

# Genioglossus Muscle Responsiveness to Chemical and Mechanical Stimuli during Non-Rapid Eye Movement Sleep

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Previous studies have suggested that during non-rapid eye movement (NREM) sleep, neither large short-duration resistive loads nor sustained normoxic hypercapnia alone leads to increased genioglossus muscle activation. However, in normal individuals during stable NREM sleep, genioglossus activity rises above baseline as  $P_{CO_2}$  rises and airway resistance increases. We therefore hypothesized that combinations of chemical ( $P_{CO_2}$ ,  $P_{O_2}$ ) and mechanical stimuli during NREM sleep would lead to increased genioglossal activation. We studied 15 normal subjects (9 males, 6 females) during stable NREM sleep, measuring genioglossus electromyogram, epiglottic/choanal pressure, and airflow under six conditions: (1) baseline, (2) inspiratory resistive loading ( $-5$  to  $-15$  cm  $H_2O$ /L/second), (3) increased  $P_{CO_2}$  (5–10 mm Hg above baseline), (4) combined resistive loading and increased  $P_{CO_2}$ , (5) hypoxia ( $Sa_{O_2}$  80–85%), and (6) combined hypoxia/inspiratory resistive loading. Only the combined condition of hypercapnia and resistive loading led to significantly increased genioglossal activation,  $3.91 \pm 0.77\%$  to  $9.64 \pm 1.96\%$  of maximum. These data suggest that the genioglossus muscle is less responsive to either chemical stimuli (hypercapnia, hypoxia) or inspiratory resistive loading alone during NREM sleep at the degrees tested. When hypercapnia is combined with resistive loading, the muscle does respond. However, the possibility that higher levels of  $P_{CO_2}$  or greater resistive loading alone could activate the muscle cannot be excluded.

**Keywords:** genioglossus; hypercapnia; resistive loading; sleep

The mechanisms that control the pharyngeal musculature during non-rapid eye movement (NREM) sleep are likely important in the pathophysiology of obstructive sleep apnea (OSA), a disorder characterized by repetitive pharyngeal collapse during sleep. This disorder is quite common and is associated with important morbidity (1–3). In normal individuals, during the transition from wakefulness to NREM sleep, after an initial decline, there is a progressive rise in genioglossal muscle activation (4–6). This increased muscle activity is believed to be a compensatory response to the increased upper airway resistance and rising  $P_{CO_2}$  that occur during supine NREM sleep in normal humans. The mechanisms driving this increased muscle activity are likely important in preventing upper airway collapse (7).

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Considerable investigation has been directed at defining the stimuli modulating upper airway dilator muscle activation. Identified modulators of muscle activity include intrapharyngeal negative pressure,  $P_{O_2}$ ,  $P_{CO_2}$ , lung volume, inspired air temperature, sleep-wake states, blood pressure, and sex-specific hormones (8–16). Evidence suggests that the upper airway mechanoreceptor, responsive to intrapharyngeal negative pressure, is the primary stimulus to genioglossal activation whether activated by rapid negative pressure pulses to the upper airway (17–19) or by the application of inspiratory resistive loads (20–22). Despite this robust relationship between genioglossal muscle activation and negative pharyngeal pressure during wakefulness, during NREM sleep muscle responsiveness to even large inspiratory resistive loads (25 cm  $H_2O$ /L/second) is substantially attenuated if not absent (22). This suggests that during NREM sleep upper airway mechanoreceptors are much less active and that a combined chemoreceptor plus mechanoreceptor input is required to activate the genioglossus muscle and stabilize the upper airway.

Chemoreceptor activation has been shown to substantially modulate genioglossus muscle activity during wakefulness. Investigations in humans who are awake have demonstrated linear increments in diaphragmatic and genioglossal electromyogram (EMG), during both normoxic hypercapnia and isocapnic hypoxia (10, 11). These studies suggest that increased central output to the pump muscles concomitantly augments upper airway muscle activation. However, genioglossus muscle responsiveness to exogenous chemical stimuli (normoxic hypercapnia) has recently been observed to be attenuated during stable NREM sleep. Normoxic hypercapnia (5 mm Hg above baseline sleeping values) failed to substantially activate the genioglossus muscle during NREM sleep, despite substantial activation when awake (23). In addition, Parisi and coworkers have shown in goats subjected to isocapnic hypoxia during NREM sleep that genioglossus muscle activation only occurs after reaching a low  $Sa_{O_2}$  threshold (24).

Collectively, these studies suggest that genioglossus muscle activation during NREM sleep is not importantly modulated by either mechanoreceptor or chemoreceptor inputs alone, at least at the degrees tested. However, as stated previously, muscle activity does increase in normal individuals after the transition from wakefulness to NREM sleep and over the course of an apnea in patients with OSA. We therefore speculated that during NREM sleep, upper airway muscle activation is dependent on the combined effects of mechanoreceptor and chemoreceptor inputs and hypothesized that combinations of hypoxia, hypercapnia, and inspiratory resistive loading would lead to increased genioglossal activation in normal individuals during NREM sleep.

## METHODS

### Subjects

We studied 15 normal individuals (9 males, 6 females) with no historical evidence of a medical problem or a sleep disorder. The protocol

was approved by the Human Subjects Committee at Brigham and Women's Hospital. All subjects provided written informed consent before participation in the study. Females were studied only during the follicular phase of their menstrual cycle (9).

### Instrumentation, Measurements, and Analysis

We measured genioglossus EMG (GGEMG) using two stainless steel, teflon-coated intramuscular wire electrodes, as previously described (25). Choanal and epiglottic pressures were measured using Millar pressure-tipped catheters (Millar, Houston, TX) (25). Subjects breathed through a nasal mask (Respironics, Murraysville, PA), with airflow being measured by a pneumotachograph (Fleisch #2, Lausanne, Switzerland) and pressure transducer (Validyne, Northridge, CA). End-tidal  $P_{CO_2}$  ( $P_{ETCO_2}$ ) was sampled at the mask using a calibrated infrared  $CO_2$  analyzer. (BCI, Waukesha, WI)  $SA_{O_2}$  was measured using a pulse oximeter attached to the index finger (BCI). When desired, resistance was added to inspiration using a specially designed inspiratory resistive loading device, described previously (26). Three different loads (5, 10, and 15 cm  $H_2O/L/second$ ) were applied for three breaths each. Data were collected during NREM sleep (Stages 2, 3, and 4) using standard sleep staging techniques (27).

### Protocol

After achieving stable NREM sleep (Stages 2, 3, and 4) and with subjects in the lateral position, the aforementioned signals were recorded under the conditions described in this section. The order of conditions was randomized.

(1) Basal breathing: Baseline conditions were recorded for three minutes. (2) Inspiratory resistive loading: Loads of 5, 10, and 15 cm  $H_2O$  per L/s were applied for three consecutive breaths to the airway. Each was applied three times. (3) Normoxic hypercapnia: A 25%  $CO_2$  (balance nitrogen) gas mixture was added to the inspiratory flow of gas in the mask, to achieve an end-tidal  $CO_2$  level 5–10 mm Hg above baseline levels observed during stable NREM sleep. Recordings were taken for three minutes. (4) Combined steady-state increased  $PCO_2$  and inspiratory resistive loading: While the  $P_{ETCO_2}$  was 5–10 mm Hg above baseline, inspiratory resistive loads (5, 10, and 15 cm  $H_2O/L/second$ ) were again applied. (5) Isocapnic hypoxia: An 11% oxygen mixture (balance nitrogen) was used to fill a meteorologic balloon. Subjects were switched into this mixture to maintain the  $SA_{O_2}$  level at our goal of 80–85%. Data were recorded for three minutes. (6) Combined isocapnic hypoxia and inspiratory resistive loading: Once the goal  $SA_{O_2}$  was achieved, the inspiratory resistive loads (5, 10, and 15 cm  $H_2O/L/second$ ) were again applied. See online data supplement for additional methodologic detail.

A repeated measures analysis of variance (ANOVA) was used to compare measurements between conditions. A two-way repeated measures ANOVA was used to determine the effect of hypercapnia on GGEMG, while controlling for the presence of a resistive load. A Tukey *post hoc* test was performed to account for multiple comparisons. Standard linear regression was performed to investigate the correlation between GGEMG and epiglottic pressure. Regression slopes were compared between conditions. An  $\alpha$  level of 0.05 was considered significant (Sigma Stat software version 2.03; SPSS Corp., Chicago, IL).

### RESULTS

Thirteen subjects completed the entire protocol (mean age  $\pm$  standard error of the mean  $28.9 \pm 4.2$  years and body mass index  $22.8 \pm 1.9$   $kg/m^2$ ). Two additional individuals completed all conditions except those using isocapnic hypoxia. Figure 1 presents raw data from one subject across all conditions and Figure 2 the mean peak phasic GGEMG and epiglottic pressures across all conditions. Finally, Table 1 shows the group mean values for genioglossal EMG, respiratory mechanics, and blood gases at baseline and under all described conditions. The inspiratory resistive loading data shown in Figure 2 and Table 1 represent the 10 cm  $H_2O/L/second$  load. The results are similar for the 5 and 15 cm  $H_2O/L/second$  resistive loads that are presented in the online data supplement. As can be seen (Table 1, Figure 2), compared with baseline, none of resistive

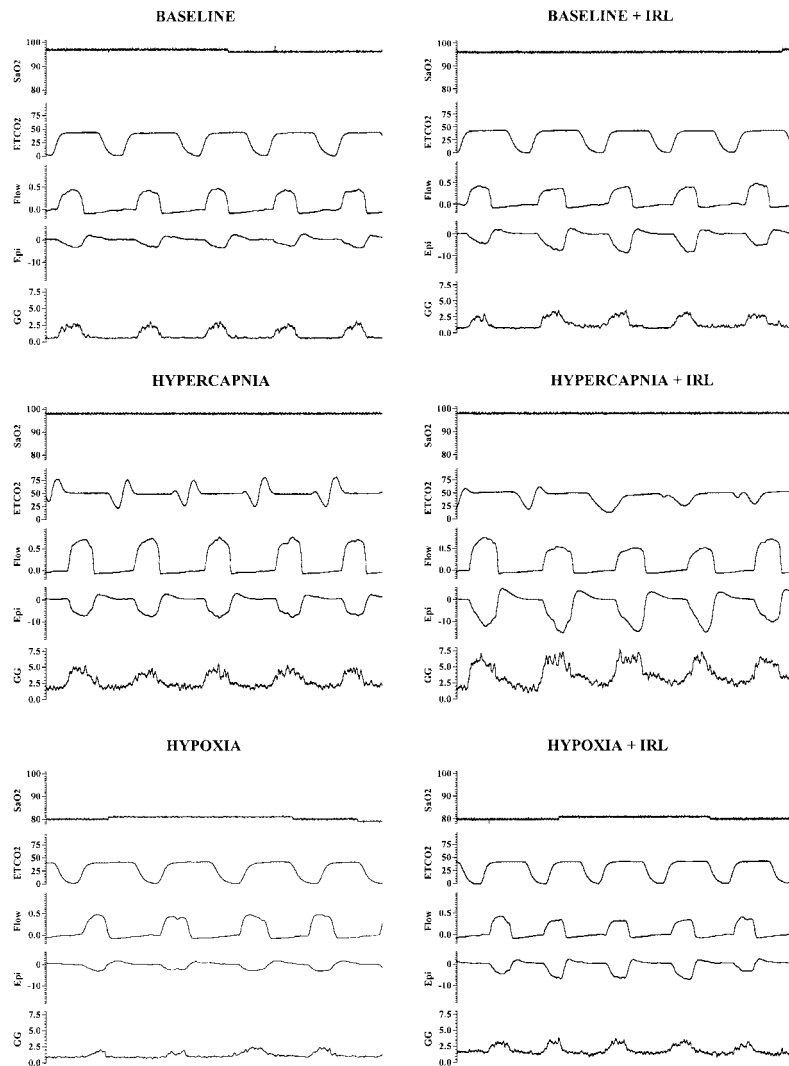
loads, hypercapnia, and hypoxemia alone substantially increased genioglossal muscle activation when compared with baseline during stable NREM sleep. This was the case despite considerable and statistically significant changes in both minute ventilation and epiglottic pressure. However, the combination of resistive loading and hypercapnia did increase the peak phasic GGEMG compared with baseline (Table 1, Figure 2). Combined hypoxia and inspiratory resistive loading did not lead to a significant increment in genioglossus muscle activation. Tonic GGEMG was not substantially different across any condition compared with baseline (Table 1). To determine the independent effects of  $CO_2$  and resistive loading on genioglossus responsiveness, we conducted a two-way repeated measures ANOVA, controlling for these two factors. After allowing for the effect of different resistive loads,  $CO_2$  had an independent effect on peak genioglossus activation that was confirmed with a Tukey multiple comparisons test ( $p = 0.016$ ).

As airway pressure has previously been observed to be such an important driver of genioglossal activation during wakefulness, we examined the relationship between epiglottic pressure and GGEMG across all conditions asleep. As can be seen in Figure 3, there is a relatively robust relationship between these two variables (GGEMG versus epiglottic pressure,  $R^2 = 0.69$ ), suggesting that 69% of the variance in GGEMG could be explained by changing epiglottic negative pressure alone. However, results of the two-way repeated measures ANOVA revealed independent effects of both  $CO_2$  and resistive loading on genioglossus activation. To further investigate this relationship, we conducted an analysis of the slopes of the regression lines comparing peak genioglossus activity and epiglottic pressure (Figure 4) across different conditions. We found that when hypercapnia and hypoxia were removed from the correlation, the slope of the relationship decreased (from 1.14 to 0.44 arbitrary units/cm  $H_2O$ ), but  $R^2$  increased (from 0.69 to 0.92). Thus, the GG–epiglottic pressure relationship seemed to be influenced by an independent stimulus (i.e., hypercapnia). To further address this, we compared the slope of the GG–epiglottic pressure relationship under the conditions of loading alone to loading plus  $CO_2$  for each individual. The mean slope of the regression line increased (from 0.56 to 1.28); however, this was not statistically significant ( $p = 0.19$ ).

### DISCUSSION

The results of this study indicate that in normal individuals, during NREM sleep, genioglossus muscle activation is importantly modulated by the combination of hypercapnia and inspiratory resistive loading. This study also confirms our previous findings that neither hypercapnia nor resistive loading alone significantly increased upper airway dilator muscle activation, despite large increases in minute ventilation and moderately greater negative epiglottic pressure (22, 28). Finally, we observed a relatively close relationship across conditions between genioglossal activation and epiglottic pressure. However, the slope of this relationship decreases with removal of the hypercapnic and hypoxic subsets, suggesting an effect that is independent of mechanoreceptor activation. Thus, chemoreceptor activation may in part regulate the GGEMG–epiglottic pressure relationship.

As noted previously, recruitment of genioglossal activity, which has been observed after the transition from wakefulness to NREM sleep and at the transition from Stage 2 to slow-wave sleep may help prevent upper airway collapse in normal sleeping individuals (4, 5). The mechanism driving this muscle activation immediately after sleep onset has been presumed to be secondary to the increasing  $PCO_2$  and airway resistance that



**Figure 1.** Raw data from one subject across conditions. Data for inspiratory resistive loading (IRL) under all conditions represented by 10 cm H<sub>2</sub>O/L/second load. GG = moving time averaged signal (GGEMG); Epi = epiglottic pressure.

occur at the transition from wakefulness to NREM sleep. Our observation that only the combination of chemical and mechanical stimuli, at least at the levels tested, led to increased genioglossus activity is consistent with the hypothesis that multiple stimuli are necessary for upper airway dilator activation during NREM sleep. This also confirms the previous observation that the genioglossus is relatively unresponsive to either chemical or mechanical stimuli alone during NREM sleep (22, 28). Therefore, multiple inputs to the upper airway may be necessary to activate upper airway muscles and maintain patency of the airway during sleep in normal individuals (18). However, the relative contribution of each receptor or physiologic stimulus to this muscle activation is not entirely clear. We propose two alternative mechanisms for the augmented GGEMG observed in this study.

First, the combined resistive load and exogenous CO<sub>2</sub> used in this experiment could have activated both chemoreceptors and mechanoreceptors, leading to increased genioglossus activation. As stated previously, there is evidence that during wakefulness both mechanoreceptor and chemoreceptor inputs influence genioglossal activity. In addition, Redline and Strohl demonstrated that exogenous CO<sub>2</sub> delivered by tracheostomy is capable of increasing GGEMG activity during wakefulness, suggesting that in the absence of upper airway mechanoreceptor influences, chemoreceptor output is a potent stimulus to genioglossal activation (29). Lastly, previous work by Malhotra

and coworkers showed that despite the application of resistive loads up to 25 cm H<sub>2</sub>O/L/second during NREM sleep, generating a negative epiglottic pressure of 9.2 cm H<sub>2</sub>O, the genioglossus was not significantly activated compared with baseline (22). Our results complement these previous studies by showing that CO<sub>2</sub> has an independent effect on peak GGEMG activation, after controlling for the effect of resistive loading. In addition, we have shown a decrement in the GGEMG–epiglottic pressure relationship (slope) with removal of the hypercapnic and hypoxic stimuli (Figure 4). Collectively, these results suggest that CO<sub>2</sub> is activating the genioglossus through a mechanism other than simply increasing respiratory drive and thus negative intrapharyngeal pressure. Thus, CO<sub>2</sub> may be up-regulating the GGEMG–epiglottic pressure relationship.

Alternatively, the combination of resistive loading and exogenous CO<sub>2</sub>, at the levels tested, may have led to increased GGEMG activity simply by leading to more negative intrapharyngeal pressure. In a recent report by Shea and coworkers, the combination of exogenous CO<sub>2</sub> and negative intrapharyngeal pressure (iron lung ventilation), did not lead to greater genioglossal EMG than the negative intrapharyngeal pressure alone when tested during wakefulness (30). As noted previously, negative pharyngeal pressure appears to be the most potent driver of genioglossus activity, and clearly, epiglottic pressure was most negative in the combined resistive loading plus hypercapnia condition (Figure 2). It is plausible that exogenous CO<sub>2</sub> in-

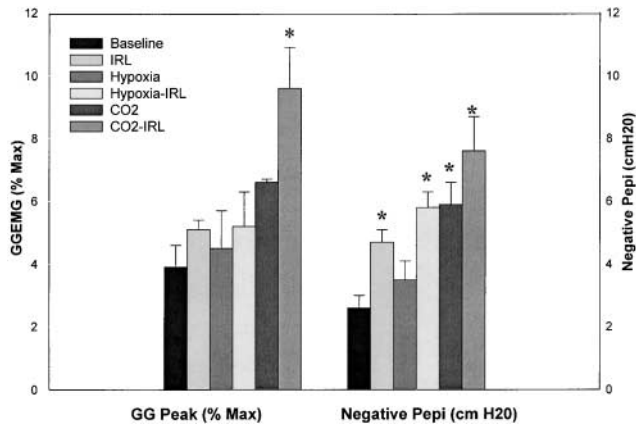


Figure 2. Group mean peak phasic genioglossus activity and negative epiglottic pressure across conditions. Data for IRL under all conditions represented by 10 cm H<sub>2</sub>O /L/second load. \*p < 0.05 compared with baseline.

creased central respiratory drive and diaphragm-driven negative intrapharyngeal pressure. When this is combined with inspiratory resistive loading, the airway negative pressure was adequate to activate the muscle during NREM sleep. This is consistent with the findings of Shea and coworkers (30) that genioglossus activation was comparably increased with either the addition of exogenous CO<sub>2</sub> or an iron lung negative pressure ventilator as long as negative intrapharyngeal pressure was comparable. This may also explain why Pillar and associates (23) did not find an increase in GGEMG from baseline with the additional exogenous CO<sub>2</sub> during NREM sleep. In that study, both GGEMG and negative epiglottic pressure only trended upward from baseline (7.7 ± 0.6% to 7.8 ± 1.4% max units, p = not significant [NS], and 3.7 ± 0.9 cm H<sub>2</sub>O to 5.5 ± 1.3 cm H<sub>2</sub>O, p = NS, respectively), despite an increase in minute ventilation of 3.6 ± 0.1 L/minute (p < 0.05) with the addition of exogenous CO<sub>2</sub> in NREM sleep. It is possible that had they achieved more negative intrapharyngeal pressure, the genioglossus activation would have been greater. Collectively, these investigations emphasize that mechanoreceptor input (i.e., epiglottic pressure) is still likely to be the most important stimulus to the genioglossus. However, the analysis of the regression slopes (Figure 4) does suggest an independent additive influence of CO<sub>2</sub> on the GGEMG–epiglottic pressure relationship.

Although we observed an increase in GGEMG with the addition of CO<sub>2</sub> alone during NREM sleep, this increment did

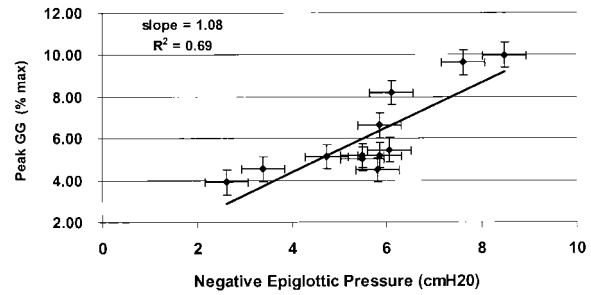


Figure 3. Simple linear regression comparing peak phasic genioglossus activity and peak epiglottic pressure across all conditions. Each point represents the mean value for all subjects under any one condition.

not reach statistical significance (Tukey p = 0.44). This was the case despite an increased minute ventilation and epiglottic pressure compared with baseline. We would argue that only by achieving a more negative intrapharyngeal pressure (as occurred with the combined hypercapnia and resistive loading condition) or by activating both chemoreceptors and mechanoreceptors did genioglossal activity increase substantially compared with baseline.

The lack of observed genioglossus muscle responsiveness during the hypoxia and combined hypoxia with inspiratory resistive loading conditions deserves comment. This lack of responsiveness may relate to the level of hypoxia chosen, as peripheral chemosensitivity is attenuated during sleep (31, 32). Thus, our level of hypoxia may have been insufficient for activation of the upper airway dilators (24). In addition, this attenuated response could have resulted from inadequate PETCO<sub>2</sub> control during the hypoxic conditions, compared with baseline (42.0 ± 3.6 mm Hg and 44.6 ± 2.6 mm Hg, respectively). This relative hypocapnia may have led to decreased central output to both the diaphragm and upper airway muscles, masking any effect of the hypoxic condition or combined condition on peak GGEMG activity. Lastly, hypoxia itself was a weak stimulator of ventilation and changed epiglottic pressure very little.

The magnitude of the changes in genioglossus activation reported here are comparable to those reported in previous studies investigating control of the upper airway musculature in normal individuals. However, extrapolating the significance of these findings to patients with OSA is difficult. Patients with OSA have greater muscle activity awake, which is largely lost during sleep (15, 33). Over the course of the ensuing apnea, large increments in EMG have been reported. These larger changes in activation are likely secondary to plasticity in the

TABLE 1. GENIOGLOSSUS ACTIVITY, PHARYNGEAL MECHANICS, AND BLOOD GAS VALUES ACROSS CONDITIONS DURING NREM SLEEP

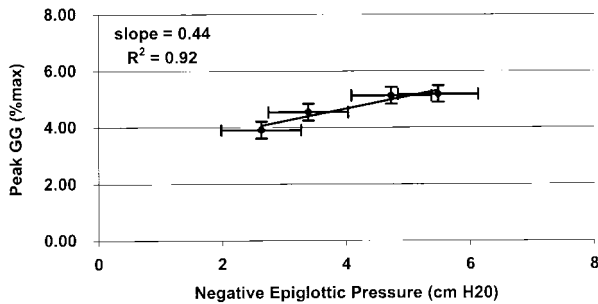
	Baseline	IRL	Hypoxia	Hypoxia + IRL	CO <sub>2</sub>	CO <sub>2</sub> + IRL
GG peak, %max	3.91 ± 0.72	5.12 ± 0.30	4.50 ± 1.21	5.20 ± 1.10	6.60 ± 1.10	9.64 ± 1.90*
GG tonic, %max	2.44 ± 0.52	2.08 ± 0.36	1.91 ± 0.30	1.73 ± 0.30	2.91 ± 0.47	2.97 ± 0.6
Rph, cm H <sub>2</sub> O per L/s	4.4 ± 1.4	6.5 ± 2.3	4.1 ± 1.6	6.3 ± 2.0	4.0 ± 1.1	4.5 ± 1.2
Pepi, cm H <sub>2</sub> O	-2.6 ± 0.4	-4.7 ± 0.4*	-3.5 ± 0.6	-5.8 ± 0.5*	-5.9 ± 0.7*	-7.6 ± 1.1*
Peak flow, L/s	0.41 ± 0.01	0.32 ± 0.02	0.49 ± 0.02	0.36 ± 0.02	0.66 ± 0.03	0.48 ± 0.03
TV, L	0.46 ± 0.02	0.37 ± 0.02*	0.51 ± 0.03*	0.39 ± 0.02*	0.74 ± 0.04*	0.58 ± 0.05*†
RR, breaths/min	16.0 ± 0.5	15.7 ± 0.5	17.0 ± 0.5	15.9 ± 0.5	16.8 ± 0.4	16.4 ± 0.5
Ve, L/min	7.37 ± 0.20	5.75 ± 0.20*	8.65 ± 0.30*	6.26 ± 0.30*	12.45 ± 0.60*	9.3 ± 0.60*†
SaO <sub>2</sub> , %	96.7 ± 1.5	97.1 ± 0.3	81.9 ± 0.5*	82.3 ± 0.17*	97.6 ± 0.2	97.7 ± 0.2
PETCO <sub>2</sub> , mm Hg	44.6 ± 0.7	44.6 ± 0.8	42.6 ± 1.0	43.3 ± 1.0	50.7 ± 0.6*	49.7 ± 0.6*

Definition of abbreviations: IRL = inspiratory resistive loading; Pepi = peak epiglottic pressure; RPH = pharyngeal resistance; RR = respiratory rate; TV = tidal volume, Ve = minute ventilation.

Values are expressed as group means ± SEM. Data for IRL under all conditions represented by 10 cm H<sub>2</sub>O per L/s load.

\* p < 0.05, compared with baseline.

† p < 0.05, compared with IRL.



**Figure 4.** Linear regression comparing peak phasic genioglossus activity and peak epiglottic pressure under conditions of baseline and mechanical loading alone.

neural systems controlling the muscle in combination with greater changes in  $P_{CO_2}$  and intrapharyngeal negative pressure. This does not, however, minimize the importance of the muscle activation observed in this study, a near tripling of GGEMG.

In conclusion, we observed increased upper airway dilator muscle activity during NREM sleep only during the combined hypercapnia plus inspiratory resistive loading condition. We speculate that a combined input of mechanoreceptor plus chemoreceptor stimulation may be required for activation of the genioglossus muscle during NREM sleep. However, we cannot exclude the possibility that either higher levels of  $P_{CO_2}$  or greater resistive loads alone could have led to more negative intrapharyngeal pressure and muscle activation in the absence of the other stimulus. We further speculate that combined activation of these receptors leads to the increase in upper airway muscle activity typically seen in normal subjects during NREM sleep and over the course of an apnea in patients with OSA.

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