Upper airway muscle activity in normal women: influence of hormonal status

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Popovic, Rainer M., and David P. White. Upper airway muscle activity in normal women: influence of hormonal status. J. Appl. Physiol. 84(3): 1055–1062, 1998.—Obstructive sleep apnea is a disorder characterized by repetitive sleep-induced collapse of the pharyngeal airway. Because upper airway patency is primarily a product of the interaction between anatomy and upper airway dilator muscle activity (13, 14), the level of activity in these pharyngeal muscles is quite important during both wakefulness and sleep.

The incidence of sleep apnea is generally recognized to be higher in men than in women (20), although recent epidemiological data suggest these gender differences may not be as great as clinical populations originally suggested (36). In addition, postmenopausal women have generally been reported to have a higher frequency of apnea than do premenopausal ones (2, 12, 26), although there is still some controversy in this area (6, 36). These observations could relate to gender differences in either ventilatory control mechanisms or the structure and function of the pharyngeal airway. Indeed, one study suggested that changes in “facial morphology” may importantly influence the incidence of apnea in pre- and postmenopausal women (6). However, there is no consistent evidence in normal subjects that either anatomy (3, 4, 30) or chemoresponsiveness (1, 21, 34) is systematically different between the genders. On the other hand, there are at least preliminary data suggesting that the female hormone progesterone may influence the genioglossal electromyogram (EMGgg) in both humans and animals (16, 17, 31). We recently observed (24) that healthy premenopausal women have significantly greater genioglossal muscle activity compared with healthy, age-matched men when assessed during wakefulness. Furthermore, these women demonstrated a significant increase in EMGgg during inspiratory resistive loading, whereas men did not. Therefore, gender-specific hormones could importantly influence pharyngeal dilator muscle activity. However, to date, neither the influence of naturally changing hormone levels nor the effect of female hormone replacement on upper airway muscle EMG in healthy women has been assessed.

To investigate the influence of female hormones on upper airway muscle activity, we measured EMGgg and upper airway resistance in healthy women in three natural hormonal states, with hormonal status being documented in each case. First, we compared EMGgg in premenopausal women in the luteal vs. the follicular phase of the menstrual cycle. Second, we compared these values with those obtained in a group of healthy postmenopausal women. In all three groups, we also determined the effect of inspiratory resistive loading on muscle activity. Finally, female hormones (estrogen and progesterone) were administered in a standard dosage to 8 of the 12 postmenopausal women for a 2-wk period, and EMGgg activity was compared before and during treatment.

METHODS

Study subjects. We studied 12 premenopausal females [mean age 32.8 ± 1.8 (SE) yr] in both the follicular and the luteal phases of the menstrual cycle. Twelve postmenopausal women [mean age 56.8 ± 2.1 yr] were also studied under basal conditions while 8 returned for repeat testing after 2 wk of oral estrogen (0.625 mg) and progesterone [5 mg medroxyprogesterone acetate (MPA)] administration. The body mass index (BMI) was normal in all participants in the premenopausal group and mildly elevated in many of the postmenopausal group (Table 1). Historical evidence of a breathing disorder during sleep, chronic snoring, or other health problems led to exclusion from this study. None of the women was on any hormonal medication for at least 6 mo before study. All premenopausal women reported a regular menstrual cycle while the postmenopausal women had been without menses for at least 3 yr. The follicular studies were always conducted between cycle days 6 and 11 (with day 1 being the first day of the menses) while the luteal studies were completed between days 17 and 21. In the premenopausal women, cycle status was confirmed by measurements of progesterone levels (low in the follicular phase with at least a fivefold increase in the luteal phase). Postmenopausal status was documented with...
Table 1. Demographic data

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<th>Premenopausal</th>
<th>Postmenopausal</th>
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<tbody>
<tr>
<td>Age, yr</td>
<td>33.3 ± 1.7</td>
<td>56.8 ± 2.1*</td>
</tr>
<tr>
<td>Height, in.</td>
<td>64.4 ± 0.7</td>
<td>64.5 ± 1.0</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>63.3 ± 3.3</td>
<td>77.8 ± 4.3*</td>
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<tr>
<td>BMI, kg/m²</td>
<td>23.8 ± 3.2</td>
<td>29.6 ± 2.6*</td>
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Values are means ± SE for 12 premenopausal and 12 postmenopausal women. BMI, body mass index. *P < 0.05, different from premenopausal.

appropriate follicle-stimulating hormone (FSH), estrogen, and progesterone levels. The study was approved by the Human Subjects Committee of the University of Colorado, with all subjects giving informed consent before participation.

Procedures and measurements. Inspiratory airflow was measured with a pneumotachometer (Fleisch model no. 2, Lausanne, Switzerland) attached to a tight-fitting face mask (covering the nose and the mouth) with a dead space of ∼75–100 ml depending on facial configuration. The mask was fitted with inspiratory and expiratory valves (Hans Rudolph, Kansas City, MO) and a sampling site for pressure determination. The pressure drop across the pneumotachometer was measured with a differential pressure transducer (Validyne, Northridge, CA). Flow calibration was accomplished with a rotameter. Pressure in the mask was determined with a second Validyne transducer, and pressure at the choanae and epiglottis was determined with two pressure-tipped catheters (Millar Instruments, Houston, TX). All three pressure transducers were calibrated simultaneously in a rigid cylinder by using a standard water manometer. There was no phase or amplitude lag between the flow and pressure signals at up to at least 2 Hz.

The Millar catheters were inserted through a decongested (oxyhematoline-HCl), anesthetized (4% lidocaine) nostril. The choanal catheter was inserted through the nose until it impacted the posterior nasal wall and then was retracted ∼0.5 cm. The epiglottic catheter was located just above the epiglottis by direct visualization through the mouth. Both catheters were carefully taped in place to avoid displacement. Nasal (mask to choanae), pharyngeal (choanae to epiglottis), and total (mask to epiglottis) resistances were determined at peak inspiratory flow.

EMGgg was measured by using a pair of unipolar intramuscular electrodes referenced to a single ground, thus producing a bipolar signal. Two 25-gauge needles containing 30-gauge, Teflon-coated, stainless steel wires were inserted perorally ∼2 cm into the body of the genioglossus muscle (at points 3 mm on each side of the frenulum and midway between the first mandibular incisor and the sublingual fold). The needles were quickly removed, leaving the wires in place. The EMG signal was amplified, rectified, and integrated by a resistance-capacitance circuit on a moving-time average basis with a time constant of 100 ms (CWE, Ardmore, PA). Both the raw and the moving time average signals were recorded simultaneously.

To allow between-subject comparisons, we used a methodology previously developed in our laboratory and described in detail elsewhere (18, 24). Briefly, subjects were asked to perform a variety of maximal maneuvers (e.g., swallowing, tongue protrusion against the maxillary ridge, and a negative inspiratory force maneuver against an occluded airway) to scale the EMG signal from electrical 0 to 100% (corresponding to the highest single value obtained during any of the maximal maneuvers). Therefore, basal activity and the muscle response to inspiratory loading (see EMGgg and Response to inspiratory loading) could be defined as a percentage of maximum on this scale (Fig. 1). All measurements were recorded on a Grass polygraph (model 78E, Grass Instruments, Quincy, MA) and analyzed manually.

Study protocol. All studies were conducted with the subjects in the supine position and breathing exclusively through their nose. After all monitoring equipment was attached (see Procedures and measurements), no data were recorded until 45 min had elapsed from the time of local anesthesia. After all signals were recorded during 5 min of tidal breathing, a near-linear inspiratory resistive load (25 cmH2O·l−1·s−1) was applied for 5 min. This procedure (5 min of basal recording followed by 5 min of inspiratory loading) was immediately repeated.

As stated in Study subjects, premenopausal women were studied in the follicular and luteal phases of the menstrual cycle while postmenopausal women were studied before (n = 12) and during (n = 8) estrogen and progesterone administration.

Data analysis. The basal inspiratory peak phasic and expiratory tonic (lowest value observed during expiration) EMGgg values were determined on every breath during 4 min of both 5-min baseline periods before inspiratory load applications. However, any obviously artifactual breath (e.g., near swallowing, during a sigh, or a tidal volume below 200 ml) was excluded. To assess the EMG response to loading, both phasic and tonic EMG for each of the first five breaths after load application (immediate response) as well as the mean of five consecutive breaths after minutes 1, 2, and 3 (sustained response) were determined and compared with the basal EMG values obtained previously.

All resistances were measured at peak inspiratory flow on every other breath during the first baseline period before the load application.

Statistical analysis. All data are presented as group means ± SE. To assess group differences in baseline EMGgg activity, resistances, age, height, weight, BMI, and progesterone levels, Student's t-tests were performed. Paired t-testing was used to compare follicular- and luteal-phase premenopausal women, and unpaired t-testing was used to compare postmenopausal women with both premenopausal groups. If the normality test failed, the Mann-Whitney rank-sum test was performed. To identify a possible correlation between progesterone level and EMG activity, the Spearman rank-order correlation was utilized.

One-way repeated-measures analysis of variance (ANOVA) was used to assess the immediate and sustained EMG responses to load. If data were not normally distributed, a nonparametric test (Friedman's repeated-measures ANOVA on ranks) was performed. If there was a statistically significant difference detected, a multiple-comparison procedure (Dunnett's test) was performed. Data analysis was performed with J andel's Sigma Stat software. A P value < 0.05 was considered significant.

RESULTS

Adequate data were obtained in all subjects under all conditions with two exceptions. In two premenopausal women, results during inspiratory resistive loading during the follicular menstrual phase were technically poor and thus were excluded from data analysis. All other data sets were complete.

Hormone levels. Progesterone levels were significantly different between each group (P < 0.01). As
expected, there was at least a fivefold increase in progesterone levels (Table 2) in the luteal compared with the follicular phase of the menstrual cycle, indicating that ovulation occurred in each subject. Postmenopausal status was confirmed by elevated blood FSH levels and low estrogen and progesterone levels (Table 2). However, two postmenopausal women had lower FSH levels than expected but also low estrogen and progesterone levels. As a result, they were included in this study.

There was a significant increase in serum estrogen (from $30.8 \pm 13.1$ to $88.0 \pm 19.9$ pg/ml) and progesterone (from $0.22 \pm 0.09$ to $2.71 \pm 0.24$ ng/ml) in the postmenopausal women during hormone replacement therapy.

**Table 2. Measured variables**

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<thead>
<tr>
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<th>Premenopausal</th>
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<tr>
<td></td>
<td>Luteal phase</td>
<td>Follicular phase</td>
<td>Postmenopausal</td>
</tr>
<tr>
<td>EMG$_{gg}$ peak phasic, % of maximum</td>
<td>$23.9 \pm 3.8$</td>
<td>$15.5 \pm 2.2^*$</td>
<td>$11.3 \pm 1.6^+$</td>
</tr>
<tr>
<td>EMG$_{gg}$ expiratory tonic, % of maximum</td>
<td>$10.2 \pm 1.0$</td>
<td>$7.3 \pm 0.8^*$</td>
<td>$5.0 \pm 0.6^*$</td>
</tr>
<tr>
<td>Rn, cmH$_2$O·l$^{-1}$·s$^{-1}$</td>
<td>$1.36 \pm 0.25$</td>
<td>$1.35 \pm 0.22$</td>
<td>$1.09 \pm 0.17$</td>
</tr>
<tr>
<td>Rphar, cmH$_2$O·l$^{-1}$·s$^{-1}$</td>
<td>$1.81 \pm 0.30$</td>
<td>$1.68 \pm 0.29$</td>
<td>$2.35 \pm 0.54$</td>
</tr>
<tr>
<td>Rt, cmH$_2$O·l$^{-1}$·s$^{-1}$</td>
<td>$3.18 \pm 0.52$</td>
<td>$3.01 \pm 0.45$</td>
<td>$3.44 \pm 0.61$</td>
</tr>
<tr>
<td>Peak flow, l/s</td>
<td>$0.38 \pm 0.02$</td>
<td>$0.36 \pm 0.01$</td>
<td>$0.46 \pm 0.04$</td>
</tr>
<tr>
<td>Progesterone, ng/ml</td>
<td>$11.55 \pm 2.39$§</td>
<td>$0.46 \pm 0.07$§</td>
<td>$0.19 \pm 0.06$§</td>
</tr>
<tr>
<td>Estrogen, pg/ml</td>
<td>$24.2 \pm 9.4$</td>
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<tr>
<td>FSH, mIU/ml</td>
<td>$57.4 \pm 8.3$</td>
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Values are means ± SE for 12 premenopausal and 12 postmenopausal women. EMG$_{gg}$, genioglossus EMG; Rn, nasal resistance; Rphar, pharyngeal resistance; Rt, total resistance; FSH, follicle-stimulating hormone. *P < 0.05, different from luteal phase. †P < 0.01, different from follicular phase. §P < 0.05, each group different from both others. §§P < 0.01, each group different from both others.

Fig. 1. Genioglossal EMG (EMG$_{gg}$) signals [raw and moving time average (MTA)] from 1 premenopausal subject (J H) in follicular (top) and luteal phase (middle) of menstrual cycle. EMG was recorded under basal conditions and during maximal maneuvers. Similar data from a postmenopausal subject (DF) are also provided (bottom).
(10.2 ± 1.0%), somewhat lower in the follicular phase (7.3 ± 0.8%), and lowest in the postmenopausal group (5.0 ± 0.6%).

Response to inspiratory loading. The application of an inspiratory resistive load (Fig. 4) led to a significant increase of peak phasic EMGgg activity in the premenopausal women in the follicular and luteal phases (P < 0.05). Both an immediate (first 5 breaths after load application) and a sustained (after 1, 2, and 3 min of loading) response were observed. There was no change in expiratory tonic activity. In the postmenopausal women, there was a similar response in peak phasic EMGgg activity (P < 0.05). However, there was also a minimal but significant increase in expiratory tonic activity during load application after minutes 1, 2, and 3.

Airway resistance. The airflow resistance values are shown in Table 2. There was no significant difference in either peak airflow or nasal, pharyngeal, or total upper airway resistance between the three groups.

Relationship between progesterone levels and genioglossal muscle activity. There was a weak but significant positive correlation observed between inspiratory peak phasic EMGgg and blood progesterone levels (r =
There was a somewhat stronger positive correlation between expiratory tonic EMGgg and progesterone levels ($r = 0.57$, $P < 0.01$).

Effect of short-term female hormone replacement on EMGgg and upper airway resistance in postmenopausal women. In the eight postmenopausal women restudied after 2 wk of hormone treatment (estrogen and progesterone), there was a significant ($P < 0.01$) increase of inspiratory peak phasic (pretreatment 13.8 ± 3.5%, posttreatment 29.4 ± 5.4%) as well as expiratory tonic (pretreatment 5.6 ± 1.2%, posttreatment 12.0 ± 2.4%) EMGgg activity (Fig. 5). However, pharyngeal resistance did not change significantly (pretreatment 2.3 ± 0.7 vs. 2.3 ± 0.5 cmH2O·l−1·s during treatment).

DISCUSSION

The overall objective of this study was to determine the influence of female hormonal status on upper airway dilator muscle activity. Our data demonstrate that in premenopausal women EMGgg (expressed as percentage of maximum) is greater in the luteal compared with the follicular phase of the menstrual cycle. Furthermore, compared with the premenopausal group, postmenopausal women demonstrated significantly less EMGgg activity, which increased with hormone replacement. However, these differences in EMGgg did not translate into obvious differences in upper airway resistance. The application of an inspiratory resistive load resulted in an augmentation of inspiratory peak phasic EMGgg activity in all participants, whereas in the postmenopausal women we also observed a minimal but significant augmentation in expiratory activity. Finally, there was a weak but significant positive correlation between progesterone levels and EMGgg.

In trying to explain the male predominance in sleep apnea, one must examine the primary variables determining upper airway patency. These are anatomy and upper airway dilator muscle activity. Previous studies addressing upper airway size have failed to consistently demonstrate significant differences between men and women (3, 4), particularly when corrected for body size (3). In addition, if anything, women tend to have a smaller airway than do men, which would likely increase rather than decrease the incidence of apnea. Therefore anatomy does not appear to be protective in the women. On the other hand, diminished upper airway muscle activity could be responsible for the higher incidence of obstructive sleep apnea in men and postmenopausal women compared with premenopausal women. This conclusion is supported by the observations of this study and by previous findings that demonstrated young healthy premenopausal women to have significantly higher peak phasic and tonic EMGgg activity compared with men (24). However, both studies were conducted during wakefulness, allowing no real conclusions about sleep. If these observations during wakefulness do apply to sleep, the upper airway of the premenopausal women could be less collapsible and apnea less common. What is driving this muscle activity has not been investigated before this study during wakefulness or sleep, but certainly it could relate to sex hormones.

The influence of sex hormones on ventilation, ventilatory control, and sleep-related breathing disorders has been investigated previously. Physiological changes in hormonal status during pregnancy (9, 23) and the luteal phase of the menstrual cycle (23, 29, 35) invoke an increase in alveolar ventilation. In both circumstances, serum progesterone levels are elevated, and the increase in alveolar ventilation has been attributed to this hormone. In addition, an increase in the ventilatory responsiveness to hypercapnia (10, 29) and hypoxia (29, 34) during the luteal compared with the follicular phase of the menstrual cycle has been reported. Similarly, the administration of MPA, a synthetic progesterone, to healthy young men yielded a significant increase in the hypercapnic and hypoxic ventilatory responses as well as an increase in resting minute ventilation (37). Therefore, progesterone does stimulate ventilation and respiratory responsiveness to hypoxia and hypercapnia.

Because of the observations described above, progesterone has also been investigated in the treatment of a variety of breathing disorders awake and asleep. In the obesity-hypoventilation syndrome, progesterone administration led to improved oxygenation and reduced hypercapnia, although sleep was not investigated (33). However, the use of MPA in the management of obstructive sleep apnea has been disappointing. Administration of MPA alone over 1–4 wk did not significantly alter apnea frequency in patients with moderate to severe obstructive sleep apnea (8, 19, 25, 32). On the other hand, in a case report, a combination of hormones (estrogen and progesterone) abolished moderate sleep apnea, whereas stopping these hormones led to a return of symptoms and polysomnographic findings (11). In addition, Pickett et al. (22) reported fewer sleep-disordered breathing episodes and a shorter duration of hypopneas in healthy postmenopausal women after 1 wk of combined estrogen and progesterone treatment. However, Cistulli et al. (7) administered estrogen alone or in combination with progesterone to postmenopausal women with moderate obstructive sleep.
apnea and observed only a minimal reduction in the apnea-hypopnea index during rapid-eye-movement sleep. In summary, the use of female hormones in the management of obstructive sleep apnea has produced conflicting and often disappointing results. It could be argued that female hormones can stimulate upper airway muscle activity, but not adequately, in many cases, to overcome anatomic limitations.

The actual interaction between female hormones and pharyngeal muscle activity is poorly understood at this time, although there is an increasing body of evidence that female sex hormones can have a clear effect on upper airway dilator muscle activity. First, combined estrogen and progesterone administration reduced upper airway collapsibility in healthy young men awake and asleep (5). Second, the depressant effects of alcohol on genioglossal activity were more prominent during the follicular than the luteal phase of the menstrual cycle (17). Finally, animal studies revealed that pretreatment of male cats with MPA lessens this depressive effect of alcohol (31). Therefore, it seems quite likely that progesterone may have a direct effect on upper airway dilator muscle activity. The observations of our study certainly support this concept, with clear differences in muscle activity being observed between hormonally different groups and a direct (but weak) correlation between progesterone level and muscle activity being noted.

Our failure to observe a clear relationship between genioglossal muscle activity and airflow resistance was somewhat disappointing. However, airflow resistance at any given moment is likely the product of airway anatomy, pharyngeal wall collapsibility, intrapharyngeal negative pressure, and the activity of multiple muscles. Therefore, our inability to correlate resistance with the activity of one muscle measured during wakefulness is neither terribly surprising nor indicates that this muscle, the genioglossus, is not an important pharyngeal dilator. In addition, we only measured airflow resistance at one point in the pressure-flow curve (at peak flow). It is certainly possible that differences in resistance could have been observed at lower flow rates where flow is predominantly laminar. However, the techniques used in this study do not allow for completely accurate resistance determinations at those flow rates, and thus this was not attempted. As a result, there are likely multiple explanations for our failure to observe a clear relationship between EMGg and airflow resistance.

Although there was only a weak positive correlation between progesterone levels and EMGg, we were able to demonstrate a clear effect on EMGg of female hormone replacement in postmenopausal women. Whether this effect can be attributed to either estrogen or progesterone or may be the result of combined influences cannot be discerned. However, this increase in muscle activity was not reflected in upper airway resistance. Again, this may be due to behavioral influences or “noise” during wakefulness. Furthermore, one may not expect a further decrease in upper airway resistance in healthy subjects with a low baseline resistance.

The application of an inspiratory resistive load was undertaken to determine whether hormonal status influenced the responsiveness of the genioglossus muscle to a standard stimulus. As shown in Fig. 4, under all three conditions there was an immediate and sustained response to this load, with no clear differences being detectable between groups. Therefore, hormonal status seemed to influence basal activity more directly than stimulated activity. However, loaded muscle activity showed a similar pattern to basal activity, being highest in the luteal phase and lowest in the postmenopausal group. Why the postmenopausal group also demonstrated an increased tonic EMG during loading is unclear. However, this may relate more to behavioral than physiological influences.

Finally, several demographic and technical issues must be addressed in interpreting our results. First, the postmenopausal women were heavier (greater BMI) than were the premenopausal ones. Because rising weight increases the likelihood of apnea, it could influence muscle activity. However, most data suggest that increasing weight leads to an anatomically small pharyngeal airway, which, in apnea patients, leads to increased muscle activity (18). Our observed decreased EMGg in postmenopausal women suggests that such mechanisms were not active or that the relatively small differences in BMI in our groups did not influence airway anatomy. Second, we did not document, in the laboratory, that our subjects (particularly the postmenopausal women) were free of sleep apnea. Because some of these women had an elevated BMI, this possibility certainly exists. However, none of these women snored regularly, had witnessed apnea, or had any symptoms of daytime somnolence. In addition, our previous work (as stated above) suggests that apnea patients have elevated pharyngeal muscle EMG, not depressed levels.
as were observed in the postmenopausal women. As a result, we believe it is unlikely they had substantial apnea. Third, as can be seen in Figs. 2 and 3, two or three of the premenopausal women had quite high EMG activity, driving up the mean values for the group. However, EMG levels fell in virtually all women in the follicular compared with the luteal menstrual phase (with one obvious exception), and the lowest EMGs were encountered in the postmenopausal group. As a result, we do not believe these two or three women disproportionately influenced our results, nor was there a physiological reason to exclude their data. As a result, these data were included.

Fourth, to allow between-subject EMG comparisons and EMG assessments on multiple occasions in the same subject, we have developed the methodology whereby the EMG is expressed as a percentage of maximum. We have previously observed such measurements to be reproducible (18) and, more recently, have studied a larger group of normal male subjects with repetitive EMG measurements being made over a period of 1–2 wk (Fig. 6). We observed no trend for the EMG to increase or decrease over repetitive determinations. Therefore, we believe the clear group differences observed in this study and the increased EMG in the follicular compared with the follicular cycle phase represent genuine physiological events. This is not to imply that there is not variability when this measurement is made on multiple occasions. This is almost certainly the case on the basis of electrode position, effort, etc. However, this variability did not prejudice our ability to demonstrate hormonal differences in muscle activity.

In conclusion, this study provides direct evidence supporting the concept that female hormones (potentially progesterone) have a substantial and significant impact on genioglossus muscle activity. If present during sleep, this hormonal influence may be physiologically relevant, rendering the female airway less collapsible and thereby protecting women from the development of obstructive sleep apnea. However, further work will be necessary to document this.

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