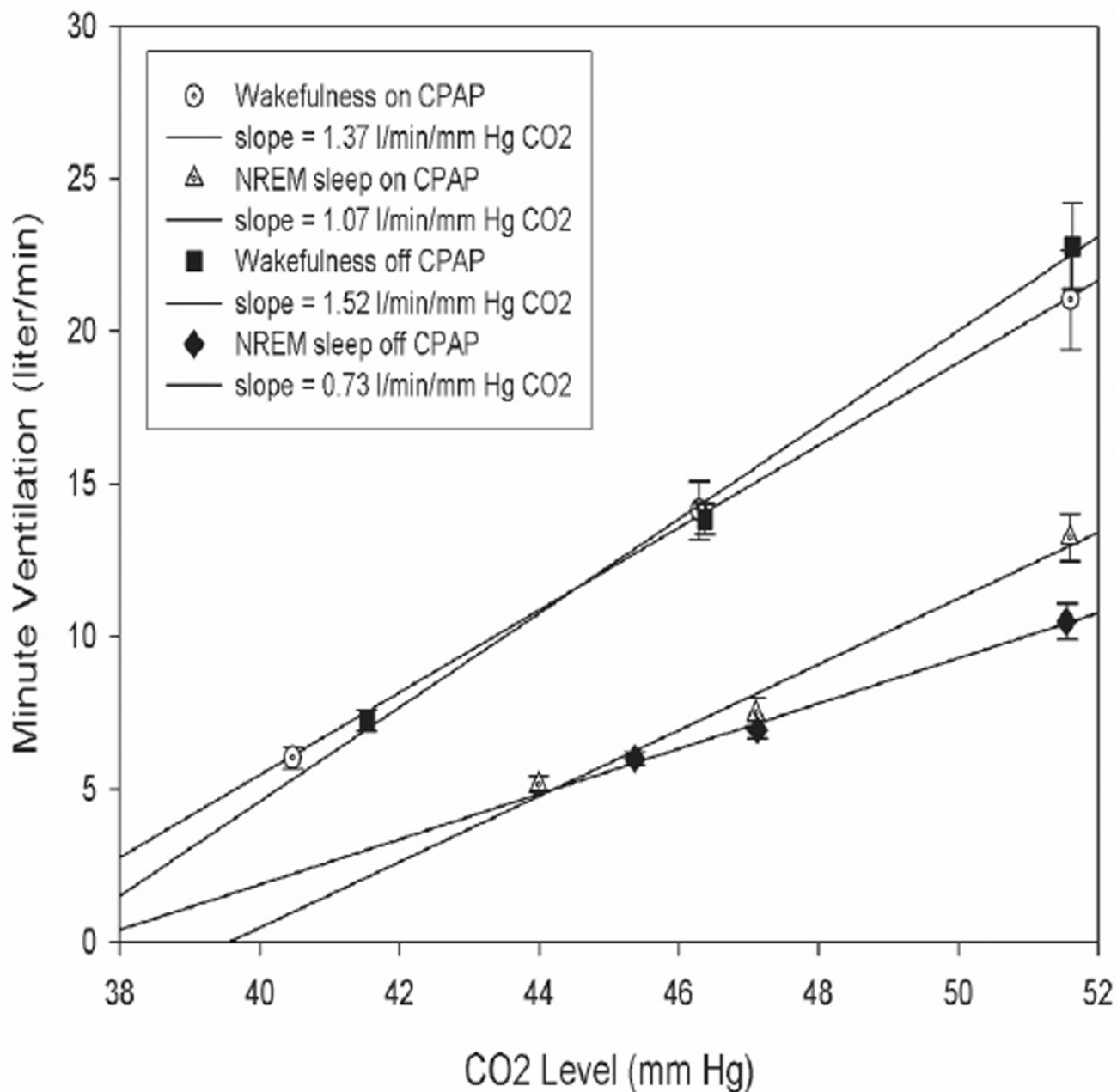


Figure 4. Both on and off continuous positive airway pressure (CPAP), non-rapid eye movement (NREM) sleep shifted the waking hypercapnic ventilatory response to the right and downward. During NREM sleep, CPAP increased the slope of the hypercapnic ventilatory response.

Table 1Ventilation and Muscle Response to CO₂ Stimulation during NREM Sleep

	Off CPAP				On CPAP			
	Baseline	5 mm Hg	10 mm Hg	p Value	Baseline	5 mm Hg	10 mm Hg	p Value
PETCO ₂	45.7 ± 0.7	47.1 ± 0.7	51.6 ± 0.6		44.0 ± 0.8	47.1 ± 0.7	51.6 ± 0.7	
Genioglossal activity, % max								
Peak	13.0 ± 4.9	14.6 ± 5.6	17.3 ± 6.3	.011	4.6 ± 1.6*	7.1 ± 2.3*	10.1 ± 3.1*	.005
Tonic	4.4 ± 1.6	5.1 ± 1.9	5.4 ± 1.8	.273	1.9 ± 0.7*	2.6 ± 0.8	3.4 ± 1.2*	.036
Phasic	8.6 ± 3.5	9.4 ± 4.0	11.9 ± 4.8	<.001	2.7 ± 1.0*	4.5 ± 1.7*	6.8 ± 2.3*	<.001
Ti, s	1.8 ± 0.1	1.8 ± 0.1	1.7 ± 0.1	.06	1.6 ± 0.1*	1.6 ± 0.1*	1.5 ± 0.1*	.307
Te, s	2.5 ± 0.2	2.5 ± 0.1	2.3 ± 0.1	<.001	2.8 ± 0.2*	2.6 ± 0.1	2.3 ± 0.1	<.001
RR, breath/min	14.2 ± 0.6	14.5 ± 0.6	15.3 ± 0.6	<.001	14.0 ± 0.6	14.7 ± 0.5	15.8 ± 0.5	<.001
TV, L	0.4 ± 0.0	0.5 ± 0.0	0.7 ± 0.0	<.001	0.4 ± 0.0*	0.5 ± 0.0	0.8 ± 0.1*	<.001
VE, L/min	6.0 ± 0.2	6.9 ± 0.3	10.5 ± 0.6	<.001	5.1 ± 0.3*	7.5 ± 0.5	13.2 ± 0.8*	<.001
SaO ₂ , %	96.5 ± 0.2	97.1 ± 0.2	97.6 ± 0.3	<.001	96.8 ± 0.3	97.3 ± 0.3	97.6 ± 0.3	<.001

NREM refers to non-rapid eye movement; CPAP continuous positive airway pressure; PETCO₂ end-tidal PCO₂; Peak, highest activity of genioglossus during inspiration; Tonic, lowest activity of genioglossus during expiration; Phasic, difference between genioglossal peak and tonic; Ti, inspiratory time; Te, expiratory time; RR, respiratory rate; TV, tidal volume; VE, minute ventilation; SaO₂, O₂ saturation.

* p < .05, off CPAP vs on CPAP

Table 2Ventilation and Muscle Response to CO₂ Stimulation during Wakefulness

	Off CPAP				On CPAP			
	Baseline	5 mm Hg	10 mm Hg	P Value	Baseline	5 mm Hg	10 mm Hg	P Value
PETCO ₂	41.5 ± 0.8	46.4 ± 0.7	51.6 ± 0.7		40.5 ± 1.0*	46.3 ± 0.6	51.6 ± 0.7	
Genioglossal activity, % max								
Peak	7.8 ± 2.1	12.0 ± 3.6	17.4 ± 5.4	<.001	5.2 ± 1.4*	9.1 ± 2.1	12.5 ± 3.1	.003
Tonic	2.3 ± 0.4	2.5 ± 0.5	2.9 ± 0.6	.067	1.4 ± 0.3*	2.9 ± 0.6	2.9 ± 0.8	.02
Phasic	5.6 ± 1.8	9.5 ± 3.3	14.5 ± 4.8	<.001	3.8 ± 1.3*	6.9 ± 1.8	9.6 ± 2.8	.001
Ti, s	1.5 ± 0.1	1.5 ± 0.1	1.8 ± 0.3	.336	1.5 ± 0.1	1.5 ± 0.1	1.6 ± 0.1	.761
Te, s	2.2 ± 0.1	1.9 ± 0.1	1.9 ± 0.2	.029	2.7 ± 0.2*	2.2 ± 0.1*	2.1 ± 0.2	<.001
RR, breath/min	16.7 ± 0.7	18.1 ± 0.9	18.5 ± 1.5	.086	15.1 ± 0.7*	17.0 ± 1.0	17.3 ± 1.2	.035
TV, L	0.4 ± 0.0	0.8 ± 0.1	1.3 ± 0.1	<.001	0.4 ± 0.0*	0.9 ± 0.1	1.2 ± 0.1	<.001
VE, L/min	7.3 ± 0.3	13.8 ± 0.5	22.8 ± 1.4	<.001	6.0 ± 0.4*	14.1 ± 1.0	21.0 ± 1.6	<.001
SaO ₂ , %	97.4 ± 0.3	98.1 ± 0.2	98.1 ± 0.2	.012	97.7 ± 0.3	98.3 ± 0.2	98.1 ± 0.2	.006

CPAP refers to continuous positive airway pressure; PETCO₂ end-tidal PCO₂; Peak, highest activity of genioglossus during inspiration; Tonic, lowest activity of genioglossus during expiration; Phasic, difference between genioglossal peak and tonic; Ti, inspiratory time; Te, expiratory time; RR, respiratory rate; TV, tidal volume; VE, minute ventilation; SaO₂, O₂ saturation.

* p < .05, off CPAP vs on CPAP