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Oral Breathing Increases P_{th} and Vocal Effort by Superficial Drying of Vocal Fold Mucosa

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Summary: Oral breathing superficially dehydrates the airway lumen by decreasing the depth of the sol layer in humans and animals. Conversely, nasal breathing can increase the humidity of inspired air. We compared the effects of short-term oral and nasal breathing on P_{th} and perceived vocal effort in 20 female subjects randomly assigned to two groups: oral breathing (N = 10, age 21–32 years); nasal breathing (N = 10, age 20–36 years). We hypothesized that short-term oral breathing, but not nasal breathing, would increase P_{th}, and that subjects would perceive this change as an increase in vocal effort. Following 15 minutes of oral breathing, P_{th} increased at comfortable and low pitch ($p < 0.01$) with 6 of 10 subjects reporting increased vocal effort. Nasal breathing reduced P_{th} at all three pitches ($p < 0.01$), and 7 of 10 subjects reported decreased vocal effort. Over all subjects, 49% of the variance in treatment-induced change in P_{th} was accounted for by change in vocal effort (R = 0.70). We posit that obligatory oral breathing places healthy subjects at risk for symptoms of increased vocal effort. The facilitatory role of superficial hydration on vocal fold oscillation should be considered in biomechanical models of phonation and in the clinical prevention of laryngeal dryness. **Key Words:** Larynx—Oral breathing—Superficial dehydration—Sol layer—Phonation threshold.

INTRODUCTION

Superficial vocal fold hydration is thought to be essential for maintaining a healthy voice and preventing laryngeal pathologies.[1–4] Superficial dehydration induced by poorly conditioned inspired air, mouth breathing, and drying medication could result in laryngeal dryness. Accordingly, the intake of humid air and nasal breathing are often recommended as strategies to facilitate laryngeal hydration.

Wet epithelia of the airway are hydrated by surface water called the sol layer. Likewise, the water overlying vocal fold epithelia provides superficial hydration to the vocal folds. Though the sol layer of the upper airway is not more than 10 μm in depth,[5] it is necessary for vocal fold oscillation to occur as demonstrated in ex vivo animal tissue.[6,7] Whether the sol layer could play an important role in sustaining human phonation has not been addressed. Phonation threshold (P_{th}) is the minimum pressure required to initiate and sustain vocal fold oscillation.[8,9] Biomechanical models predict the direct dependence of P_{th} on a lumped element representation of the viscoelas-

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tic properties of the mucosa. Analytically P_{th} was shown to increase when frictional energy losses (the viscous modulus) of the mucosa increase relative to the energy stored by the mucosa (the elastic modulus).[8,10] In these accounts, the effects of the sol layer in superficial hydration were not explicitly considered, although water is transported from the sol layer into the lamina propria,[11] and the presence of the sol layer may reduce surface drag and adhesiveness of the vocal folds.

In order to appreciate the role of the sol layer in sustaining phonation, it is important to induce superficial dehydration of the vocal folds. This study induced superficial dehydration by using 15 minutes of oral breathing, a natural task of short duration thought not to desiccate the underlying lamina propria. Oral breathing, but not nasal breathing, causes superficial dehydration of healthy tracheal and bronchial airway epithelia by decreasing the depth of the sol layer in vivo.[12,13] Data to show that oral breathing, but not nasal breathing, increases P_{th} and perceived vocal effort would support the recognition of the sol layer in molecular models of mucosal rheology. Oral breathing is common during singing, teaching, exercising, and loud speaking. Results from this study may lead to the development of new strategies for preventing voice symptoms in healthy speakers from at-risk populations.

The present study examined the effects of oral and nasal breathing on P_{th} and vocal effort. We hypothesized that short-term oral breathing, but not nasal breathing, would increase P_{th}, and that subjects would perceive this change as an increase in vocal effort. Such data would provide support for the role of sol layer in regulating P_{th}.

METHODS

Subjects

Twenty female students (ages 21–36 years, mean = 25 years) with perceptually normal speech and voice participated in this study (Table 1). All reported good general health, normal hearing, and no upper respiratory tract infection. Subjects presented a normal larynx as indicated by rigid oral videolaryngostroboscopy (Kay Elemetrics 9100, Lincoln Park, NJ). None were taking medications other than birth control. All were naïve to the purposes of the study.

The subjects were randomly assigned to one of two groups (oral breathing and nasal breathing). Oral-breathing subjects ranged in age from 21 to 32 years (mean = 25 years). One subject in the oral-breathing

TABLE 1. *Subject Characteristics*

Nasal Breathing			Oral Breathing		
Subject #	**Age (years)**	**Training (years)**	**Subject #**	**Age (years)**	**Training (years)**
1	24	10	11	23	0
2	26	0	12	29	0
3	27	0	13	23	0
4	25	0	14	22	0
5	20	1.5	15	31	0
6	24	0	16	32	0
7	26	0	17	21	0
8	22	0	18	23	3
9	36	0	19	26	0
10	25	0	20	25	0
Mean	25.5	1.15		25.5	0.3
SD	4.2	3.14		3.9	0.94

Abbreviation: SD, standard deviation.

group had received vocal training for a period of three years (S8). In the nasal-breathing group, ages ranged from 20 to 36 years (mean = 25 years). One subject in the nasal breathing group (S1) had received vocal training for a period of 10 years. An unpaired Student's *t*-test confirmed that groups did not differ by age ($t = 0.00$, $p = 1.00$), and vocal training ($t = 0.76$, $p = 0.45$).

Instrumentation

A vented pneumotachograph mask was fitted with low-bandwidth and wide-bandwidth differential pressure transducers (Glottal Enterprises PTL-1, PTW-1, Syracuse, NY). The low-bandwidth transducer, housed on the left side of the face mask, was coupled to a disposable 1-inch plastic tube placed translabially at a 45° angle inside the subject's mouth to collect oral pressure. The wide-bandwidth transducer was housed on the right side of the face mask to collect the combined oral-nasal airflow. The output from both transducers was led to a two-channel airflow system (Glottal Enterprises, MSIF-2) where the airflow signal was manually inverse filtered in order to generate the glottal volume velocity airflow. The volume velocity flow and pressure signals were amplified and digitized at 2 kHz and 200 Hz, respectively using a MacLab/8s AD (ADInstruments, Castle Hill, Australia) converter and a Power Macintosh 7500/1000 computer (Apple Computers Inc., Cupertino, CA). MacLab/s version 3.5 (ADInstruments) software was used to display and store the data. Flow and pressure signals were calibrated prior to data collection for each subject.

Protocols

All measurements were carried out in a quiet room with ambient humidity of 20% ± 2%. To determine the maximum vocal pitch, subjects performed a pitch glide on vowel /ɑ/ ascending from a comfortable speaking pitch to their highest pitch. The subject was prompted to produce higher pitches until a maximum pitch was reached. The highest musical note that a subject could sustain for 1 s was taken as the maximum vocal pitch. An electronic keyboard was used to match the highest pitch produced to the nearest semitone. Likewise, to determine the minimum vocal pitch, each subject produced a downward glide on /ɑ/ beginning at a comfortable pitch and ending on the lowest pitch that could be sustained for 1 s. The vocal pitch range was then calculated as the difference in semitones between the lowest and highest musical notes sustained for 1 s. The 10th, 20th, and 80th percentile pitches were calculated relative to the semitone range,[4,14,15] and are hereafter called low, comfortable, and high target pitches, respectively. These three target pitches were controlled for the remainder of the experiment due to the direct dependence of P_{th} on vocal F_0.

Subjects were taught to perform the threshold task.[4,16] Each subject was instructed to produce a five-syllable string of /pi/ "as smoothly as possible on a single breath." The subject was instructed to speak "as softly as possible but not to whisper." The investigator modeled a syllable repetition rate of 1.5 syllables per second. The flow mask was placed snugly over the subject's nose and mouth. The comfortable target pitch was provided on the keyboard prior to each task and the experimenter modeled the target pitch once. At the comfortable pitch, the subject practiced a suprathreshold syllable series, and then an "even softer" syllable series just above a whisper. The subjects viewed the practice trials on the computer monitor for feedback from the experimenter. Subjects were instructed to make the glottal volume velocity waveform "as small (in amplitude) as possible, but not flat." Subjects practiced until they could produce oral pressure peaks consistently near a minimum threshold below which oscillation in the glottal volume velocity waveform was absent. Subthreshold trials (with absent or intermittent voicing oscillation in the glottal volume velocity) were excluded and the subject was reinstructed to "speak softly but not to whisper." Trials were excluded and repeated if they differed from the targeted pitches by ≥ 1 ST. Trials were also excluded if oral flow > 12 mL/s was observed during the /p/, suggestive of either poor lip seal or velopharyngeal opening.[16]

Following three to five practice trials, each subject executed the pretreatment tasks. Subjects produced five /pi/ syllables at comfortable pitch and this constituted one trial. Subjects produced five such trials. This protocol was replicated at low pitch and the high target pitch resulting in five syllable strings at the three pitch conditions.

To obtain an estimate of patient-perceived vocal effort, subjects next sang "Happy Birthday" in their

quietest voice starting at the 50th percentile of their pitch range. After singing, subjects rated their vocal effort on a magnitude estimation scale, where a value of 100 represented usual effort, a value of 50 represented half the usual effort and a value of 200 represented twice the usual effort.[14] The same procedures were used to obtain posttreatment pressures and effort ratings. Subjects were shown their previous vocal effort ratings to reduce scale drift.

Following the pretreatment measures, subjects performed either oral breathing or nasal breathing for 15 min. To enforce oral breathing, a subject's nostrils were occluded with snugly fitting, hypoallergenic, nontoxic, disposable putty (Santa Barbara Medico, Inc., Santa Barbara, CA) The flow mask was placed over the subject's nose and mouth. The subject sniffed strongly through the nose. Absence of inspiratory nasal flow during the sniff confirmed adequate nasal occlusion. The flow mask was removed, and the subject sat at rest, breathing orally, and not speaking for 15 minutes. A 15-min challenge was expected to result in a reduction of sol layer depth and respiratory tract fluid output,[17] as well as to potentially affect voice production[18] but was not expected to dessicate the mucosa in vivo. The experimenter visually monitored the subject to confirm open lip posture consistent with oral breathing. After fifteen minutes of oral breathing, the putty was removed and P_{th} and vocal effort protocols were replicated. Subjects in the nasal-breathing group were instructed to close their lips, breathe nasally, and not speak for 15 minutes. The experimenter visually monitored the subject for closed-mouth posture. After 15 minutes of nasal breathing the P_{th} and vocal effort protocols were replicated. No subject spoke during the assigned breathing task and upon questioning, all indicated that they had not changed from the assigned breathing mode.

The entire P_{th} protocol consisted of P_{th} completed before and after the breathing treatment [3 pitches $\times$ 5 syllable strings $\times$ 2 assessment times (pretreatment and posttreatment) to yield a corpus of 30 /pi/ syllable strings per subject].

Data extraction, analysis, and reliability

The dependent variables were P_{th} and vocal effort ratings. As P_{th} relates to the subject's self-perceived phonatory effort[15] subject-perceived vocal effort was selected as an additional measure.

To estimate P_{th} the experimenter manually selected the three middle /p/ occlusions from each trial after discarding the first and the last /pi/ syllable.[16] Pairs of adjacent oral pressure peaks were averaged to approximate the subglottic pressures during the two intervening /i/ vowels. This procedure was used to obtain the estimated P_{th} at each target pitch. The experimenter and a second experimenter independently reanalyzed 10% of the syllable strings to estimate intrarater and interrater reliability, respectively. The first and second measures of P_{th} were strongly correlated (R_{intra} = 0.99; R_{inter} = 0.99) and yielded a mean absolute difference less than 0.1 cm H_2O, suggesting measurement reliability adequate for the purpose of the study.

Statistical analyses

Data were summarized as means ± standard deviation (SD). Raw data were further inspected for normality, skewness, and kurtosis. P_{th} at high pitch was transformed (square root transformation) to fulfill assumptions of normality. Tests of normality confirmed that P_{th} at high pitch was normally distributed following transformation (p = 0.62). To assess any difference in the effects of oral and nasal breathing on P_{th}, three separate repeated measures of analysis of variance were applied to the three different pitch conditions with breathing mode (oral $\times$ nasal) as the between factor and assessment time (before $\times$ after) as the repeated factor. Unpaired Student's *t*-tests were applied to ascertain that the two groups did not differ prior to the treatment conditions. Paired Student's *t*-tests were applied to describe the nature of any significant interaction. In order to understand the predictive relation between P_{th} and effort ratings, we applied a correlation analysis to the effort rating differences (pretreatment–posttreatment) and cumulative P_{th} differences (pretreatment–posttreatment, averaged across all the three pitch levels). Henceforth, the effort rating difference measures and P_{th} difference measures will be called Δeffort and ΔP_{th}, respectively.

RESULTS

The effects of oral and nasal breathing on P_{th} differed over assessment time as indicated by the sig-

nificant interactions at low pitch ($F = 52.37$, $p < 0.01$, Figure 1), comfortable pitch ($F = 21.23$, $p < 0.01$, Figure 2) and high pitch ($F = 14.93$, $p < 0.01$, Figure 3). For the low-pitch condition (Figure 1), oral breathing increased the mean P_{th} by 0.8 ± 0.4 cm H_2O ($t = 6.30$, $p < 0.01$) while nasal breathing decreased mean P_{th} by 0.7 ± 0.4 cm H_2O ($t = 4.10$, $p < 0.01$). This finding was replicated at comfortable pitch (Figure 2) where oral breathing increased the mean P_{th} by 0.8 ± 0.4 cm H_2O ($t = -6.49$, $p < 0.01$)

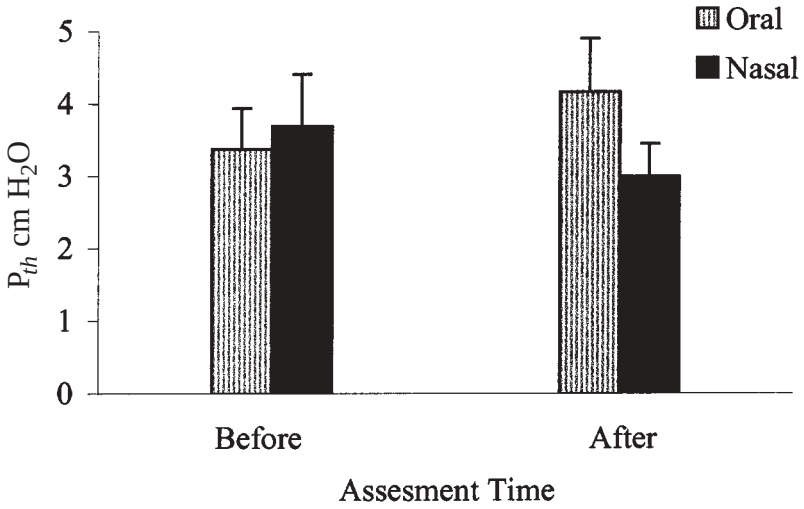

FIGURE 1. The interaction plot for assessment time versus breathing mode at low pitch. Filled bars represent mean P_{th} ± SD for nasal breathers and hatched bars represent mean P_{th} ± SD for oral breathers before and after the breathing task.

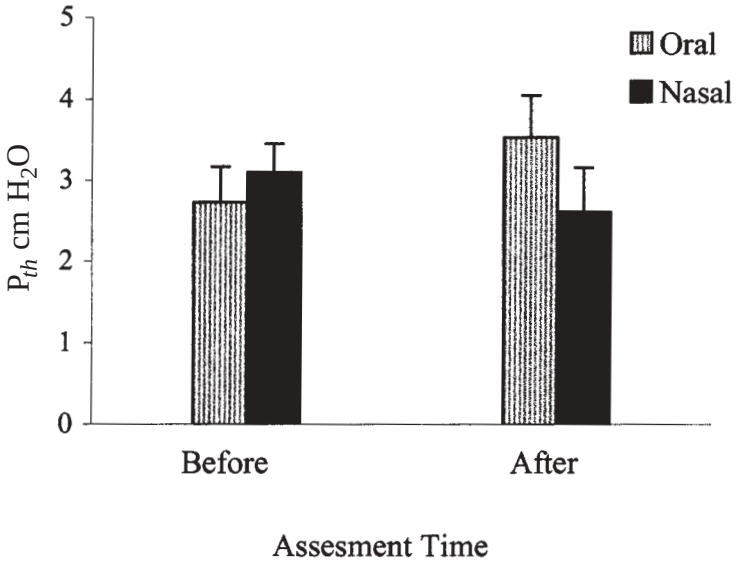

FIGURE 2. The interaction plot for assessment time versus breathing mode at comfortable pitch. Filled bars represent mean P_{th} ± SD for nasal breathers and hatched bars represent mean P_{th} ± SD for oral breathers before and after the breathing task.

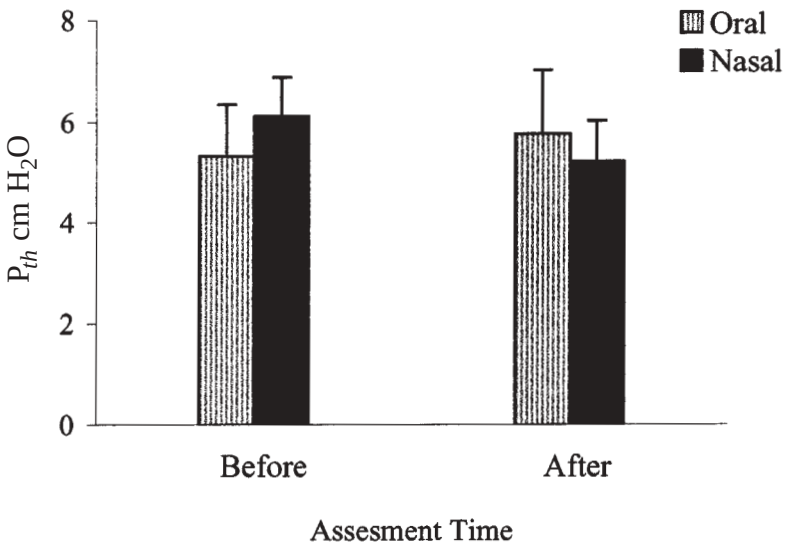

FIGURE 3. The interaction plot for assessment time versus breathing mode at high pitch. Filled bars represent mean P_{th} ± SD for nasal breathers and hatched bars represent mean P_{th} ± SD for oral breathers before and after the breathing task.

while nasal breathing decreased mean P_{th} by 0.5 ± 0.3 cm H_2O ($t = 5.73$, $p < 0.01$). At high pitch (Figure 3) nasal breathing significantly decreased mean P_{th} by 0.9 ± 0.5 cm H_2O ($t = 3.68$, $p < 0.01$); but the slight increase in mean P_{th} with oral breathing (0.7 ± 0.6 cm H_2O) was not significant ($t = 0.10$). Comparisons of pretreatment P_{th} at the three pitches confirmed that the two groups did not differ in P_{th} prior to treatment ($p > 0.10$).

Effort ratings for individual subjects are depicted in Figure 4. Subjects in the oral-breathing group (filled bars) reported a mean increase in vocal effort of 24 ± 13.5, while subjects in the nasal-breathing group (stippled bars) reported a mean decrease in vocal effort by 20 ± 17. Figure 5 shows the relation between ΔP_{th} and Δeffort. For each subject Δeffort is represented on the ordinate, while the ΔP_{th} value is represented on the abcissa. Data for oral-breathing subjects are denoted by filled squares. Data for 6 of 10 oral breathers are in the lower left quadrant; that is, increased P_{th} with oral breathing was associated with a perceived increase in vocal effort during singing. Two oral-breathing subjects (S15, S17) reported a decrease in vocal effort following oral breathing (Figure 5, upper left quadrant), and two oral-breathing subjects (S18, S19) did not report a change in effort. The majority of nasal breathers (unfilled circles) are in the upper right quadrant; that is, decreased P_{th} with nasal breathing was associated with a perceived reduction in vocal effort during singing. Three of the 10 nasal breathers (S4, S6, S9) did not report a change in vocal effort. Over all subjects, Pearson product-moment correlation of 0.70 suggested that 49% of the variance in Δeffort was predicted by ΔP_{th}.

DISCUSSION

This study confirmed the hypothesis that oral breathing, but not nasal breathing, would increase P_{th}. Consistent with prior research, increased P_{th} was perceptually salient to the majority of subjects as an increase in vocal effort.[14,19] That superficial drying of the vocal folds increases P_{th} has been demonstrated elsewhere in vitro.[6,7,20] In human studies the dependence of P_{th} on hydration was demonstrated by using methods that potentially reduce both systemic and superficial hydration simultaneously.[4,14,15,19] The study reported here is the first to show adverse effects of short-term (15 minute) oral breathing on

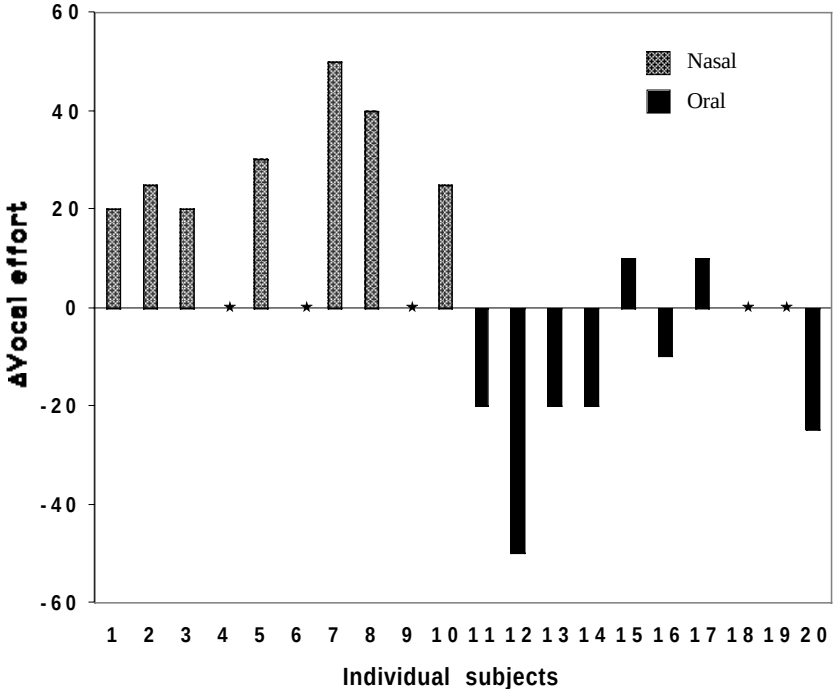

FIGURE 4. Mean Δeffort ratings (pretreatment–posttreatment) for individual subjects. Nasal breathers and oral breathers are represented by *stippled* and *filled bars*, respectively. *Filled stars* represent subjects who showed no change in their vocal effort.

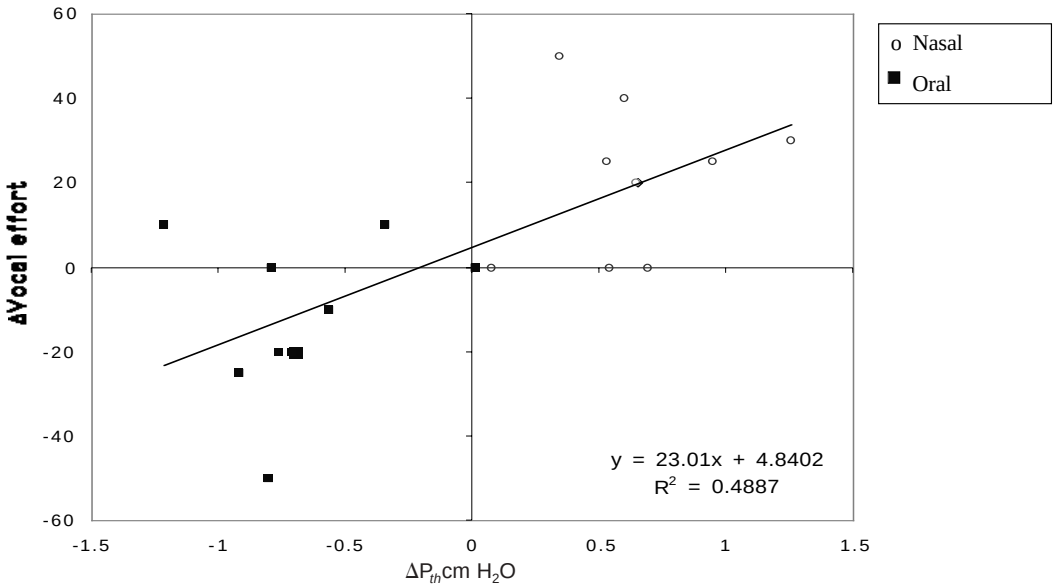

FIGURE 5. Scatterplot of relation between ΔP_{th} (pretreatment–posttreatment) and Δeffort ratings (pretreatment–posttreatment) for individual subjects. Oral and nasal breathers are represented by *filled squares* and *unfilled circles*, respectively. Overall, 49% of the variance in ΔP_{th} was represented by the variance in effort.

normal healthy subjects, effects that are most likely the result of superficial dehydration. The humidity level of 20% used in this study was less extreme than that reported previously (e.g., dessicated air and 100% humidity)[18] and was typical for the environment in which the subjects spent most days.

We propose that the increased P_{th} and vocal effort with oral breathing may be due to reduced depth of the sol layer (luminal surface water) from the vocal fold as in the airway in general.[21–23] In humans, oral breathing reduced the depth of the sol layer, decreased mucociliary clearance and impaired ciliary beat frequency in tracheal epithelia[21,22,24,25] In studies of dogs in vivo[12,13] and guinea pig tracheal epithelium in vitro,[26] oral breathing or dry air inhalation, respectively, reduced the depth of the sol layer increasing its osmolarity. Other animal studies demonstrated that water loss from the sol layer, induced by oral breathing, increased the viscosity of respiratory epithelia and overlying mucus,[12,13,27,28] increased tracheal mucus velocity, decreased mucociliary clearance,[29–32] and increased spinnability of mucus.[23] That oral breathing causes superficial dehydration of the sol layer has been widely acknowledged.

The sol layer is believed to be responsible for protecting the vocal folds, maintaining them in a mobile state, and assisting vibration by lubrication.[11,33] The mechanisms by which superficial dehydration maintains low P_{th} remains a matter of speculation. In 1988, Titze[8] proposed a model relating high P_{th} to increased viscosity and stiffness of vocal fold mucosa. The separate and important role of the sol layer overlying the mucosa has not been included. We consider that superficial hydration may smooth the vocal fold surface, thereby reducing drag and thus glottal resistance. Conversely, loss of superficial hydration might be expected to increase resistance particularly when flow rates are high (reducing the boundary layer depth) and the vocal folds are thicker, thereby allowing any superficial roughness to be "seen" by flow. This might be supported by the larger effect size observed at low and comfort pitches, rather than at high-pitch phonation. Adhesion of mucus also increases with dehydration of the sol layer.[34] Increased work of adhesion of respiratory tract mucus following oral breathing would be expected to reduce the ease of vocal fold separation requiring greater P_{th}. While short-term oral breathing potentially induced only superficial dehydration of the sol layer, this resulted in a magnitude of increase in P_{th} comparable to previous studies that potentially challenged both systemic and superficial hydration simultaneously.[4,15] The present study thus highlights the need for phonatory theory to include the separate and important role of the sol layer in lowering P_{th}.

P_{th} showed frequency dependent behavior, with greater P_{th} at 80th percentile pitch than P_{th} at 10th and 20th percentile pitches. This finding is in agreement with the biomechanical model associating higher P_{th} with increased stiffness, mucosal wave velocity, and decreased thickness of vocal fold tissue, occurring at higher pitch. It also replicates other reports that subglottal pressures tend to be greatest for high pitches in humans.[4,15]

Individual subjects varied in the magnitude of ΔP_{th} and Δeffort. The individual variation could be due to differences in homeostatic mechanisms that maintain vocal fold hydration. Studies on excised canine respiratory airway epithelia have shown increases in osmolality of the airway surface liquid by 50 mosm following oral breathing.[12,13] It has been proposed that increased luminal osmolality draws water from the epithelium toward the airway surface.[35–38] Airway epithelia must regulate water and ion transport[26,39,40] and can compensate for increases in luminal osmolality. In the vocal folds Na^+-K^+-ATPase contributes to bidirectional, transepithelial water fluxes providing a mechanism for regulating the superficial hydration of the vocal folds.[11] Additionally, transepithelial water flux of the airway may be regulated by sympathetic and parasympathetic tone[41] which also likely varies across individuals.

A related finding was the decrease in P_{th}, and associated vocal effort induced by nasal breathing. While the effects of motor learning on lowering P_{th} cannot be excluded, nasal breathing may also decrease P_{th} by hydrating the air above the ambient level supplied to the subjects. A study in humans who breathed orally or nasally showed 95% humidity of air at the pharynx during nasal breathing compared to 75% humidity of air at the pharynx following oral breathing.[42] Based on a mathematical model,[21]

Daviskas et al[22] also demonstrated greater water loss for mouth breathing compared to nasal breathing. Generally, one role of the nasal mucosa is to condition the hydration of inspired air. A lowering of P_{th} decreases expiratory effort to initiate phonation, potentially decreasing amplitude, collision, and shear stress of oscillation. Reduction of collision and shear stresses may aid prevention of voice pathologies. The ability to breathe nasally could have important implications in preventing voice disorders. It has been believed that pharmacological agents such as antihistamines and sympathomimetics result in laryngeal dryness.[43] Some speakers avoid such drying medication. It is possible, however, that these medications obviate oral breathing thereby facilitating nasal breathing and better conditioning of inspired air.

We considered that the decrease in P_{th} following nasal breathing could be attributed partly to motor learning. If this is the case, repeated assessment could have led to the decrease in P_{th}. If motor learning contributed to the decreased P_{th} observed with nasal breathing, the adverse effects of oral breathing become even more significant, with oral breathing increasing P_{th} despite the effects of motor learning in the opposite direction.

CONCLUSIONS

The results from the present study indicate that P_{th} is potentially regulated by the sol layer, which maintains superficial hydration of the vocal folds. Currently, models of phonation do not consider the significant but separate role of the sol layer in regulating P_{th}. Professional voice users engage in prolonged periods of oral breathing, which increases P_{th} and could thereby predispose individuals to vocal pathology. Our findings emphasize the important role of oral breathing in superficial laryngeal dryness and the need for developing strategies to prevent these adverse effects.

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