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Awake negative pressure reflex response of the genioglossus in OSA patients and normal subjects

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Berry, Richard B., David P. White, John Roper, Giora Pillar, Robert B. Fogel, Michael Stanchina, and Atul Malhotra. Awake negative pressure reflex response of the genioglossus in OSA patients and normal subjects. *J Appl Physiol* 94: 1875–1882, 2003. First published January 17, 2003; 10.1152/japplphysiol.00324.2002.—We hypothesized that the response of the genioglossus to negative pressure during wakefulness should be intact in obstructive sleep apnea (OSA) patients despite published evidence showing impairment of the response of palatal muscles (Mortimore IL and Douglas NJ. *Am J Respir Crit Care Med* 156: 867–873, 1997). Thus the response of the genioglossus to brief nasal negative pressure applications (NPAs) in early inspiration was compared between OSA patients and an age-matched group of normal subjects at two study sites ($n = 11$ per group in Long Beach, $n = 14$ per group in Boston). Subjects were studied in the sitting (Long Beach) or supine (Boston) posture, and the genioglossus electromyogram (EMGgg) was measured with an intraoral surface electrode (Long Beach) or intramuscular electrode (Boston). The response of the EMGgg was expressed as the percent change from baseline where the baseline EMGgg was the value at the onset of the NPA. In Long Beach, the EMGgg response was significantly higher in the OSA patients at a lower suction pressure of ~10 cmH_2O (75.2 ± 8.4 vs. 37.4 ± 4.0% increase; $P < 0.001$) but not at a higher suction pressure of ~20 cmH_2O. In Boston, the response in the OSA patients was also greater (107.2 ± 25.9 vs. 46.3 ± 8.3%; $P < 0.05$) at a suction pressure of ~13 cmH_2O. We conclude that the response of the genioglossus to NPA during wakefulness is not impaired in OSA patients compared with normal subjects and is greater at low suction pressures.

upper airway; sleep apnea syndrome; obstructive sleep apnea

PATIENTS WITH OBSTRUCTIVE sleep apnea (OSA) appear to have higher than normal basal genioglossus muscle activity during wakefulness (5, 17). The higher activity is thought to be a compensatory mechanism that maintains upper airway patency despite unfavorable upper airway anatomy (21, 22). Loss of muscle activity at sleep onset is thought to be a major factor in the development of airway closure in patients with OSA (18, 21). The mechanisms mediating the higher upper airway muscle activity in OSA patients during wakefulness are unknown. The augmentation of genioglossus activity by negative upper airway pressure is one possible mechanism. Patients with OSA tend to have smaller upper airways (22) and higher upper airway resistances than normal subjects during wakefulness. The more negative upper airway pressures and higher tonic activity may explain the greater genioglossus activity in OSA patients (5).

A reflex augmentation of genioglossal activity has been demonstrated in animals (16) and awake humans when negative pressure is applied suddenly to the upper airway (8–11, 25). Augmentation of activity can be demonstrated with a latency shorter than a voluntary response. A similar reflex augmentation has been demonstrated for the palatal muscles in humans during wakefulness (20, 26). The increase in genioglossus activity by negative pressure is decreased by topical upper airway anesthesia (6, 9, 27), suggesting that topical mechanoreceptors mediate at least part of the effect. The reflex is also decreased during stable sleep (10, 25), although it may be intact at wake-sleep transitions (23). Topical lidocaine also diminishes genioglossus activity during obstructive apneas, implying that augmentation of genioglossus activity by negative pressure still occurs during sleep but may require higher pressures (1) or combinations of stimuli (24).

If negative upper airway pressure is one explanation for augmented genioglossus activity during wakefulness in OSA patients, then one would expect the negative pressure reflex to be intact. In contrast, a previous study comparing the reflex response of palatal muscles (levator palatini, palatoglossus) to sudden application of negative pressure with subjects in the seated posture found the response in OSA patients to be reduced compared with a group of normal subjects (20). The genioglossus responses to negative pressure in normal subjects and OSA patients have not been compared.

Given the previous findings of augmented genioglossus activity in OSA compared with normal subjects, we hypothesized that the response of this muscle to neg-

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ative upper airway pressure during wakefulness should not be impaired. Because there is considerable variability in the activity of the genioglossus in both normal and OSA populations (6, 17) and the technical aspects of measuring this activity [posture (supine or seated) and instrumentation of the airway] can affect the results, we chose to combine data from two centers that used different experimental approaches. In one center (Long Beach), subjects were studied in the sitting posture with an intraoral surface electromyogram (EMG) electrode but without instrumentation of the upper airway. Two different suction pressures goals were studied (-10 and -20 cmH_2O). In the second center (Boston), subjects were studied with intramuscular electrodes in the supine position. The upper airway was instrumented to detect the magnitude of upper airway pressure changes. By combining the findings obtained with the use of the two different methodologies, we hoped to minimize the possibility that the findings would be based on the methodological approach rather than the biological characteristics of the genioglossus in normal subjects vs. patients with OSA.

METHODS

The projects were approved by the Human Studies Subcommittee of the Long Beach Veterans Affairs Medical Center and the Human Subjects Committee of the Brigham and Women's Hospital. All subjects signed an informed consent before participating in the studies. The normal subjects were all determined to be nonsnoring by thorough history, and none gave a history of daytime sleepiness. A history was taken and physical examination was performed to exclude subjects with nasal deformity, nasal obstruction, and those on medications that might affect genioglossus activity (narcotics, serotonergic agents). All patients with OSA had an apnea + hypopnea index >40/h (Long Beach) or ≥25/h (Boston) on a prior sleep study. None of the patients with OSA had undergone previous upper airway surgery. The patients with OSA were recruited from patients referred to the sleep laboratories of the Long Beach Veterans Affairs Medical Center or the Brigham and Women's Hospital. Premenopausal women (Boston site) were studied during the follicular phase of the menstrual cycle (between *days 5* and *11*, with *day 1* being the first day of menses). At the Long Beach site, 6 of 11 patients were using continuous positive airway pressure (CPAP) at the time of the study. At the Boston site, all patients were reportedly on CPAP, although objective adherence information was not obtained at either site.

Long Beach study site. Negative pressure applications (NPAs) were applied without warning to a tightly fitting nasal CPAP mask (Contour, Respironics, Pittsburgh, PA) while the subjects were seated upright in a chair. This posture minimized any tendency for the upper airway to collapse. Subjects breathed only through the nose. Constant observation via a video camera allowed verification of wakefulness (eyes open) and breathing with the mouth closed during the entire procedure. Airflow was measured with a pneumotachograph inserted into the mask. The pneumotachograph was calibrated with a rotameter. Mask pressure was measured by connecting a port to a pressure transducer (Validyne, Northridge, CA) calibrated with a water manometer. Mask pressure, airflow, raw EMG, and integrated moving-time-average (MTA) EMG were all recorded by using a Grass model 78 D polygraph (Grass Instruments, Quincy, MA). Data were acquired (J6 output jack) by using an analog-to-digital board at a sampling rate of 1,000 samples/s. All measurements were made manually from the digitally acquired data by using a computer program (Windaq, Akron, OH).

The genioglossus EMG (EMGgg) was measured with an intraoral surface electrode mouthpiece with the use of the method of Doble et al. (3) and Horner and co-workers (7). The mouthpiece was constructed from flexible polypropylene sheets (Pressform, Ellman International, Hewlett, NY) pulled down under pressure over a stone model of each subject's lower teeth and anterior floor of the mouth. The stone model was fabricated from a dental impression. The mouthpiece fitted snugly over the teeth and the anterior floor of the mouth. Two wires (Teflon-coated stainless steel; 30 gauge) were sewn through the material, and a small area of the Teflon coat was stripped so that a bare portion of wire was in contact with the floor of the mouth on each side of the frenulum above that portion of the genioglossus muscle that attaches to the mandible. The two signals from the mouthpiece electrode and a ground electrode produced a bipolar recording. The raw EMG signal was then amplified, band-pass filtered between 50 and 1,000 Hz, rectified, and electronically integrated to obtain a MTA EMGgg by using an integrator (model MA821RSP, CWE, Ardmore, PA) with a time constant of 50 ms. To allow comparisons between different subjects, the EMGgg was scaled (%max) by using the maximum value during three voluntary maximal tongue protrusions as 100 with *electrical 0* being assigned a value of 0 (17, 25).

NPA. The method similar to that of Horner and co-workers (11) was used. The tight-fitting nasal mask was connected via T piece to a one-way expiratory valve and an inspiratory circuit that could be opened to either a negative pressure source or room air by a two balloon controller (Hans Rudolph, Kansas City, MO). NPAs were triggered by a computer circuit that monitored the flow signal. When armed, the computer circuit was triggered 150 ms after the onset of inspiration. At this time, the balloon controller deflated one balloon, which opened the circuit to a negative pressure source, and inflated the other balloon, which sealed the path to the atmosphere. The negative pressure source consisted of a negative pressure reservoir (50 liters) that was connected to a vacuum source. A threshold valve in the inspiratory line limited how negative pressure could become. Threshold pressure valves of 10 and 20 cmH_2O (Vital Signs, Totowa, NJ) were utilized with vacuum settings of -40 and -80 mmHg, respectively. At the end of the brief (200 ms) negative pressure pulse, the balloon leading to the negative pressure source was inflated and the balloon leading to the atmosphere was again deflated, which allowed the subject to breathe room air. At least 10 satisfactory applications of NPAs at each suction pressure level were obtained. A satisfactory NPA was defined as one in which the prestimulus EMG was stable and the mask pressure during the negative pressure pulse approximated a square wave. The -10- and -20-cmH_2O NPAs were alternated randomly. The timing between NPAs was alternated so that subjects could not predict when a NPA would occur. The baseline value of the EMGgg (at the onset of the negative pressure pulse) and the maximum value of the EMGgg within 150 ms of the negative pressure pulse onset (before onset of voluntary changes), both expressed as %max (voluntary maximum); the change in EMGgg (maximum EMGgg within 150 s − baseline); and the percent change (%change; as change in EMGgg × 100/baseline) were computed. The plateaus of the negative mask pressure achieved during NPAs were also measured. The baseline EMGgg, maximum EMGgg, %change EMGgg, and mask pressure for the 10- and 20-cmH_2O suction runs were

determined for the normal and OSA groups. The means were compared by using a *t*-test or Mann-Whitney rank sum test depending on whether the data passed a normality test (Sigma Stat, SPSS, Chicago, IL). A value of $P < 0.05$ was considered statistically significant.

Boston site. Breathing was monitored with a nasal CPAP mask (Healthdyne Technologies, Marietta, GA) connected to a two-way valve partitioning inspiration and expiration. Inspiratory flow was determined with a pneumotachograph (Fleish, Lausanne, Switzerland) and differential pressure transducer (Validyne, Northridge, CA). This instrument was calibrated with a rotameter, and the inspiratory flow signal was integrated to produce tidal volume (model 7P10 integrator, Grass). Subjects were instructed to breathe exclusively through the nose and were carefully monitored by video camera to ensure that the mouth was completely closed. Wakefulness was carefully documented by using the video camera because subjects were required to maintain their eyes wide open throughout the study. Any data collected during episodes of drowsiness (eyes closing) were discarded.

The EMGgg was recorded by using two stainless steel, Teflon-coated, 30-gauge wire electrodes. Each was inserted 15–20 mm into the body of the genioglossal muscle by using a 25-gauge needle, which was quickly removed, leaving the wire in place. The two electrodes were inserted on opposite sides of the muscle, each just lateral to the frenulum and close to the genioglossal insertion onto the mandible as previously described (17, 26). Each electrode was referenced to a common ground (placed on the forehead) to yield a bipolar recording. The raw EMG was amplified, band-pass filtered (between 30 and 1,000 Hz), rectified, and electronically integrated on a MTA basis with a time constant of 100 ms (CWE). The EMGgg was quantified as a percentage of the maximal signal for the muscle established during three maneuvers: swallowing, maximal negative inspiratory force against an occluded airway, and maximal tongue protrusion against the front teeth (17, 18). The highest single EMG value was considered to be 100%, and *electrical 0* was defined as 0% activity.

Pressures were monitored in the nasal mask and in the airway at the level of the choanae and epiglottis. One nostril was decongested (oxymetazalone HCl) and anesthetized (lidocaine HCl), and two pressure-tipped catheters (model MPC-500, Millar, Houston, TX) were inserted through this nostril to determine choanal and epiglottic pressures. One was located at the choanae by advancing it through the nostril until it impacted the posterior nasopharyngeal wall. It was then extracted 0.5 cm and taped to the nose to ensure stability. The second catheter tip was located at the level of the epiglottis by visual inspection through the mouth. It also was taped to the nose. Mask pressure (just external to the nares) was determined with a differential transducer (Validyne) referenced to the atmosphere. All of the three pressure signals were calibrated simultaneously in a rigid cylinder before insertion by using a standard water manometer. These three signals, plus flow, were demonstrated to be without amplitude or phase lags up to 50 Hz.

Negative airway pressure stimuli were generated by using a partially evacuated 50-liter canister and a solenoid valve, as described previously (10, 14). The canister was pressurized to between −60 and −80 cmH_2O, and negative pressure could be quickly applied to the upper airway at a predetermined point in early inspiration by triggering the solenoid valve at a preset inspiratory flow threshold. Each NPA had a rapid onset and offset.

The EMGgg was quantified during early inspiration before the pulse (prestimulus), and the maximum EMGgg was observed within 150 ms after the onset of the stimulus. This time window was chosen to avoid any behavioral influences on muscle activity, as has previously been shown (9, 11). Each NPA generated −8- to −14-cmH_2O pressure at the choanae, with a goal of −10 to −12 cmH_2O.

Each subject reported to the laboratory during the day, having been without food intake for at least 4 h. After we obtained the subject's informed consent, the pressure catheters, EMG wires, and nasal CPAP mask were attached, and the subject lay in the supine posture. The subject was initially monitored during tidal breathing for ~15 min and then during repeated trials of NPA until ~40 NPAs had been collected.

All signals [EMGgg (raw and MTA), airway pressure (mask, choanal, epiglottic), inspiratory flow, and tidal volume] were recorded on a 16-channel Grass model 78 polygraph. Certain signals [(raw EMGgg, MTA EMGgg), airway pressures, and inspiratory flow] were also recorded onto computer by using signal-averaging software (SIG-AVG, Cambridge Electronic Design, Cambridge, UK).

During NPA, all signals were digitized at 1,000 Hz, and signal averaging was performed with the waveform centered around the start of the NPA as detected on the choanal pressure signal. The onset of negative pressure at the choanae was considered *time 0*. The peak EMGgg response was determined from the signal-averaged waveform of many breaths. The EMGgg was quantified during early inspiration at the onset of the pulse (*time 0*), and the maximum EMGgg observed was within 150 ms after the onset of the stimulus. This time window was chosen to avoid any behavioral influences on muscle activity, as has previously been shown (9, 11). This increase in EMGgg was determined (within each condition in each subject) in several ways: *1*) as the peak EMGgg value obtained (in % of maximum units), *2*) as the change in EMG from *time 0* to the peak value within 150 ms (in % of maximum units), and *3*) as the %change from baseline (*time 0*). The %change was calculated by using the following formula: [(peak EMGgg − baseline EMGgg)/baseline EMGgg] × 100 (25, 27).

All statistical analyses were performed with commercially available software (STATVIEW V.4.5, Abacus Concepts). For variables that were normally distributed, comparisons between the controls and the patients with apnea were performed by using the Student's paired *t*-test. For those variables where the data were not normally distributed, the Mann-Whitney rank sum test was used. For all analyses, α was set at 0.05.

RESULTS

The demographics of the subjects at the Long Beach site are shown in Table 1. Subjects with OSA were

Table 1. *Subject demographics at the Long Beach study site*

	Normal Subjects	OSA Patients	*P* Value
n	11	11	
Age, yr	42.4 ± 10.4	47.3 ± 7.5	NS
Sex	M = 11, F = 0	M = 11, F = 0	
Height, in.	70 ± 1.8	71.4 ± 3.0	NS
Weight, lb.	207.1 ± 38.2	276.8 ± 53.8	<0.01
BMI, kg/m^2	29.7 ± 4.7	38.3 ± 6.6	<0.01

Values are means ± SD; *n,* no. of subjects. OSA, obstructive sleep apnea; BMI, body mass index; M, male; F, female; NS, not significant.

heavier, as evidenced by a higher body mass index (BMI). However, the populations were age matched by design. A sample tracing of the response of the EMGgg to −20 cmH_2O is shown in Fig. 1. In Fig. 2, a longer time duration with baseline breathing is shown. The change and %change in the EMGgg were significantly greater in the OSA patients at −10-cmH_2O suction (Table 2). However, neither the change nor the %change in the EMGgg at −20 cmH_2O differed between normal subjects and patients with OSA. The mean values of the %change in the EMGgg for each individual subject are shown in Fig. 3. The actual mask pressures at −10- and −20-cmH_2O suction were close to the target pressures and did not differ between normal subjects and OSA patients (Table 2).

At the Boston site, 14 age-matched subjects were studied in each group (Table 3). The %change in the EMGgg was significantly higher in the OSA patients (Table 4). The change was also higher in the OSA patients but did not reach statistical significance. The individual mean values for the %change in the EMGgg are shown in Fig. 4. The actual mean choanal and epiglottic pressures were not statistically different between the groups. The epiglottic pressure was slightly more negative in the normal group, although the mask suction pressure was slightly more negative in the OSA group, suggesting greater collapse in the apnea patients.

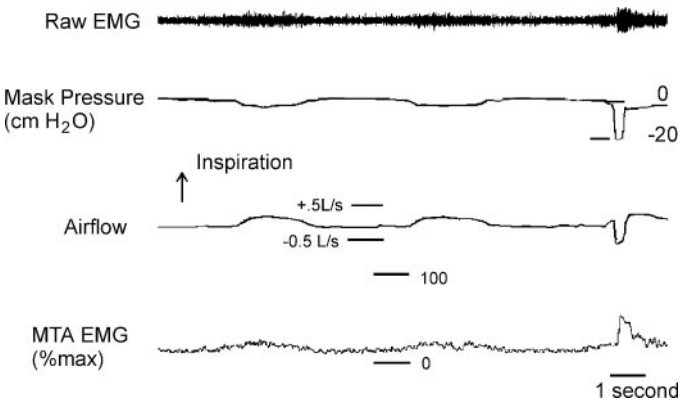

Fig. 2. Sample tracing showing a much longer time period than Fig. 1. Some phasic activity in the MTA EMG was seen before negative pressure application.

DISCUSSION

In this study, we compared the response of the genioglossus to brief NPAs in age-matched groups of normal subjects and patients with OSA during wakefulness by using different methodologies at two study sites. Our results strongly suggest that the response of the genioglossus to negative pressure is not impaired in patients with OSA.

In fact, at a negative pressure of ~10 cmH_2O, the patients with apnea showed a greater response. Although the application of sudden and brief negative pressure is certainly different from the normal generation of negative pressure during tidal breathing, finding an intact response is consistent with a role for negative pressure in mediating augmented genioglossus activity in patients with OSA (6).

Mechanoreceptors at multiple sites in the upper airway, including the nasopharynx and larynx, appear to contribute to the negative reflex (8, 9, 11). In comparing the activity of the OSA patients and normal subjects, one must ask whether *1*) equivalent areas were stimulated and *2*) the amounts of negative pressure at those receptors were similar. Studies have shown that awake OSA patients in the supine posture have more collapsible airways than normal controls (14). For a given amount of negative mask pressure, the epiglottic pressure will therefore be less negative in OSA pa-

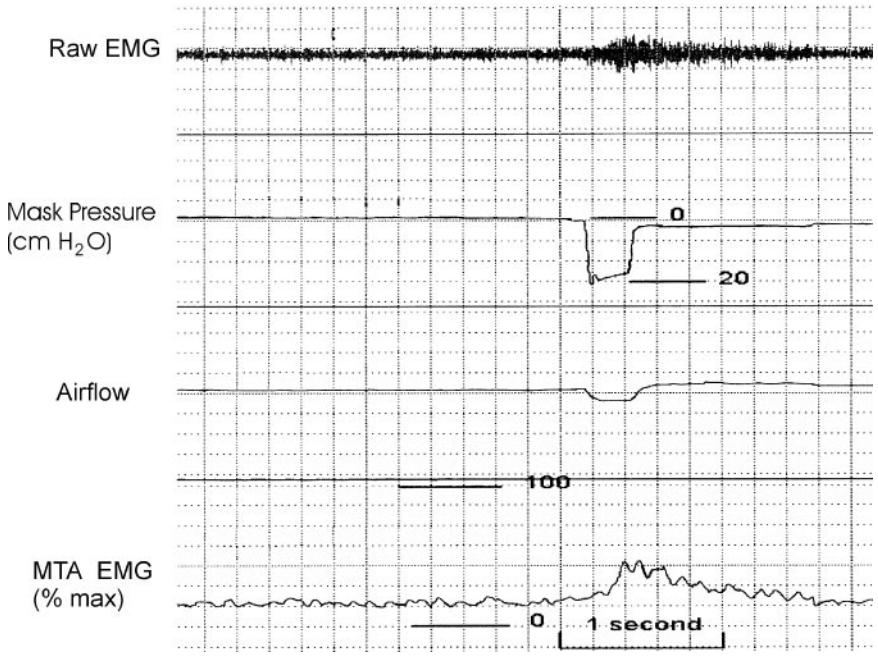

Fig. 1. Sample tracing for negative pressure application in a normal subject at the Long Beach site. EMG, electromyogram; MTA, moving time average; %max, percentage of maximum voluntary tongue protrusion.)

Table 2. *Effects of negative pressure on EMGgg at the Long Beach study site*

	Normal Subjects (n = 11)	OSA Patients (n = 11)	P Value
	10 cmH_2O		
EMGgg baseline, %max	16.3 ± 1.5	19.7 ± 1.6	NS
EMGgg change, %max	6.1 ± 1.1	14.8 ± 1.8	<0.01
EMGgg, %change	37.4 ± 4.0	75.2 ± 8.4	<0.001
Actual mask pressure, cmH_2O	10.4 ± 1.7	10.3 ± 0.6	NS
	20 cmH_2O		
EMGgg baseline, %max	16.2 ± 1.5	21.3 ± 3.5	NS
EMGgg change, %max	13.1 ± 2.5	19.9 ± 2.6	NS
EMGgg, %change	81.1 ± 10.1	93.4 ± 12.5	NS
Actual mask pressure, cmH_2O	18.7 ± 0.8	18.6 ± 0.8	NS

Values are means ± SE; *n,* no. of subjects. EMGgg, genioglossus electromyogram; baseline, EMGgg at start of negative pressure; change, maximum EMGgg within 150 ms − baseline EMGgg; %change; [(change in EMGgg − baseline EMGgg) × 100/baseline EMGgg]; %max, percentage of maximal voluntary EMGgg.

tients compared with normal subjects. This would tend to diminish the reflex response to a given amount of negative mask pressure (14). To minimize this possibility, subjects were studied in the sitting position at the Long Beach site. However, greater collapse in the OSA patients, especially at the higher suction pressure, could have yielded a lower laryngeal pressure stimulus for a given mask pressure. This could explain why we found a greater difference in the responses between normal controls and OSA patients only at the lower suction pressure. At the Boston site, at which subjects were studied supine, the amount of pressure was measured at the choanal and epiglottic regions. The amount of negative pressures at these upper airway locations in the OSA patients were slightly but not

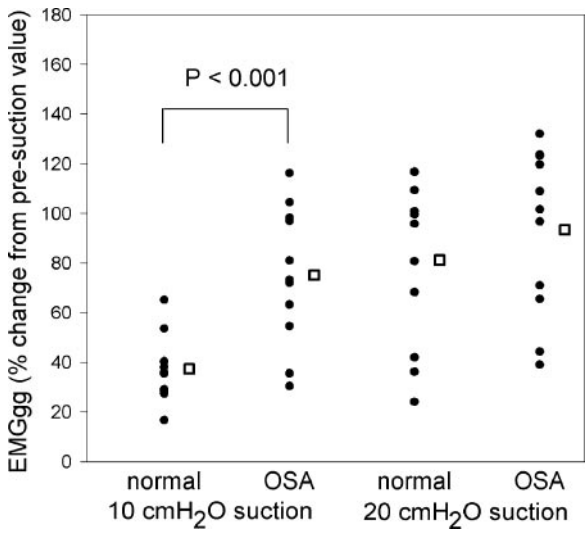

Fig. 3. Mean value for each normal and obstructive sleep apnea (OSA) subject of the percent change {%change; [maximum genioglossus electromyogram (EMGgg) within 150 ms − baseline EMGgg) × 100]/baseline EMGgg} at the 2 levels of suction pressure (Long Beach site). □, Group means. The %change was significantly higher in the OSA patients at 10-cmH_2O suction ($P < 0.001$).

Table 3. *Subject demographics at the Boston study site*

	Normal Subjects	OSA Patients	P Value
n	14	14	
Age, yr	35.5 ± 5.8	38.8 ± 8.7	NS
Sex	M = 9, F = 5	M = 10, F = 4	
Height, in.	67.5 ± 2.2	68.4 ± 4.1	NS
Weight, lb.	154 ± 22.4	243 ± 55.0	<0.0001
BMI, kg/m^2	23.6 ± 3.3	37.3 ± 10.9	<0.0001

Values are means ± SE; *n,* no. of subjects.

significantly less negative than in normal subjects. If anything, a slightly less negative pressure should reduce the response. Because the response of OSA patients was actually higher, it is unlikely that a slightly lower epiglottic pressure affected our conclusions. That is, diminished negative laryngeal pressure in OSA patients would bias toward the null hypothesis.

At the Long Beach site, the genioglossus response to negative pressure was studied at both a low- and high-pressure range of ~10 and ~20 cmH_2O, respectively. At the higher suction pressure range, the response of patients with OSA was slightly but not significantly higher than that of normal subjects. We speculate that one explanation for the finding of a significant difference only at the lower pressure is that patients with OSA may have more upper airway collapse at the higher suction pressure. Thus less pressure would have been transmitted to the laryngeal mechanoreceptors in OSA patients compared with controls. However, because epiglottic pressure was not measured, this assertion cannot be proven. It is also possible that, at the higher stimulus range, the genioglossus response is really not different i.e., a true "ceiling effect." Of note, the finding still suggests that the response is not impaired in OSA patients.

As shown in Figs. 3 and 4, there was a greater range of responses in OSA patients than normal controls. The reason that only some OSA patients showed a larger genioglossus response to negative pressure than normal controls is not clear. In addition, our groups were age but not weight matched. It is possible that obesity itself by predisposing to a smaller awake upper airway could be associated with an augmented genioglossus response. We did not attempt to weight match our

Table 4. *Effect of negative pressure on EMGgg at the Boston study site*

	normal Subjects (n = 14)	OSA Patients (n = 14)	P Value
EMGgg baseline, %max	14.4 ± 2.7	15.3 ± 3.9	NS
EMGgg change, %max	5.7 ± 1.1	8.9 ± 1.8	NS
EMGgg, %change	46.3 ± 8.3	107.2 ± 25.9	<0.05
P_{mask}, cmH_2O	12.7 ± 0.9	13.5 ± 0.7	NS
$P_{choanae}$, cmH_2O	11.2 ± 0.9	11.4 ± 0.6	NS
$P_{epiglottic}$, cmH_2O	8.1 ± 0.5	6.9 ± 0.6	NS

Values are means ± SEM; *n,* no. of subjects. P_{mask}, pressure at mask; $P_{choanae}$, pressure at choanae; $P_{epiglottic}$, pressure at epiglottic location. Pressures are negative with respect to atmospheric pressure maximum.

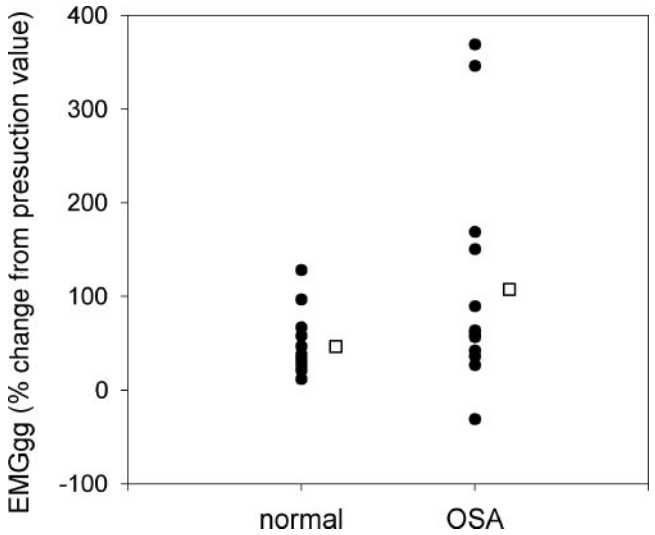

Fig. 4. Mean value for each normal and OSA subject of the %change in the EMGgg after suction (Boston site). □, Group means. The %change was significantly higher in the OSA patients ($P < 0.05$).

groups because of the difficulty in finding a group of heavy individuals without OSA. However, we did perform a subgroup analysis. We selected the seven normal subjects from each site with the highest BMI (14 total) and compared them with a group composed of the seven OSA patients from each site with the lowest BMI. The BMI in the OSA group was only slightly larger (OSA: 32.2 ± 1.2 kg/m^2 vs. normal: 28.9 ± 1.1 kg/m^2; $P < 0.05$), but the %change in the GG (using the lower suction pressure at Long Beach) was more than twice as large in the OSA group [103.1 ± 23.2 vs. 50.5 ± 8.3% (SE); $P = < 0.01$]. Thus, even when the groups were more closely matched for weight, there was still evidence for a higher response in the OSA patients. However, we accept that obesity is a potential confounding variable.

We can only speculate on possible mechanisms for an augmented response of the genioglossus to negative pressure in the OSA patients. One possibility is that more negative pressures (higher upper airway resistance) during tidal breathing might augment the response in patients with OSA. We did not measure epiglottic pressure during tidal breathing in our patients. However, a recent study comparing a group of normal subjects and OSA patients found the epiglottic pressure during tidal breathing to be about two times more negative in OSA patients (5). Another possibility is neuronal plasticity in the brain stem from chronically high upper airway resistance (negative pharyngeal pressures) during wakefulness and sleep. However, future studies in this area will be needed to clarify this issue.

Previous studies have suggested that the resting genioglossus activity is higher in patients with OSA than normal controls (5, 17). In our study, the baseline (prestimulus) EMG activity at both sites was slightly but not significantly higher in OSA patients. However, this value was measured in early inspiration and not at the peak of inspiratory activity. The goal of the present study was to compare the upper airway reflexes of OSA patients and controls rather than to reconfirm previous observations during basal breathing.

Our results for the genioglossus differ from a previous study of the negative pressure reflex in palatal muscles (levator palatini, palatoglossus) that reported an impaired response in OSA patients compared with normal subjects (20). The palatal muscle response in that study could differ because of *1*) differences in experimental technique, *2*) differences in the central control of the reflex response between palatal and genioglossal muscles, or *3*) differential effects of OSA or years of heavy snoring on the control or physiology of genioglossus and palatal muscles. There were clearly technical differences, because, in the study of palatal muscles, subjects were studied in the erect sitting posture, NPA occurred at end expiration, and the negative pressure ranged from −2.5 to −12.5 cmH_2O. Upper airway size is smallest at end expiration (22) (more collapsible); therefore, it is possible that less pressure was transmitted to the entire group of mechanoreceptors than would have occurred if the negative pressure was applied during inspiration. In terms of central control, to our knowledge, there is no information about differences in central modulation of the negative pressure reflex between palatal muscles and the genioglossus. There is evidence that OSA can cause changes in upper airway sensation (2-point discrimination, vibration, temperature) in the palatal or tonsillar areas (7, 12, 13) and produce pathological damage to both palatal muscles and the genioglossus (2, 4, 28). However, there is no evidence to date that these changes preferentially impair the palatal muscle response to negative pressure compared with the geniolgossus. Histological changes also do not necessarily mean function is impaired. For example, one study found that tongue protrusion strength and fatigability did not differ in vivo between normal controls and OSA patients (19). In summary, because our study did not directly compare the response of genioglossus and palatal muscles with the use of identical experimental techniques in the same patient groups, we can only speculate that OSA might affect the response of palatal muscles to negative pressure differently from the genioglossus.

A number of technical issues with respect to our methods require discussion. The use of nasal CPAP could have affected the response of our OSA patients to negative pressure. CPAP use did increase the response of palatal muscles to negative pressure in a previous report (20). At the Boston site, all the OSA patients studied were offered CPAP, but the adherence with this treatment was not objectively documented. At the Long Beach site, 6 of the 11 OSA subjects were reportedly using CPAP. Their adherence was also not objectively measured. However, the %change in the EMG at a suction pressure of −10 cmH_2O was 77.7 ± 11.5% (SE) in the CPAP users and 72.2 ± 13.5% in the non-CPAP users. The response in both groups was greater than that of the normal subjects. The power of

such a small sample size to exclude an effect of CPAP use on the genioglossus response is low. Thus it is beyond the scope of this study to make any conclusions about CPAP use on the genioglossus response to negative pressure. Further studies are required to address this issue. However, we do not believe studying a group of patients of both CPAP users and nonusers altered our conclusions.

We did not record EEG during the study. The awake state was documented at both sites by continuous video monitoring of the face (eye closure and head nodding). However, we cannot eliminate the possibility that some episodes of microsleeps did occur in our OSA patients. However, sleep has been shown to decrease the response of the genioglossus to negative pressure (10, 25). Thus microsleeps could have reduced the OSA responses. Because the responses were equal or higher than those observed in normal controls, microsleeps would not affect our conclusion that the genioglossus response is not impaired and may be augmented in OSA patients.

Some technical differences between the study sites also warrant discussion. At the Boston site, intramuscular electrodes were utilized, whereas, at Long Beach, the EMGgg was measured with an intraoral electrode. To our knowledge, there have been no comparisons between techniques on the response of the EMGgg to negative pressure. At the Boston site, subjects were studied supine, whereas the sitting posture was utilized in Long Beach. There are only preliminary data regarding the effect of posture on the response of the genioglossus to negative pressure pulses. Comparison of genioglossal responses to negative pressure pulses in normal subjects during non-rapid eye movement sleep revealed a higher response in the supine than lateral decubitus position (15). Because the normal controls were older and heavier in Long Beach than in Boston, some could have had mild asymptomatic OSA. We did not perform sleep studies in our normal subjects, although none gave a history of snoring or bedmate-observed apnea. Undiagnosed sleep apnea among the normal subjects would again minimize any observed differences between normal subjects and patients with apnea.

In summary, using two different methodologies, we have demonstrated that the awake response of the genioglossus to negative airway pressure in OSA patients is not impaired compared with age-matched groups of normal controls. Indeed, the response to relatively low amounts of negative pressure in both the supine and sitting postures appears to be enhanced in OSA patients compared with age-matched controls. The mechanisms mediating the augmentation in OSA patients remain to be determined.

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REFERENCES

1. **Berry RB, McNellis M, Kouchi K, and Light RW.** Upper airway anesthesia reduces genioglossus activity during sleep apnea. *Am J Respir Crit Care Med* 156: 127–132, 1997.
2. **Carrera M, Barbe F, Sauleda J, Tomas M, Gomez C, and Agusti AG.** Patients with obstructive sleep apena exhibit genioglossus dysfunction that is normalized with continuous positive airway pressure. *Am J Respir Crit Care Med* 159: 1960–1966, 1999.
3. **Doble EA, Leiter JC, Knuth SL, Daubenspeck JA, and Bartlett D.** A noninvasive intraoral electromyographic electrode for genioglossus muscle. *J Appl Physiol* 58: 1378–1382, 1985.
4. **Edstrom L, Larsson H, and Larsson L.** Neurogenic effects on the palatopharyngeal muscle in patients with obstructive sleep apnoea: a muscle biopsy study. *J Neurol Neurosurg Psychiatry* 55: 916–920, 1992.
5. **Fogel RB, Malhotra A, Pillar G, Edwards JK, Beauregard J, Shea SA, and White DP.** Genioglossal activation in patients with obstructive sleep apnea versus controls subjects. *Am J Respir Crit Care Med* 164: 2025–2030, 2001.
6. **Fogel RB, Malhotra A, Shea SA, Edwards JK, and White DP.** Reduced genioglossal activity with upper airway anesthesia in awake patients with OSA. *J Appl Physiol* 88: 1346–1354, 2000.
7. **Friberg D, Ansved T, Borg K, Carlsson-Nordlander B, Larsson H, and Svanborg E.** Histological indications of a progressive snorers disease in an upper airway muscle. *Am J Respir Crit Care Med* 157: 586–593, 1998.
8. **Horner RL.** Motor control of pharyngeal musculature and implications for the pathogenesis of obstructive sleep apnea. *Sleep* 19: 827–853, 1996.
9. **Horner RL, Innes JA, Holden HB, and Guz A.** Afferent pathways for pharyngeal dilator reflex to negative pressure in man. *J Physiol* 436: 31–44, 1991.
10. **Horner RL, Innes JA, Morrell MJ, Shea SA, and Guz A.** The effect of sleep on reflex genioglossus muscle activation by stimuli of negative airway pressure in humans. *J Physiol* 476: 141–151, 1994.
11. **Horner RL, Innes JA, Murphy K, and Guz A.** Evidence for reflex upper airway dilatory muscle activation by sudden negative airway pressure in man. *J Physiol* 436: 15–29, 1991.
12. **Kimoff RJ, Sforza E, Champagne V, Ofiara L, and Gendron D.** Upper airway sensation in snoring and obstructive sleep apnea. *Am J Respir Crit Care Med* 164: 250–255, 2001.
13. **Larsson H, Carlsson-Nordlander B, Lindblad LE, Norbeck O, and Svanborg E.** Temperature thresholds in the oropharynx of patients with obstructive sleep apnea syndrome. *Am Rev Respir Dis* 146: 1246–1249, 1992.
14. **Malhotra A, Pillar G, Edwards J, Beauregard J, Fogel R, and White DP.** Upper airway collapsibility: measurement and sleep effects. *Chest* 120: 156–161, 2001.
15. **Malhotra A, Trinder J, Stanchina M, Fogel RB, Edwards JK, Schory K, and White DP.** Tonic and phasic upper airway muscle reflexes: the influence of posture (Abstract). *Am J Respir Crit Care Med* 165: A39, 2002.
16. **Mathew OP, Abu-Osba YK, and Thach BT.** Influence of upper airway pressure changes on genioglossus and muscle respiratory activity. *J Appl Physiol* 52: 438–444, 1982.
17. **Mezzanotte WS, Tangel DJ, and White DP.** Waking genioglossal electromyogram in sleep apnea patients versus normal controls (a neuromuscular compensatory mechanism). *J Clin Invest* 89: 1571–1579, 1992.
18. **Mezzanotte WS, Tangel DJ, and White DP.** Influence of sleep onset on upper-airway muscle activity in apnea patients versus normal controls. *Am J Respir Crit Care Med* 153: 1880–1887, 1996.

19. **Mortimore IL, Bennett SP, and Douglas NJ.** Tongue protrusion strength and fatiguability: relationship to apnoea/hypopnoea index and age. *J Sleep Res* 9: 389–393, 2000.
20. **Mortimore IL and Douglas NJ.** Palatal muscle EMG response to negative pressure in awake sleep apneic and control subjects. *Am J Respir Crit Care Med* 156: 867–873, 1997.
21. **Remmers JE, Degroot WJ, Sauerland EK, and Anch AM.** Pathogenesis of upper airway occlusion during sleep. *J Appl Physiol* 44: 931–938, 1978.
22. **Schwab RJ, Gefter WB, Hoffman EA, Gupta KB, and Pack AI.** Dynamic upper airway imaging during wake respiration in normal subjects and patients with sleep disordered breathing. *Am Rev Respir Dis* 148: 1385–1400, 1993.
23. **Shea SA, Edwards JK, and White DP.** Effect of wake-sleep transitions and rapid eye movement sleep on pharyngeal muscle response to negative pressure in humans. *J Physiol* 520: 897–908, 1999.
24. **Stanchina M, Malhotra A, Fogel R, Edwards JK, Schory K, Ayas N, and White DP.** Genioglossal muscle responsiveness to chemical and mechanical loading during NREM sleep. *Am J Respir Crit Care Med* 165: 945–949, 2002.
25. **Wheatley JR, Mezzanotte WS, Tangel DJ, and White DP.** The influence of sleep on genioglossal muscle activation by negative pressure in normal men. *Am Rev Respir Dis* 148: 597–605, 1993.
26. **Wheatley JR, Tangel DJ, Mezzanotte WS, and White DP.** Influence of sleep on response to negative airway pressure of tensor palatini muscle and retropalatal airway. *J Appl Physiol* 75: 2117–2124, 1993.
27. **White DP, Edwards JK, and Shea SA.** Local reflex mechanisms: influence on basal genioglossus activation in normal subjects. *Sleep* 21: 719–728, 1998.
28. **Woodson BT, Garancis JC, and Toohill RJ.** Histopathologic changes in snoring and obstructive sleep apnea syndrome. *Laryngoscope* 101: 1318–1322, 1991.