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Gender Differences in Genioglossus Muscle Response to the Change in Pharyngeal Airway Patency

Jeffrey J. Blasius

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GENDER DIFFERENCES IN GENIOGLOSSUS MUSCLE RESPONSE TO THE
CHANGE IN PHARYNGEAL AIRWAY PATENCY

Jeffrey J. Blasius

B.S., University of Connecticut, 1994
D.M.D., University of Connecticut, 1998

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GENDER DIFFERENCES IN GENIOGLOSSUS MUSCLE RESPONSE TO THE CHANGE IN PHARYNGEAL AIRWAY PATENCY

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ABSTRACT

Differences in genioglossal (GG) basal activity and in GG muscle response to the change in pharyngeal patency were studied using bipolar surface EMG electrodes in age-matched, historically healthy, 16 males and 15 females. To minimize hormonal influences on ventilatory activity, all female EMG records were obtained during the follicular phase of the menstrual cycle or during the placebo days for individuals using oral contraceptives. Two experimental conditions were applied: a miniature balloon was placed in the retroglossal pharynx, and three positive airway pressures were applied to the nose to observe the change in GG basal tone. The EMG activity was expressed in both arbitrary units (au) and percentage of maximum activity. The maximum GG activity was defined as an average of peak tongue activities during swallowing and maximum protrusion. The measurements were recorded in both upright and supine positions. The results show that GG activity in females during natural breathing was 9 au (p< 0.05) or 5% of the maximal activity (p< 0.01) higher than that of males. All measurements in females did not change upon inflation of the balloon or positive pressure changes to the nose. Contrarily, when the balloon was inflated the GG basal activity increased 9 au (p < 0.05) in the upright position and 15 au (p< 0.01) in the supine position in males. When positive airway pressure was applied at 2, 4, and 6 cm H₂O, GG activity was increased in the upright position significantly in males (p< 0.05). However, in the supine position, GG activity was significantly increased only at 2 cm H₂O compared to the baseline (p < 0.05). We conclude that females show a higher GG basal activity during spontaneous breathing at rest. Males may be more vulnerable to the change in pharyngeal airway resistance.
ACKNOWLEDGEMENT

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Introduction

Over the last 15 years, there has been an abundance of literature researching the human upper airway. Many of the current advancements have taken place in the newly recognized disorder, obstructive sleep apnea (OSA). Obstructive sleep apnea is characterized by repetitive episodes of respiratory interruption that occur during sleep and generally are caused by a periodic collapse of the pharyngeal airway during sleep. Obstructive sleep apnea has been shown in numerous studies to demonstrate a strong male predominance, with men demonstrating a 2- to 10- fold greater incidence of sleep apnea than women.

The degree of patency of the pharyngeal upper airway during breathing is determined by the interaction between various anatomical and physiological factors. Ventilatory control mechanisms have been studied in both genders with highly variable results. Several studies have shown that women have lower hypoxic and hypercapnic ventilatory response than men. Albeit so, these differences appear to have been attributed to the larger physical size of men rather than intrinsic respiratory control differences between genders.

Many research methods have been used to study the structure and physiologic properties in the upper airway in patients demonstrating obstructive sleep apnea. Most studies have concluded that the single most common predictor in the development of obstructive sleep apnea is an anatomically small airway. Pharyngeal size or patency is importantly influenced by the activity of a variety of pharyngeal dilator muscles. One of the most important pharyngeal dilator muscles in the maintenance of airway size is the genioglossal muscle.
Tongue muscle activity, especially the genioglossus, is one of the principal determinants of airway size. Popovic and White concluded that women have, under basal conditions during wakefulness, a higher genioglossal muscle activity compared with men. If this augmented activity is maintained across all states, the female airway may be more stable and less collapsible than their male counterparts. If this is true, genioglossus response to an upper airway partial obstruction in males would be different than those in females.

This may also suggest that dental openbites in males should be managed differently from those in the female population, as openbite etiology is thought to be associated with airway size and tongue activity. This study aims to examine the differences in the relationship between upper airway size changes and the genioglossal muscle response. This study, in addition, will help establish a ground for our long-term goal of understanding the causal relationship between airway inadequacy and facial growth patterns. Moreover, the result of this study may also contribute to our understanding relating to the diagnosis, retention and stability of openbite patients.
LITERATURE REVIEW

1. **Anatomy and physiology of the upper airway**

   Exchange of respiratory gases is essential to life. Respiratory adaptations assume two crucial roles: the transport of respiratory gases and the development of specialized areas for gaseous exchange. 14 “The patency of the airway can only be threatened above the level of the hyoid bone. Below this it is protected by the cartilages of the larynx and the trachea. The interest of the orthodontist has been chiefly focused on the anterior part of the tongue, because of its implication in the crimes of thrusting, resting, biting, and lisping. Actually, the back of the tongue is usually the villain in these and other nefarious activities. Nearly all of them are related in various degrees to interference with the airway, and such interference is most often attributable to the tongue.” 15

   The upper airway, which runs from the nares or lips to the extrathoracic trachea, can be discretely divided into three segments: the nose, the pharyngeal airway (from chonae to epiglottis), and the larynx. 16 The upper portion of the pharynx, or velopharynx, is bounded anteriorly by the posterior surface of the soft palate and uvula, and posteriorly by the superior pharyngeal constrictor muscle and the cervical spine. Laterally, this portion of the pharynx is surrounded by the various muscular structures of the palate, the pharyngeal constrictor series of muscles, and the muscles extending from the base of the skull to attach on the hyoid bone and other pharyngeal structures.
The superior and middle pharyngeal constrictors and the cervical spine bind the oropharynx posteriorly. The lateral wall structures are similar to those mentioned for the velopharynx. The tongue, consisting of the genioglossus muscle, forms the anterior border of the oropharynx. The lowest portion of the pharyngeal airway, or hypopharynx, is bounded anteriorly by the epiglottis and posteriorly and laterally by the middle constrictor and thyropharyngeal muscles, but also is substantially influenced by the location of the hyoid bone.

Patency of each airway segment is dependent on several variables, including anatomy (cross sectional area), the compliance of the lumen walls, and the pressure-flow characteristics within the airway. Thus, the form of each segment is directly related to the function of each individual segment. The human pharynx is surrounded primarily by muscle tissue, with little bony or cartilaginous support. Although the cervical spine does provide some posterior support, patency of the pharynx is considered to be largely dependent on muscle tonicity, rather than rigid structures. Patency of the velopharynx is largely a product of the activity of the superior constrictor muscle and the position of the soft palate and uvula, under direct muscular control. The patency of the oropharynx is largely a product of the activity of the pharyngeal constrictor muscles plus the genioglossus muscle, although side-wall thickness and shape may also be relevant. Lastly, the patency of the hypopharynx is largely determined by the activity of the middle constrictor and thyropharyngeal muscles, but may also be substantially influenced by the position of the hyoid bone.

The muscles of the respiratory system, with physiologic or anatomic defects, cannot function normally, but may compensate for one another either functionally or
anatomically. In such instances as a deviated nasal septum, tonsillar hypertrophy, or mandibular retrognathia, the form of each individual airway segment may subjectively or objectively affect the airway patency of that segment.
1.1 Neural control of the upper airway

The muscles of the tongue are divided into two types, intrinsic and extrinsic. The intrinsic muscles are confined to the tongue, with no bony attachments. These intrinsic muscles consist of longitudinal, transverse, and vertical fibers that serve to alter the physiologic shape of the tongue. The extrinsic muscles of the tongue suspend the tongue between the mandible, the styloid process of the basicranium, the hyoid bone, and the soft palate. The genioglossus muscle is a fan-shaped muscle that originates on the anterior portion of the mandible and widens out as it extends backward into the tongue. The genioglossus muscle is the only muscle that protrudes the tongue, and along with the hypoglossal muscle also depresses the tongue. The genioglossus has been considered as the safeguard of the upper airway. With the exception of the palatoglossus muscle (which is innervated by the pharyngeal plexus, a branch of the vagus nerve), all of the tongue muscles are innervated by the hypoglossal nerve (cranial nerve XII).

The genioglossus muscle has an inspiratory phasic activation pattern, assumedly dilating and stiffening the upper airway on inspiration when intrapharyngeal pressure is negative. Sauerland and Mitchell, in 1975, first demonstrated phasic genioglossal activity during inspiration by means of bipolar needle electrodes. A series of their observations speculated about the neural relationship between the maintenance of the airway and genioglossal muscle activity. Normal muscle activity of the upper airway during respiration is precisely synchronized. The respiratory control mechanism is also
designed to save energy expenditure of the upper airway muscles by suppressing their activity when not needed. Normal breathing control is based on several respiratory reflexes such as the stretch reflex of the lung, i.e. Hering-Breuer reflex, and a reflex of the pump muscles and of other upper airway muscles, which are interrelated to each other. Several studies indicate that there are clear vagal effects on the genioglossal and hypoglossal activity in animals. Lung inflation produces a sustained graded inhibition of genioglossal EMG activity or hypoglossal neural activity. Agostoni et al. investigated the time-coursed effect of the stretch receptor in the bronchii on genioglossal muscle activity. They found that bronchial input facilitates genioglossus activity at end-expiratory volume and inhibits it at larger volumes. Van Lunteren et al. examined the effect of vagally mediated volume-related feedback on the activity of the upper airway muscles. They reported that the amount of depression for the upper airway muscles at the end of inspiration is greater than that for the diaphragm. When lung inflation is prevented by airway occlusion, or vagal transmission is blocked, genioglossal activity increases.

The genioglossus muscle clearly responds to many standard respiratory stimuli, such as hypoxia and hypercapnia, with increased activity. St. John et al. found that the activity of phrenic and hypoglossal nerves increases or decreases in parallel fashion in hypercapneic conditions in cats. It was suggested by Kuna that the hypoglossal nerve is more sensitive than the recurrent laryngeal nerve to suppression by phasic volume feedback after studying the interaction of hypercapnea and phasic volume feedback on the motor control of the upper airway. Several studies have reported that inspiratory activity of the upper airway precedes that of the diaphragm
under normal conditions. This synchronized order may be extremely critical in the rhythmic feedback of respiratory control.

The genioglossus muscle has the ability to respond to intrapharyngeal negative pressure. It has been demonstrated, in anesthetized animals with an isolated upper airway, that intrapharyngeal negative pressure leads to an increased genioglossal muscle response, particularly the inspiratory component.\textsuperscript{37} The afferent pathway for this reflex is almost certainly through the superior laryngeal nerve. Two groups of investigators have reported that rapid-onset pulses of negative pressure applied to the human upper airway led to increased genioglossal EMG activity over a time period compatible with the neural reflex.\textsuperscript{38,39} Hence, the genioglossus muscle seems able to respond to both general respiratory stimuli (hypoxia and hypercapnia) and to events occurring in its immediate vicinity (negative airway pressure).

Salamone et al. demonstrated in anesthetized animals, that increments in blood pressure lead to a preferential decrement in hypoglossal compared with phrenic neural activity.\textsuperscript{40} Therefore, increased baroreceptor output in the blood vessel seems to inhibit genioglossal activity. Garpestad et al.\textsuperscript{41} demonstrated in humans that phenylephrine-induced increments in mean blood pressure leads to substantial decrements in genioglossal EMG activity (to 53% of baseline) in normal men. Similarly, Wasicko et al.\textsuperscript{42} reported that rapid movement from a near upright posture to the supine or head-down position leads to decreases in genioglossal activity, presumably secondary to changes in blood pressure and, therefore, baroreceptor output. As a result, baroreceptor output seems to have a strong inhibitory influence on genioglossal activity.
1.2 Histology of the upper airway

Upper airway muscles are easily fatigable. Histologically, both type I and type II fibers can be identified in the extrinsic tongue muscles in cats. The fiber type nomenclatures of the human skeleton are based on fiber identification with the myofibrillar adenosinetriphosphatase (ATPase) reaction at pH 9.4. The muscle fibers markedly predominating in the red muscles are type I, whereas the muscles occurring exclusively in white muscles are type II. All type II fibers are fast-twitch units that are generally fatigable, whereas type I units are all slow-twitch and fatigue resistant. The type II fibers are subdivided further into two types: a fast fatigue-resistant type IIA fiber and fast fatigable type IIB fiber. Compared with the white muscle, biochemical assays of tissue homogenates show that the red muscle has more myoglobin, succinate dehydogenase, and cytochrome oxidase and has less myosine ATPase, lactate dehydogenase, diphosphopiridine nucleotide-linked alpha glycerophosphate dehydrogenase, phosphorylase, and mitochondrial alpha glycerophosphate dehydrogenase. Hellstrand found that 75-81% of the tongue extrinsic muscles consists of type II fibers in the feline population.

In humans, the genioglossal muscle is proportionately larger than in other mammals. Although the classification based on contraction velocities is useful, their velocities show a wide range of variability within the same fiber category. For example, the upper airway muscles have contraction times of 3 to 5 msec, which is equivalent to the quickness of the eye muscles. This suggests that the upper airway muscles seem to be limited in their ability to sustain contractions despite having a high
oxidative capacity, high capillary density, and high blood flow rate. Furthermore, the upper airway muscles are composed of a small number of very fine fibers. Due to their small size and small number of motor units, the upper airway muscles must depend primarily on an increase in stimulus frequency to increase the tension output or to sustain tension. Although low-frequency fatigue is the common type of fatigue during most voluntary contractions, the upper airway muscles may be susceptible to high-frequency fatigue due to their recruitment pattern. A study, undertaken by Scadella et al., concluded that genioglossus endurance is reduced as the contraction force increases and is significantly reduced by short-term activation that is insufficient to fatigue the diaphragm.
2. Respiration and the upper airway

The respiratory control system of the human body consists of five components: (1) the lungs, (2) inspiratory and expiratory muscles, (3) the upper airway, (4) sensory elements of the central nervous system, and (5) central nervous system neurons that integrate information. The prime function of the lungs is to exchange gas between the inspired air and the venous blood. The upper airway is a compliant tube extending from the nose and mouth to the trachea, which along with the bronchial tree, conducts respired gases to and from the lungs. The sensory elements of the central nervous system and the peripheral nervous system provide information about the status of the respiratory system. The central nervous system neurons integrate the information about respiration and control the activity of the muscles of the pump and tube.  

Ventilation is the process of moving inspired air into the alveolar gas exchange region where exchange with the blood occurs. Effective ventilation depends on the coordination of the activities of the primary muscle pump and the upper airway. This is accomplished through a complex set of interrelated functions involving the brain and specialized sensory receptors, the neuromuscular apparatus, the chest wall, the lungs, and the airway. Upper airway muscles try to stabilize the proper lumen size against inspiratory and expiratory muscle pumps in a synchronized fashion.
2.1 Obstructive sleep apnea

Obstructive sleep apnea (OSA) was first recognized thirty years ago when it was discovered that some individuals were unable to maintain pharyngeal patency during sleep despite normal respiratory function while awake. Obstructive sleep apnea is now recognized as a common disorder, affecting one to four percent of the adult population. The prevalence of undiagnosed breathing leading to daytime hypersomnolence is high among men and is much higher than previously suspected among women.

The principal problem in the patient with obstructive sleep apnea is the collapse of the pharyngeal airway, which characteristically occurs at the onset of sleep in these individuals. Although respiratory effort generally continues, the obstructed (or partially obstructed) pharyngeal airway prevents effective ventilation producing apnea (total respiratory cessation) or hypopnea (substantial reduction in ventilation). In either situation, hypoxic and hypercapnic episodes develop quickly necessitating arousal to re-establish airway patency and normal ventilation. The obstruction is virtually always located in the velopharynx or the oropharynx, or some combination of the two.

The pathogenesis of upper airway collapse in OSA is complex and multifactorial. Male sex, age and obesity are major risk factors. The anatomy of an individual pharyngeal airway varies from individual to individual. Individual airway variability is generally poorly understood. However, airway size can be affected by a number of individual variables. Obesity is one important variable that influences the anatomy of the upper airway and increases airway resistance. Whether this is a product
of direct fat deposition around the pharyngeal airway or relates to less obvious events remains undetermined. Albeit so, the relationship between obesity and size of airway seems clear. 60,61 The second important variable to be considered is aging. Aging may influence the quality of the airway, with older individuals tending to have anatomically smaller and possibly more collapsible airways. 62 The third variable resides in the genetically-driven individual variability in jaw position, tonsillar tissue, tongue size, and other anatomical differences that all influence the size of the pharyngeal airway. 63 All of these variables, solely or in combination, aid in determining the size and patency of an individual pharyngeal airway.
2.2 Gender differences in Obstructive Sleep Apnea

The ratio of men to women relative to the prevalence affected with sleep disordered breathing has varied widely.\textsuperscript{64,65} Most estimates of the male to female ratio in the general public range between 2:1 and 4:1. It has also been suggested that sleep apnea is uncommon in pre-menopausal women but increases in prevalence after menopause.\textsuperscript{66} However, data supporting this conclusion has been mixed. Bixler et al.\textsuperscript{67} investigated the prevalence of sleep disordered breathing in a large sample of women, compared with men, from the general population across a wide age range while controlling for age, obesity, and, (in women) menopause and hormone replacement therapy. The results of their study indicated that for clinically defined sleep apnea (apnea/hypopnea index $>10$ and daytime symptoms), men had a prevalence of 3.9\% and women 1.2\%. The resulting overall ratio of sleep apnea for men to women was 3.3:1 in this large two-phase study. Further, in women the presence of sleep apnea appeared to be associated exclusively with obesity (Body Mass Index $>32.3$ kg/m$^2$). Hormone replacement therapy also appeared to be associated with a lower prevalence of sleep apnea. There was no significant difference identified between the prevalence of sleep apnea in pre- and post-menopausal women taking hormone replacement therapy.\textsuperscript{65}

In women, it is believed that progesterone levels may play a role in protecting against the development of sleep apnea.\textsuperscript{68} Women experience an increase in ventilatory drive during the luteal phase of the menstrual cycle when progesterone
levels are highest. Oral progesterone has been associated with slight but definite improvement in ventilatory indices during sleep in both male and female sleep apnea patients. It has also been suggested that estrogen may increase the sensitivity of ventilatory centers to the stimulant effect of progesterone.

One of the major common links that drives the expression of sleep apnea in both genders is obesity. It is recognized that even small changes in body mass index (BMI) may significantly alter upper airway collapsibility and the severity of the sleep apnea. Small decrements in weight in moderately obese individuals can reduce the collapsibility of the airway to the point of eliminating airway occlusion, even though ideal body weight is not achieved. Thus, it is conceivable that small differences in BMI could account for some of the gender difference. Interestingly, for a given severity of apnea, women have a higher BMI suggesting that the type and pattern of obesity may play an important role. The male upper body pattern of obesity compared to the female lower body fat distribution may offer important clues in the gender differences observed in sleep apnea.

A significant amount of anatomic data has previously shown that the airway in patients is narrower at various locations, has a different shape, and has various patterns of fat deposition. The upper airway is either similar, or smaller, in women than in men. This would indicate that the female airway should be more collapsible than men. Currently, no strong evidence exists that anatomical factors can account for increased susceptibility of the male sex to pharyngeal collapse during sleep.

The functional properties of the upper airway have been quantified by the degrees of static (pressure-area) and dynamic (pressure-flow) collapsibility during
sleep. Subtle changes in collapsibility can describe a continuous, but discrete, range of collapsibility that distinguishes between completely normal people who snore and patients with severe sleep apnea.

A result study by Pillar et al., found that healthy men adapt significantly less well than women to resistive loading during sleep. They found that the male pharyngeal airway is considerably more collapsible than the female one, when exposed to greater intra-luminal negative pressure. The pressure equivalent to a few centimeters of water separates the collapsing pressure of each of these groups. Nonetheless, the data convincingly demonstrates that the upper airway of men collapses more easily when stressed by a subtle upper airway resistive load. Pharyngeal muscles respond robustly to increasing $P_{CO2}$ during wakefulness. Hypercapnia-induced pharyngeal dilator muscle activation alone is unlikely to explain the paucity of sleep disordered breathing events during slow wave sleep. The static and dynamic studies indicate that there appears to be a fundamental difference between the two sexes.
3. The role of the genioglossus muscle in maintaining pharyngeal patency

Since the dorsal surface of the tongue contributes to the anterior wall of the pharynx, the tongue participates in the respiratory function in maintaining the pharyngeal airway. Popovic and White $^{80}$ indicated that during wakefulness, both peak phasic and expiratory tonic genioglossal EMG activities are greater in men than in women. There were also clear differences in dilator muscle activity. Pharyngeal and supraglottic airway resistances were also comparable between the genders. During application of an inspiratory load of 25 cm H20/L/s, only the female subjects showed a significant increase in phasic inspiratory genioglossus EMG.

Upper airway size and patency in both males and females likely represents a dynamic interaction between dilator muscle activity and anatomy, with reduced pharyngeal anatomy being a strong predisposing variable in the development of obstructive sleep apnea. $^{81,82}$ As a result, the reduced prevalence of sleep apnea in women is most likely a product of either a structurally unique airway or higher genioglossus muscle activity. Augmented genioglossal basal activity in females could compensate for a deficient anatomy to protect against developing sleep apnea.
OBJECTIVES AND RATIONALE:

While the prevalence of obstructive sleep apnea differences between the sexes has been confirmed, there are few previous studies addressing gender effects on pharyngeal muscle activation. The purpose of this study was to examine the gender differences in the relationship between upper airway size changes and the genioglossal muscle response.

Hypotheses:

Ho: No statistical gender differences can be attributed to the genioglossal muscle as it relates to upper airway patency.

Ho: No statistical difference can be attributed to various morphologic variables evaluated on the cephalometric radiograph, when evaluated with EMG activity.

Ho: No statistical difference exists between the intramuscular technique and the surface technique in measuring EMG signals from the genioglossus muscle.

The specific aims of this study were multi fold. The first objective was to compare the two techniques, wire and surface, of measuring the EMG signals of the genioglossus muscle. The second objective was to determine the maximal genioglossal muscle EMG activity as it relates to baseline genioglossal activity during natural breathing and at rest. Third, we wanted to determine the genioglossal response when 2, 4, and 6 cm H$_2$O positive airway pressure was applied with a nasal CPAP. Fourth, we sought to identify the variations in genioglossus EMG activity with and without airway...
resistance. The fifth objective was to compare the gender differences that may exist
with an experimentally induced increased pharyngeal resistance applied with a small
balloon. Lastly, we wanted to compare the cephalometric characteristics between
males and females, and responders (those whose basal GG EMG activity increased with
an increased airway resistance) and non-responders to increased airway resistance.
The significance of the findings may help improve our understanding of the
relationship between airway competency and tongue activity in a normal population.

Evidence has accumulated during recent years that support the view that
environmental and neuromuscular influences may alter facial and dental structures. An
augmented genioglossal activity in females may result in significant alterations in
dental structures. The altered tongue posture that arises as the tongue moves superiorly
and anteriorly may be a contributing etiologic factor in dental open bite. It may
represent one factor in a multi-factorial complex that influences the dentition and the
morphogenetic facial pattern.
MATERIALS AND METHODS

The current work was designed as an experimental comparison study. Subjects were selected from volunteers willing to participate. Sixteen males and 15 females volunteered to participate in the study.

Experimental apparatus and experimental conditions:

The experimental studies were performed on the MicroTrons SARS™ Perci speech- aeromechanics research system (Perci- SARS) in the dental clinics of the Department of Orthodontics, School of Dental Medicine, University of Connecticut Health Center. The Perci- SARS was used to assess and record the nasal pressure, nasal flow, respiratory rate, and electromyographic data acquired during data acquisition. A nasal mask was employed which was connected to a pneumotachometer (Hans- Rudolph, Inc.). The pneumotachometer consists of airflow sensors that convert airflow into proportional differential pressures. The pressure transducer converts the differential pressure supplied by the pneumotachometer into an analog electrical value that is interfaced with the Persi- SARS interface computer. A heater on the pneumotachometer was employed to reduce the water and vapor concerns involved in assessing human respiration.

A PERSI-SAR interface program was designed to measure and record the changes in airflow and muscle activity (see figure 6). Baseline activity of the genioglossus muscle was first assessed during natural breathing at rest. Genioglossal
(GG) activity during rest and at task was expressed as raw values and as a percentage with respect to its maximal value. The maximal genioglossal EMG activity was obtained by pushing the tongue forward in its furthest anterior position, and additionally with voluntary swallowing, for reference. Three separate GG EMG readings were analyzed for maximal values; maximal protrusion, swallowing, and the average value of both activities. Nasal pressure, nasal flow, and respiratory rate were recorded during each activity. Next, two experimental conditions were applied. The first of which was completed to evaluate the physical properties of the pharynx. A nasal- CPAP mask was then positioned and sealed around the midface and three unique positive pressures applied. Second, a miniature balloon was positioned in the oropharynx with the subject in an upright position. After the balloon position was confirmed visually, the subject was brought into the supine position and signals of the genioglossus were recorded with the balloon inflated and deflated during natural breathing.

Procedure Protocol:

On the first visit, subjects were examined intraorally and extraorally to evaluate the suitability to participate in the experiment. General physical conditions, respiratory and circulatory systems were assessed. Signatures for consent were obtained if the subject met the inclusion criteria (see table 1). A lateral head film was obtained with the patient in the upright position with natural head posture at rest. A Reprosil® matrix (vinyl siloxane impression material) was constructed using the lower arch as a template.
This matrix was later modified to fit two surface electrodes, and trimmed to not interfere with the patient’s freeway space.

On the second visit, electromyographic (EMG), nasal pressure, nasal flow, and respiratory rate signals were recorded. The first five subjects volunteered to participate in a crossover experiment that assessed the reliability of the surface electrodes. In the first five subjects, two methods of EMG recording were used. The first method employed used two surface electrodes carried in a Reprosil® matrix (vinyl siloxane impression material). The entire protocol (see figure 9) was assessed using the two surface electrodes. Upon completion of the initial protocol, the left side of the polyvinylsiloxane was cored out where the previous surface electrode was placed (see figure 1). Topical anesthesia with 4% lidocaine was applied to the left side of the tongue, approximating the genioglossus muscle. The matrix was then re-inserted onto the patient’s lower dentition with the right surface electrode in place and the left side cored out. A single wire electrode needle (Life- Tech®, Inc.)(30-gauge Teflon-coated stainless steel wires)(see figure 3) carried in a 27-gauge hypodermic needle was inserted under the tongue about 1.5-2.0 cm into the body of the tongue, approximating the region of the previous surface electrode placement. The needle was quickly withdrawn, leaving the stainless steel wire in place (Sauerland and Mitchell, 1975). To confirm the position of the electrodes, protrusion and lateral movements of the tongue were performed. A modified protocol design was assessed with the wire electrode in place.
The previously described procedure (complete procedure for wire electrode protocol in following material) was completed on the first five patients only to compare the EMG signals of the intramuscular electrodes with the surface electrodes. Statistical tests were performed to compare the types of electrodes. Surface readings only were carried out on the 26 subsequent participants. We did not use the intramuscular electrode for the subsequent readings, nor use the wire electrode EMG data in any further data analysis.

The surface electrode protocol consisted of many steps (see Figure 9). The nasal flow, nasal pressure, and GG EMG measurements were all evaluated and recorded during each step of the procedure. All measurements were recorded for a thirty second duration. The initial EMG readings were evaluated with no nasal mask placed on the face (no pressure and flow data could be obtained) with the patient in an upright position 3-5 minutes after mouthpiece placement. GG EMG readings were recorded with the patient in a relaxed state, with the tongue in maximal protrusion, and during voluntary swallowing. The nasal mask fitted and placed on the face. Leakage around the mask was assessed. The nasal mask was evaluated to permit maximal comfort while demonstrating no pressure leaks. Adjustments were made as necessary to alleviate any problems before any further measurements were taken. The pneumotachometer was placed at the end of the nasal mask. With the patient still in the upright position, GG EMG resting levels, as well as GG EMG during maximal protrusion and swallowing were assessed. The initial measurements taken with the nasal mask off were compared to the measurements taken with the nasal mask on to ensure that the nasal mask did not alter the GG activity.
The patient was then placed in a supine position and allowed to rest for 3-5 minutes. Resting GG EMG, maximal protrusion EMG, and swallowing were then assessed with the patient in the supine position. The first experimental condition was then applied. Positive airway pressure was applied to the subject in the supine position. The positive pressure was applied at the pneumotach using a Remedy™ Renew Continuous Positive Airway Pressure (CPAP) system. The Remedy Renew CPAP supplied pressurized air through a nasal mask to the subjects, maintaining continuous positive airway pressure during experimentation and all phases of respiration. The experimental protocol consisted of measuring the resting GG EMG activity at 2, 4 and 6 cm H₂O.

With the subject in the supine position, the second experimental condition was applied to simulate an increased airway resistance. Lidocaine spray (Hurricane®) was sprayed on the posterior palate and oropharyngeal airway space. The patient was given approximately 3-4 minutes to allow the anesthesia to have effect. A miniature non-inflated angioplasty balloon (1.25 cc of air within approximately 1 cm diameter balloon) was then placed in the pharyngeal airway with the patient in the supine position. GG EMG recordings were made. The angioplasty balloon was then inflated, and resting GG EMG recordings were made. The subjects were permitted to remove the balloon if they experienced discomfort. For those subjects that did not tolerate the balloon initially, the spray anesthesia was re-applied and the balloon replaced. Recordings comparing baseline GG EMG to balloon deflated GG EMG activity were compared to ensure that balloon placement had minimal effect on GG activity.
The patient was then placed in the upright sitting position and allowed a few minutes to relax. Resting GG EMG recordings were then made with the artificially induced increased airway resistance (balloon) while the subjects gag reflex was still inhibited by the spray anesthesia with the balloon deflated and inflated. The final resting GG EMG recordings were completed with the patient in the upright position at 2,4 and 6 cm H₂O positive airway pressures.

For the first five volunteers, the mask was removed for 5 minutes and the patient was allowed to relax. The polyvinylsiloxane matrix was modified ("cored out", see Figure 1) and topical anesthesia was placed on the left side of the tongue. The mouthpiece was then re-inserted, and the wire electrode was placed to closely approximate the position of the surface electrode from the previous surface recording. The nasal mask was placed back on the subjects. Resting GG, protrusive GG, and swallowing EMG recordings were completed with the subjects in the upright position. Analysis was completed to compare the wire and surface electrodes and to ensure accurate readings (raw and % of maximal activity) were made with the surface electrode technique.

The wire electrodes were fabricated using re-usable surface electrodes purchased from The Electrode Store™ ground down to the bare wire. The bare wire was then soldered using standard silver solder into approximately a 2.5 mm round ball. The bare wire and attached 2.5 mm circular soldered end of the wire were coated with heat-shrink tubing, leaving approximately one-half of the round solder end exposed.
The surface electrode was embedded into the polyvinylsiloxane mouthpiece and held in place by a light body polyvinylsiloxane.

GG activity was recorded in arbitrary units (au) in raw numbers and also expressed in percentage to the average of maximum protrusion and swallowing activity. The average of the maximal activity was derived from three separate EMG recordings (upright, no nasal mask; upright with nasal mask; and supine with nasal mask). The three individual measurements of maximal activity were time averaged for all three trials and quantified in arbitrary units/sec (for comparison between participants). The GG basal activity was quantified for at least a 5-second period and time averaged within each trial and averaged. In the event that a participant had multiple measurements (data acquisition was repeated (as was the case in many participants)), the GG EMG measurements were quantified and time averaged (arbitrary units/sec).
Cephalometric Imaging and Analysis

Lateral cephalometric radiographs were taken from all individuals in the Department of Radiology (Dental Clinic #8) at the University of Connecticut Health Center. All lateral cephalograms were taken with the patient in the standing natural head position at end-expiration and not swallowing. Maximum lower airway viewing was achieved. Tracings of the radiographs were made on acetate sheets (0.05 mm pencil) by the same individual, blinded to the clinical and electromyographic results.

The following cephalometric measurements assessed as angular (degrees) and linear (millimeters) were obtained. SNA (angle measurement from sella [S] to nasion [N] to point A [subspinale]; SNB (angle measurement from sella to nasion to point B [supramentale]; ANB (angular measurement from point A to point B to nasion); SN-C4 (angular measurement from sella to nasion to C4; MP- FH (angular measurement of the border of the mandibular plane to Frankfort horizontal); LFH (linear measurement from ANS [anterior nasal spine] to Me [menton] perpendicular to Frankfurt Horizontal); UFH (linear measurement); total FH (linear measurement); Base of epiglottis- PNS (linear measurement); MP- H ([Vertical hyoid position] linear measurement of the distance from the mandibular plane [MP] to hyoid bone [H]; Retrognathion- H ([Horizontal hyoid position] linear measurement of the distance from retrognathion to the superiormost position of the hyoid); IAS ([inferior airway space] the distance between the base of the tongue and the posterior pharyngeal wall parallel a line intersecting B point to Gonion); SPL ([soft palate length] the distance from PNS to the tip of the soft palate); MAS ([middle airway space] the distance between the base of
the tongue and the posterior pharyngeal wall parallel to FH at the level of the soft palate); and UAS ([upper airway space]).
Statistical Analysis:

All measurements were inputted into a Microsoft Excel database and then transferred into an SPSS statistical analysis program (SPSS Version 10) and analyzed. Statistical analysis was performed using the paired t-test for all the EMG and nasal pressure data. The cephalometric measurements were analyzed using the non-paired t-test.
RESULTS:

Subjects

The age of the male participants (n=16) was an average of 27 years with a standard deviation of 2.73 years. The body mass index (BMI = weight [kg] / height $^2$ [m]) was 25.4 with a standard deviation of 2.69. The female participants (n=15) had an average age of 26.5 years with a standard deviation of 3.23 years. The body mass index of the female participants was 21.2 with a standard deviation of 3.04. When male and female subjects were compared, a significant gender difference (male 25.4 vs. female 21.1 at $p < 0.01$) was evident in body mass index. A summary of the anthropometric data may be found in table 2.

Wire vs. surface

The comparisons between wire and surface electrodes during natural breathing at rest in the upright position are shown in table 3. Figure 10 demonstrates a representative example of recordings from the genioglossus EMG signals using both the surface electrodes and the fine wire electrodes during baseline, protrusion, and swallowing for a 30 second recording. The similarity between these two measurements is quite accurate.

The results showed that the basal activity of the GG muscle recorded using surface electrodes was approximately 18.5% of maximum protrusion or swallowing
activity. In contrast the basal activity expressed as a percentage of maximal protrusive or swallowing activity was 6% higher using the intramuscular wire electrodes (~24.5%) but consistent between all samples. The standard deviation of the mean difference between the surface and wire electrodes was 1.2% (see figure 11).

Although, there were differences in absolute amount of activity measured (raw data), the percentage of activity that was measured during protrusion and swallowing using both techniques was comparable.

Comparison of GG basal activity

GG basal activity was recorded in arbitrary units (au) in raw numbers and also expressed in percentage to the average of maximum protrusion and swallowing activity. The basal activity of male and female genioglossal activity differed between the genders. The GG basal activity (upright) in males was 0.38 au with a standard deviation of 0.09 au. The male genioglossal activity (upright) when expressed as a percentage of the average of maximal activity was 20.01% with a standard deviation of 0.47%. The GG basal activity of females (upright) was 0.47 au with a standard deviation of 0.095 au. The female genioglossal activity (upright) when expressed as a percentage of maximal activity was 25.4% with a standard deviation of 0.47%. In the supine position, both genders GG EMG activity increased approximately 0.04 au. The male GG EMG activity increased from 0.38 (sd 0.09) au to 0.42 (sd 0.15) au. The female GG EMG activity increased from 0.47 (sd 0.09) au to 0.51 (sd 0.16) au.
The results indicate that GG activity in the female population appears to be significantly higher (0.09 au or 5% of maximum activity) at \( p < 0.05 \) in the upright seating position. However, the gender difference in the supine position was not statistically significant (male 0.42 au vs. female 0.51 au at \( p = 0.059 \)). When body position changed from upright to supine, GG activity increased (male, 0.38 au \( \rightarrow \) 0.42 au; female, 0.47 \( \rightarrow \) 0.51 au) significantly in both genders at \( p < 0.01 \). The genioglossal activity in both genders is summarized in table 4.

**Balloon study**

The miniature angioplasty balloon placed in the oropharynx was also evaluated. The resting (basal) raw GG EMG values did not show significant differences for either gender during natural breathing when compared to balloon deflated values in either the upright or supine positions. The male GG EMG values increased slightly in the upright position and remained the same for the supine position (upright, 0.38 au (sd 0.09) \( \rightarrow \) 0.40 au (sd 0.1); supine, 0.42 au (sd 0.15) \( \rightarrow \) 0.42 au (sd 0.11)). The female GG EMG values decreased slightly in the upright position and increased slightly in the supine position (upright, 0.47 (sd 0.09) \( \rightarrow \) 0.45 au (sd 0.11); supine, 0.51 au (sd 0.16) \( \rightarrow \) 0.52 au (sd 0.22)).

Upon inflation, or increased respiratory resistance, only the male participants increased basal GG EMG activity. The male EMG values increased significantly in both the upright and supine positions when the balloon was inflated (upright, 0.040 au (sd 0.1) \( \rightarrow \) 0.51 au (sd 0.10); supine, 0.42 au (sd 0.11) \( \rightarrow \) 0.57 au (sd 0.22)). The
female EMG values increased in both upright and supine positions, but were not statistically significant (upright, 0.45 au (sd 0.11) → 0.49 au (sd 0.18); supine, 0.52 au (sd 0.22) → 0.57 au (sd 0.18)). The resultant values of both genders in both body positions closely approximated one another quite accurately. The miniature balloon data is summarized in table 4.

CPAP study

The continuous positive airway pressure (CPAP) of 2, 4, and 6 cm H\(_2\)O was also evaluated in both upright and supine positions in both genders. Only the male participants showed statistically significant differences when the positive airway pressures were applied. The male upright GG EMG values increased slightly from natural breathing to 2 cm H\(_2\)O, increased more from 2 to 4 cm H\(_2\)O, and decreased slightly from 4 to 6 cm H\(_2\)O (0.38 au (sd 0.09) → 0.41 au (sd 0.10) → 0.46 au (sd 0.14) → 0.41 au (sd 0.10)). The male supine position demonstrated a statistically significant increase from natural breathing to 2 cm H\(_2\)O, slight decrease to 4 cm H\(_2\)O, and a slight decrease to 6 cm H\(_2\)O (0.42 au (sd 0.15) → 0.48 au (sd 0.11) → 0.47 au (sd 0.17) → 0.45 au (sd 0.22)). The female upright GG EMG values decreased slightly from natural breathing to 2 cm H\(_2\)O, increased more from 2 to 4 cm H\(_2\)O, and decreased slightly from 4 to 6 cm H\(_2\)O (0.47 au (sd 0.09) → 0.45 au (sd 0.11) → 0.53 au (sd 0.24) → 0.49 au (sd 0.18)). The female supine position demonstrated a slight increase from natural breathing to 2 cm H\(_2\)O, slight decrease to 4 cm H\(_2\)O, and a slight increase to 6 cm H\(_2\)O.
The continuous positive airway pressure data is summarized in Table 5.

Standardized data with respect to the maximum protrusion and swallowing showed more significant results. However, to be more conservative, raw measurements were used for analysis except for the comparison in natural breathing shown in Table 3.

Cephalometric Study

The cephalometric analysis was completed comparing the male and female populations. Eighteen unique data sets were completed for all individual subjects. The cephalometric variables included: age, body mass index, mandibular plane – Frankfurt horizontal angle, lower facial height, upper facial height, total facial height, base of epiglottis – posterior nasal spine length, hyoid – mandibular plane length (vertical hyoid position), retrognathion – hyoid length (horizontal hyoid position), inferior airway length, soft palate length, middle airway length, upper airway length, SNA angle, SNB angle, ANB angle, and SN-C4 Angle. When the male participants were compared to the female participants, only four variables were found to be statistically significant. These four variables included an increased body mass index for the males (males, 25.5 (sd 2.69); females, 21.0 (sd 3.04)), an increased lower facial height for males (males, 74.13 mm (sd 5.03); females, 67.92 mm (sd 6.78)), an increased horizontal hyoid length (RGN- HY) for males (males, 43.94 mm (sd 5.54); females, 37.92 mm (sd 8.72)), and
an increased vertical airway length in males (males, 78.56 mm (sd 6.74); females, 64.33 mm (sd 19.44)).

The cephalometric variables were then evaluated by subgroup using the GG EMG data. Two groups were differentiated on the basis of response to the increased airway resistance in the upright position. The two unique groups were categorized into male and female groupings, and subcategorized in responders and non-responders. The responders were those individuals that demonstrated an increase of 0.1 au in response to the inflation of the balloon in the upright position. The male responders GG EMG were 0.63 au (sd 0.26) and the male non-responders average GG EMG was 0.44 (0.17). Female responders GG EMG were 0.64 au (sd 0.21) and the female non-responders average GG EMG was 0.41 (0.10). Based on the cephalometric comparisons of the 18 variables within the male population, the only statistically significant cephalometric variable was horizontal airway length (responders, 20.67 au (sd 7.2); non-responders, 12.60 (sd 6.4)). All other variables of the male group were not statistically significant. There were no statistically significant cephalometric variables in the female population of responders and non-responders. A complete summary of most cephalometric variables is located in table 6 (comparisons within gender).

The final step in the analysis included comparing the male and female responders to each other and the male and female non-responders to each other. Thus, the two different groups were the responders and the non-responders. Within each group, male and female comparisons were made. Among the responders, the males and females differed statistically significantly for 8 variables. The body mass index was
higher in male responders, the vertical hyoid position (MP-H) was lower in male responders (males, 20.67 (sd 7.2); females, 13.40 (sd 4.39)), the horizontal hyoid position (RGN-H) was longer in male responders (males, 45.67 (sd 6.02); females, 34.80 (sd 6.06)), and the vertical airway length was longer in the male responders (males, 82.50 (sd 6.89); females, 65.80 (sd 2.59)). The remaining variables were either not statistically significant in either the responders or non-responders, or statistically significant in both response type populations (i.e. total facial height, lower facial height, and ANB angle). The summary of the male and female responders and non-responders comparisons between gender are located in table 7.
Discussion:

The results of the current study confirmed previous findings. In short, females show a higher genioglossal basal activity than that of males. When body position was changed from upright to supine, GG activity increased in both genders. When the pharyngeal airway was partially obstructed, GG EMG activity increased significantly in only males in both body positions. When positive pressure was applied to the airway, again, only males responded.

Obstructive sleep apnea patients are known to exhibit a high body mass index and constricted pharyngeal airway. Therefore, GG compensatory activity in the awake natural breathing state could be explained by the narrowing of the pharyngeal airway due to obesity. We failed to match weight during the sampling process, however the low mean values of BMI in female subjects (and subsequent higher BMI found in the male population) would hypothetically only strengthen our findings since obese patients would increase the awake GG activity. Most data suggest that increasing weight leads to an anatomically smaller pharyngeal airway, which, in apnea patients, leads to increased muscle activity. The males exhibited a statistically significant lower GG activity than that of females in this study.

Electromyographic activity, or the electrical activity of skeletal muscles, is of considerable interest to clinicians because it is a direct representation of the outflow of motoneurons in the spinal cord to the muscle as a result of voluntary or reflex activation. In our study, there were differences in absolute amount of activity measured between the wire and surface electrode techniques, but both techniques were
comparable when measured as a percentage of the average maximal GG activity. The wire technique was shown to measure a consistent 6% higher GG EMG values, yet the size of standard deviations of both techniques was remarkably similar. The trend was statistically significant and we conclude that both methods produce equally accurate and reproducible results.

The use intramuscular wire electrode has been the “gold standard” to record EMG activity in skeletal muscles particularly because wire allows muscle movement. Intramuscular electrodes have been used to record the EMG activity of the human genioglossus (GG) muscle. However, using an intramuscular electrode in human subjects presents many potential drawbacks. It is difficult to design a protocol to assess the basal and maximal GG activity in patients without pain and fear when the electrode is inserted and left to remain in the muscle. Even with the use of a topical anesthetic, the sensation of the needle penetrating the mucosa and remaining in the muscle lingers and creates special difficulty in taking consistent, accurate records of basal and maximal GG levels.

As a result, there have been many recent studies comparing a non-invasive EMG recording technique with the wire technique. Some studies assessing the GG muscle use surface electrodes incorporated into an acrylic plate. However, the EMG recordings by the surface electrode in the plate also has several potential drawbacks, which include interference of natural tongue movement (especially during protrusion and swallowing) by the bulky, non-flexible carrier, difficulty in proper placement of the electrodes during functional tongue movements, and a lowering of volume of the oral cavity. Ishiwata, et. al, evaluated genioglossus activity using a
miniature surface electrode directly attached to the sublingual mucosa when studying the human jaw-tongue reflex. During the trial period of this project, the method described in Ishiwata's paper was tested. We found that this method also had its inherent problems. The 2 mm Ag-AgCl electrode that was attached with cyanoacrylate adhesive did not remain attached to the mucosa throughout the study protocol. As a matter of fact, we were not even able to complete more than 3 of the 16 measurements before adhesive failure. Thus, this technique had to be abandoned and the polyvinylsiloxane method was introduced prior to all participant evaluation.

Sauerland and Harper observed marked heterogeneity in the activity of the GG muscle depending on intramuscular electrode placement. If the electrodes were placed anteriorly, they found the GG to exhibit more phasic activity. Conversely, when placed more posteriorly, the GG demonstrated more tonic activity. Our study attempted to place the surface electrode in similar positions in all participants, depending on the anatomy of the lingual surface. We also attempted to quantify the EMG activity, using the Persi-Sars analysis program that has not been previously studied. However, the activity measured by the surface electrode held a constant relationship to the activity measured by the wire electrodes, even after the mouthpiece was removed and then replaced. Additionally, we sampled over half of the participants at least twice for each protocol measurement. The data sets were compared and it was determined that no significant variation occurred when the repeated measurements were taken individually. Therefore, it appears reasonable to quantitatively compare the electrical activity obtained from different sampling periods.
The acrylic mouthpiece design has been shown to reveal less contamination arising from the digastric and mylohyoid muscles.\textsuperscript{92} It is our belief, that the polyvinylsiloxane carrier acts similarly to the acrylic mouthpiece and will therefore also demonstrate little contamination from other muscles. The close contact of the electrodes to the GG muscle, high patient tolerance, ease of fabrication, consistent performance, close fit of the electrode to the teeth, and ability to make repeated quantitative measurements of EMG activity over an extended period of time, are all aspects that make the polyvinylsiloxane surface electrode carrier an excellent research tool in the assessment of genioglossal function.

In trying to explain the male predominance in sleep apnea, the primary variables determining the upper airway patency must be evaluated, including anatomy and upper airway dilator muscle activity. Previous studies have failed to demonstrate consistent upper airway pharyngeal size differences between men and women.\textsuperscript{93,94} In addition, women are consistently smaller than men, in criteria like BMI, size and pharyngeal airway size, which should then predispose them to sleep apnea, not decrease the prevalence, as is seen in the female population. Nonetheless, the anatomical size does not appear to explain the gender differences observed in sleep apnea.

Diminished upper airway muscle activity could be responsible for the increased incidence of obstructive sleep apnea in men. Previous studies have demonstrated that young, healthy, pre-menopausal women have significantly higher peak phasic and tonic GG EMG activity when compared to men.\textsuperscript{95,96} The current study supports the conclusions drawn from previous studies.
In a recent study, no sex differences were found in muscle activation in response
to inspiratory resistance loading during sleep. Neither the basal EMG level during
NREM sleep nor the response of the muscles to loading differed between men and
women. They concluded that based on current data, it appeared unlikely that
substantial sex differences in pharyngeal collapsibility result from differences in muscle
activation. One potential limitation of their study was their method of comparing
EMG's between individuals. They used the percentage of maximal activation (i.e.
swallowing, protrusion) that is probably less than ideal due to the variability in
electrode placement and effort on maximal maneuvers. However, they agreed that the
EMG measurements were not the primary goal of this study. We found, in our study,
that when individuals had the wire electrode in place, it was quite painful to move the
tongue in almost any direction. Maximal activity was therefore difficult to assess easily
with the wire electrode.

In our study, the basal activity of the GG muscle in females was approximately
5% higher than that of males in the upright body position, yet the difference was not
statistically significant (p = 0.059) in the supine position. The finding of no statistical
difference identified in the supine position may be due to the increased standard
deviations of the supine measurements. However, the mean difference between two
genders was exactly the same, 0.09 au. Interestingly, the body position changes elicited
the same amount of GG EMG activity increase (0.04 au) in both genders as well and
both increases were statistically significant at p<0.01. This finding indicates that the
inter- subject variation may be large, but intra- subject variations upon the body
position change are small. Taken together, these findings may imply that females have
a higher GG basal activity during spontaneous breathing at rest irrespective of body position.

It is interesting to note that males are much more reactive to change in the resistance of the pharyngeal airway when compared with females. We used the same size balloon in every subject. Thus, the blocked portion of the pharynx by the balloon in female subjects would be greater proportionally than the male subjects (in relation to pharyngeal size). However, the increase in GG activity in females was less than half of those in males in both body positions. This may indicate that the basal tone of the GG of the female participants is already high enough, and females may not need to change the GG basal activity against the increased airway resistance. In fact, table 3 demonstrates that the measurements with the balloon inflated in females appear to approximate those of male in both body positions.

All studies, including this one, that have demonstrated an increased GG EMG activity of the female population were conducted during wakefulness, allowing no real conclusions that could be drawn about sleep. If these observations during wakefulness do apply to sleep, the upper airway of premenopausal women hypothetically could be less collapsible than the male pharyngeal airway, and thus help explain the gender differences observed. It would have been better for us to complete this study in a sleeping state, to allow direct comparisons. However, it would have been cost prohibitive to conduct 31 overnight sleep studies in this study population and for this project. Future studies may benefit from this design.

Many have studied the influence of hormones on the GG muscle activity. The influence of hormones may contribute to the driving mechanism of the increased GG
EMG expressed in the female population. Postmenopausal women have been generally reported to have a higher frequency of sleep apnea, when compared to pre-menopausal women, although some controversy in the issue has yet to be resolved. Popovic and White evaluated the influence of female hormonal status on upper airway dilator muscle activity. They demonstrated that in premenopausal women, GG EMG (expressed as a percentage of maximum) is greater in the luteal phase compared with the follicular phase of the menstrual cycle. Furthermore, compared with the premenopausal group, postmenopausal women demonstrated significantly less GG EMG activity, which increased with hormone replacement. Progesterone does appear to stimulate ventilation and respiratory responsiveness to hypoxia and hypercapnia. Although, the actual interaction between female hormones and pharyngeal muscle activity is poorly understood at this time.

We choose to test females in their follicular phase of the menstrual cycle, to minimize the hormonal influences on GG activity. Three participants, were taking oral contraceptives. These three women were tested during the late placebo days in the oral contraception regimen, immediately prior to menses. The hormone levels of women were not evaluated and the phase of testing (which day in follicular cycle) was confirmed by participant. If we had tested the GG EMG during any other phases, there may have been even greater differences observed between the gender EMG GG levels. There were no statistically significant differences between the three women taking oral contraceptives and the remainder of the female participants.

Application of positive air pressure resulted in less consistent findings. Unexpectedly, the positive air pressure increased GG activity in males. It was expected
that positive air pressure would decrease the GG basal activity gradually as the pressure was elevated. Table 4 shows that the GG activity increases right away as the pressure was applied and gradually returned to basal tone when the pressure increased up to 6 cm H2O in both body positions. This may indicate that positive air pressure may have hindered basal breathing activity of GG in normal subjects. In contrast, females demonstrated no change in GG EMG once again.

Airflow resistance at any given period is most likely a combination of airway anatomy, pharyngeal collapsibility, intrapharyngeal pressure changes, and the activity of muscles involved in maintaining the airway. We identified that the nasal pressure measurements in the female population was statistically significantly lower throughout the experiment. Even though we measured the resistance at one point in the pharyngeal airway, at the most distal end, we were pleased to find that the pressure changes were consistent. We thought it was unlikely to observe statistically significant differences and more likely that differences in resistance would have been observed at lower flow rates where flow is primarily laminar, not where we has tested at the most distal end of the airway. However, the techniques used in our study did not allow completely accurate resistance determinations at other anatomical levels, but we did observe a clear relationship between GG EMG and nasal pressure.

The cephalometric analysis failed to delineate clear cephalometric landmarks identifying individuals who may be predisposed to sleep apnea, with the exception of hyoid bone position. The vertical hyoid length (MP-H) was found to be approximately 8 mm lower in the male responders when compared to the male non-
responders. In evaluating the differences between the male and female responders, most variables found to of statistical significance were intimately connected to the greater physical size of the male study population (increased BMI, increased vertical airway length, increased total facial height, increased lower facial height, and lower ANB angle). However, the male responders did exhibit a longer horizontal hyoid position (RGN- H) and a lower vertical hyoid position (MP-H). It still remains to be found that the responders become symptomatic in the future.
Conclusions:

The results of this study confirm previous evidence that suggest females exhibit a higher genioglossal basal activity than that of males. In addition, when the pharyngeal airway was partially obstructed, the GG EMG activity increased in only males significantly in both body positions. Males were much more reactive to change in the resistance of the pharyngeal airway when compared with females. If these observations during wakefulness apply to sleep, the upper airway of premenopausal women hypothetically could be less collapsible than the male pharyngeal airway, and thus help explain the gender differences observed. We have also demonstrated a novel GG EMG sampling technique that exhibits many positive aspects making it an excellent research tool in the assessment of genioglossal function. Additionally, two statistically significant cephalometric variables (vertical and horizontal hyoid position) were identified that may aid in the diagnosis and assessment of individuals that do not respond well to respiratory change.

The findings of this study could not explain why females show a higher GG basal tone than males. However, we displayed enough evidence to believe the gender difference in GG basal activity may explain the gender disparity in incidence of OSA.
Table 1: Inclusion and exclusion criteria for participation in the study protocol

<table>
<thead>
<tr>
<th>Inclusion Criteria</th>
<th>Exclusion Criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>a. All males and females between the age of 18 and 45</td>
<td>a. Subjects who are unable to provide informed consent.</td>
</tr>
<tr>
<td>b. Adult females show a regular menstrual cycle</td>
<td>b. Subjects with a diagnosis of craniofacial anomalies</td>
</tr>
<tr>
<td></td>
<td>c. Subjects who expect to have orthognathic surgery.</td>
</tr>
<tr>
<td></td>
<td>d. Subjects who are diagnosed to have any endocrinologic disease which can affect muscle function (i.e., hyper/hypothyroidism)</td>
</tr>
<tr>
<td></td>
<td>e. Subjects who take medications that can affect muscle activity such as a major tranquilizer or antidepressant.</td>
</tr>
<tr>
<td></td>
<td>f. Subjects with history of habitual snoring</td>
</tr>
<tr>
<td></td>
<td>g. Subjects with a strong, inhibitory gag reflex</td>
</tr>
</tbody>
</table>
Table 2: Anthropometric comparisons between male and female participants

<table>
<thead>
<tr>
<th></th>
<th>Male (n = 16)</th>
<th>Female (n = 15)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>27.0 ± 2.73</td>
<td>26.5 ± 3.23</td>
<td>0.668</td>
</tr>
<tr>
<td>BMI</td>
<td>25.4 ± 2.69</td>
<td>21.1 ± 3.04</td>
<td>0.000</td>
</tr>
</tbody>
</table>

Table 3: Wire and surface electrode raw data

<table>
<thead>
<tr>
<th>Subject</th>
<th>Wire Mean</th>
<th>Surface Mean</th>
<th>Mean difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>25.1</td>
<td>18.4</td>
<td>6.7</td>
</tr>
<tr>
<td>2</td>
<td>21.9</td>
<td>14.5</td>
<td>7.4</td>
</tr>
<tr>
<td>3</td>
<td>22.2</td>
<td>16.4</td>
<td>5.8</td>
</tr>
<tr>
<td>4</td>
<td>20.0</td>
<td>15.7</td>
<td>4.3</td>
</tr>
<tr>
<td>5</td>
<td>32.5</td>
<td>27.6</td>
<td>4.9</td>
</tr>
<tr>
<td>Average and standard deviation</td>
<td>24.3 ± 4.9</td>
<td>18.5 ± 5.2</td>
<td>6.0 ± 1.2</td>
</tr>
</tbody>
</table>
Table 4: Comparison of GG basal activity raw values between males and females during natural breathing at rest and upon application of the balloon.

<table>
<thead>
<tr>
<th></th>
<th>Natural Breathing</th>
<th>Balloon-Deflated</th>
<th>Balloon-Inflated</th>
</tr>
</thead>
<tbody>
<tr>
<td>Upright</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>0.38 ± 0.09</td>
<td>0.40 ± 0.10</td>
<td>0.51 ± 0.10</td>
</tr>
<tr>
<td>Female</td>
<td>0.47 ± 0.09</td>
<td>0.45 ± 0.11</td>
<td>0.49 ± 0.18</td>
</tr>
<tr>
<td>Supine</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>0.42 ± 0.15</td>
<td>0.42 ± 0.11</td>
<td>0.57 ± 0.22</td>
</tr>
<tr>
<td>Female</td>
<td>0.51 ± 0.16</td>
<td>0.52 ± 0.22</td>
<td>0.57 ± 0.18</td>
</tr>
</tbody>
</table>

Table 5: GG activity changes upon the positive air pressure changes to the nose

<table>
<thead>
<tr>
<th></th>
<th>Natural Breathing</th>
<th>2 cmH2O</th>
<th>4 cmH2O</th>
<th>6 cmH2O</th>
</tr>
</thead>
<tbody>
<tr>
<td>Upright</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>0.38 ± 0.09</td>
<td>0.41 ± 0.10</td>
<td>0.46 ± 0.14</td>
<td>0.41 ± 0.10</td>
</tr>
<tr>
<td>Female</td>
<td>0.47 ± 0.09</td>
<td>0.45 ± 0.11</td>
<td>0.53 ± 0.24</td>
<td>0.49 ± 0.18</td>
</tr>
<tr>
<td>Supine</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>0.42 ± 0.15</td>
<td>0.48 ± 0.11</td>
<td>0.47 ± 0.17</td>
<td>0.45 ± 0.22</td>
</tr>
<tr>
<td>Female</td>
<td>0.51 ± 0.16</td>
<td>0.52 ± 0.22</td>
<td>0.50 ± 0.13</td>
<td>0.57 ± 0.18</td>
</tr>
</tbody>
</table>
Table 6: Male versus female statistically significant different cephalometric variables

<table>
<thead>
<tr>
<th></th>
<th>Male (16)</th>
<th>Female (15)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>27 ± 2.7</td>
<td>26.5 ± 3.23</td>
<td></td>
</tr>
<tr>
<td>Body mass index</td>
<td>25.4 ± 2.69</td>
<td>21.0 ± 3.04</td>
<td>0.000 **</td>
</tr>
<tr>
<td>Vertical hyoid position</td>
<td>15.63 ± 7.62</td>
<td>15.42 ± 7.48</td>
<td></td>
</tr>
<tr>
<td>Inferior Airway Space</td>
<td>13.44 ± 4.68</td>
<td>11.33 ± 7.23</td>
<td></td>
</tr>
<tr>
<td>Lower Facial Height</td>
<td>74.13 ± 5.03</td>
<td>67.92 ± 6.78</td>
<td>0.015 *</td>
</tr>
<tr>
<td>Horizontal hyoid position</td>
<td>43.94 ± 5.54</td>
<td>37.92 ± 8.72</td>
<td>0.034 *</td>
</tr>
<tr>
<td>Vertical Airway Length</td>
<td>78.56 ± 6.74</td>
<td>64.33 ± 19.44</td>
<td>0.011 *</td>
</tr>
</tbody>
</table>

Statistically significant variable (**: p< 0.01) (*: p< 0.5)
Table 7: Male and Female responders and non-responders comparisons within gender

<table>
<thead>
<tr>
<th>Variables</th>
<th>Males (n=16)</th>
<th>Females (n=15)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Responders (n=6)</td>
<td>Non-responders (n=10)</td>
</tr>
<tr>
<td><em>Balloon inflated in upright position (au)</em></td>
<td>0.63 ± 2.74</td>
<td>0.44 ± 2.42 *</td>
</tr>
<tr>
<td><em>Age (years)</em></td>
<td>28.50 ± 1.64</td>
<td>26.10 ± 2.42</td>
</tr>
<tr>
<td><em>BMI</em></td>
<td>27.05 ± 2.17</td>
<td>24.35 ± 2.54</td>
</tr>
<tr>
<td><em>MP- Hyoid (mm)</em></td>
<td>20.67 ± 7.2</td>
<td>12.60 ± 6.4 **</td>
</tr>
<tr>
<td><em>RGN- H (mm)</em></td>
<td>45.67 ± 6.02</td>
<td>42.90 ± 5.28</td>
</tr>
<tr>
<td><em>IAS (mm)</em></td>
<td>14.67 ± 2.81</td>
<td>12.70 ± 5.52</td>
</tr>
<tr>
<td><em>MAS (mm)</em></td>
<td>17.00 ± 3.80</td>
<td>15.60 ± 4.20</td>
</tr>
<tr>
<td><em>SAS (mm)</em></td>
<td>12.83 ± 1.60</td>
<td>12.90 ± 2.64</td>
</tr>
<tr>
<td><em>VAL (mm)</em></td>
<td>82.50 ± 6.89</td>
<td>76.20 ± 5.73</td>
</tr>
<tr>
<td><em>TFH (mm)</em></td>
<td>135.00 ± 4.98</td>
<td>133.80 ± 4.40</td>
</tr>
<tr>
<td><em>LFH (mm)</em></td>
<td>75.17 ± 4.17</td>
<td>73.50 ± 5.60</td>
</tr>
<tr>
<td><em>SNA (angle)</em></td>
<td>83.5 ± 8.46</td>
<td>83.20 ± 3.36</td>
</tr>
<tr>
<td><em>ANB (angle)</em></td>
<td>2.33 ± 2.16</td>
<td>2.50 ± 1.08</td>
</tr>
<tr>
<td><em>MP (angle)</em></td>
<td>23.00 ± 5.51</td>
<td>22.40 ± 3.98</td>
</tr>
<tr>
<td><em>Head Posture (angle)</em></td>
<td>102.00 ± 12.22</td>
<td>100.10 ± 6.74</td>
</tr>
</tbody>
</table>

Statistically significant variable (**: p< 0.01) (*: p< 0.5)
### Table 8: Male and Female responders and non-responders comparisons between gender

<table>
<thead>
<tr>
<th>Variables</th>
<th>Responders</th>
<th>Non-Responders</th>
<th>Responders</th>
<th>Non-Responders</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Males (n=6)</td>
<td>Females (n=5)</td>
<td>Males (n=10)</td>
<td>Females (n=10)</td>
</tr>
<tr>
<td><strong>Balloon inflated in upright position (au)</strong></td>
<td>0.63 ± 2.74</td>
<td>0.64 ± 0.21</td>
<td>0.44 ± 2.42</td>
<td>0.41 ± 0.10</td>
</tr>
<tr>
<td><strong>BMI</strong></td>
<td>27.05 ± 2.17</td>
<td>20.16 ± 3.09*</td>
<td>24.35 ± 2.54</td>
<td>21.55 ± 3.06</td>
</tr>
<tr>
<td><strong>MP- Hyoid (mm)</strong></td>
<td>20.67 ± 7.2</td>
<td>13.40 ± 4.39*</td>
<td>12.60 ± 6.4</td>
<td>15.90 ± 7.84</td>
</tr>
<tr>
<td><strong>RGN- H (mm)</strong></td>
<td>45.67 ± 6.02</td>
<td>34.80 ± 6.06*</td>
<td>42.90 ± 5.28</td>
<td>41.50 ± 8.76</td>
</tr>
<tr>
<td><strong>VAL (mm)</strong></td>
<td>82.50 ± 6.89</td>
<td>65.80 ± 2.59*</td>
<td>76.20 ± 5.73</td>
<td>70.40 ± 7.11</td>
</tr>
<tr>
<td><strong>TFH (mm)</strong></td>
<td>135.00 ± 4.98</td>
<td>119.80 ± 7.05**</td>
<td>133.80 ± 4.40</td>
<td>123.10 ± 7.13*</td>
</tr>
<tr>
<td><strong>LFH (mm)</strong></td>
<td>75.17 ± 4.17</td>
<td>66.40 ± 6.80**</td>
<td>73.50 ± 5.60</td>
<td>68.50 ± 6.00</td>
</tr>
<tr>
<td><strong>ANB (angle)</strong></td>
<td>2.33 ± 2.16</td>
<td>5.20 ± 2.59*</td>
<td>2.50 ± 1.08</td>
<td>4.30 ± 1.49*</td>
</tr>
<tr>
<td><strong>Natural Breathing at rest (upright au)</strong></td>
<td>0.380 ± 0.10</td>
<td>0.529 ± 0.14*</td>
<td>0.374 ± 0.10</td>
<td>0.440 ± 0.08*</td>
</tr>
</tbody>
</table>

Statistically significant variable (**: p< 0.01) (*: p< 0.5)
Figure 1: Underside of the matrix carrier constructed from polyvinylsiloxane material. The right side of the picture demonstrated the carrier when both surface electrodes are in place. The left side shows the position of the wire electrode insertion.

Figure 2: Occlusal view of the matrix carrier
Figure 3: View of the surface and wire electrode. The surface electrode is approximately 2.5 mm in diameter. The wire electrode consists of a 27-gauge hypodermic needle carrying a 30 gauge Teflon coated wire.
Figure 4: Photograph of the deflated balloon

Figure 5: Photograph of inflated balloon
Figure 6: Diagrammatic representation of the Perci-SARS setup
Figure 7: Photograph of participant with nasal mask

Figure 8: Photograph of the SARS electronics
Figure 9: Study Protocol

** Basal GG EMG was recorded for a 30 second interval, with nasal pressure and nasal flow recorded as well.

Comparisons made to ensure nasal mask has no effect on GG activity

Comparisons made between male and female GG and nasal pressure activity for all measurements

Cephalometric data evaluated and compared between and within genders for responders and non-responders.

Comparisons made with baseline GG to ensure deflated balloon has no effect on basal GG activity

1. Basal GG EMG recorded
   - Measurement taken with patient upright and no nasal mask
   - Nasal Mask placed and participant again permitted to acclimate

2. Protrusive and swallowing measurements taken

3. Basal GG EMG recorded

4. Protrusive and swallowing measurements taken

5. Basal GG EMG recorded

6. Protrusive and swallowing measurements taken

7. Basal GG EMG recorded
   - CPAP placed on 2 cm H₂O

8. Basal GG EMG recorded
   - CPAP placed on 4 cm H₂O

9. Basal GG EMG recorded
   - CPAP placed on 6 cm H₂O
   - Oropharynx sprayed with Lidocaine spray and balloon placed deflated in oropharynx

10. Basal GG EMG recorded

11. Basal GG EMG recorded
   - Balloon inflated
   - Patient placed in upright position and balloon placed deflated in oropharynx

12. Basal GG EMG recorded

13. Basal GG EMG recorded
   - Balloon inflated
   - CPAP placed on 2 cm H₂O

14. Basal GG EMG recorded

15. Basal GG EMG recorded
   - CPAP placed on 4 cm H₂O

16. Basal GG EMG recorded
   - CPAP placed on 6 cm H₂O

Mouthpiece inserted and allowed to remain in place 3-5 minutes for acclimation. Test measurement performed to ensure proper
**Figure 10:** Samples of the wire and surface EMG recordings
Figure 11: Wire and surface electrode expressed as a percentage of maximal GG activity
**Figure 12:** Samples of EMG, nasal flow, and nasal pressure measurements. The top figure represents maximal protrusion (1), voluntary swallow (2), and resting basal levels (3). Measurements were time-averaged for all measurements.
Figure 13: Nasal pressures of males and females, both body positions, during natural breathing at rest and experimental conditions.
**Figure 14:** Cephalometric landmark identification

### Angular Measurements:
1. SNA
2. SNB
3. ANB
4. SN- C4
5. MP- FH

### Linear Measurements:
6. Lower Facial Height
7. Upper Facial Height
8. Total Facial Height
9. Base of epiglottis- PNS
10. Vertical Hyoid position (Hyoid perpendicular- MP)
11. Horizontal Hyoid position (Retrognathion - Hyoid)
12. Inferior Airway Space
13. Soft palate length (PNS- tip of soft palate)
14. Middle Airway Space
15. Upper Airway Space
References:

7 Patrick JM, Howard A. The influence of age, sex, body size, and ling size on the control and pattern of breathing during CO2 inhalation in caucasians. Respiratory Physiology 1972; 16: 337-350.

27 Agostoni E, Cavagna AM, Citterio G. Effects of stretch receptors of bronchi or trachea on genioglossus muscle activity. Respiratory Physiology 1987; 67: 333-345.


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