Manometric evidence for a phonation-induced UES contractile reflex

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Perera L, Kern M, Hofmann C, Tatro L, Chai K, Kuribayashi S, Lawal A, Shaker R. Manometric evidence for a phonation-induced UES contractile reflex. Am J Physiol Gastrointest Liver Physiol 294: G885–G891, 2008. First published January 31, 2008; doi:10.1152/ajpgi.00470.2007.—The mechanism against entry of gastric content into the pharynx during high-intensity vocalization such as seen among professional singers is not known. We hypothesized that phonation-induced upper esophageal sphincter (UES) contraction enhances the pressure barrier against entry of gastroesophageal contents into pharynx. To determine and compare the effect of phonation on luminal pressures of the esophagus and its sphincters, we studied 17 healthy volunteers (7 male, 10 female) by concurrent high-resolution manometry and voice analysis. We tested high- and low-pitch vowel sounds. Findings were verified in six subjects by UES manometry using a water-perfused sleeve device. Eight of the volunteers (2 male, 6 female) had concurrent video fluoroscopy with high-resolution manometry and voice recording. Fluoroscopic images were analyzed for laryngeal movement. To define the sex-based effect, subgroup analysis was performed. All tested phonation frequencies and intensities induced a significant increase in UES pressure (UESP) compared with prephonation pressure. The magnitude of the UESP increase was significantly higher than that of the distal esophagus, the lower esophageal sphincter (LES), and the stomach. Concurrent videofluoroscopy did not show posterior laryngeal movement during phonation, eliminating a purely mechanical cause for phonation-induced UESP increase. Subgroup analysis demonstrated phonation-induced UESP increases in males that were significantly greater than those of females. Phonation induces a significant increase in UESP, suggesting the existence of a phonation-induced UES contractile reflex. UESP increase due to this reflex is significantly higher than that of the distal esophagus, LES, and stomach. The phonation-induced UESP increase is influenced by sex.

upper esophageal sphincter

ANATOMIC CONTINUITY OF THE STOMACH AND LUNG necessitates the existence of elaborate defense mechanisms for prevention of retrograde aspiration. In the past several years our laboratory has been involved in studying these mechanisms. Our overall hypothesis has been that reflux-related upper gastrointestinal or respiratory events trigger a number of reflexes that prevent entry of refluxate into the aerodigestive tract and the lung. Examples of these mechanisms include upper esophageal sphincter (UES) contractile reflexes (14, 18, 22, 28, 30, 34, 36, 37), secondary esophageal peristalsis (3, 5, 7, 8, 10, 16, 19, 26), vocal cord closure reflexes (6, 21, 29, 31–33), and pharyngeal reflexive swallow (35) in response to esophageal or pharyngeal stimulation. The activation of airway-protective mechanisms in response to respiratory events with potential for causing reflux events such as phonation and cough has not been systematically studied.

Phonation requires rapid opening and closing of the vocal folds to interrupt the air stream (25), accompanied by an increase in subglottal tracheal pressure that is believed to be generated by contraction of the diaphragm, simultaneously causing an increase in intra-abdominal pressure. This minimal subglottal pressure is necessary to drive the vocal cords into vibration. The fundamental frequency of a voice depends on the rapidity of vocal fold vibration, pharyngeal dimensions, and vocal fold length (11). Whenever the subglottal pressure is increased, the vocal fold vibrates faster and fundamental voice frequency is increased (25). This phenomenon, especially during high-frequency vocalization such as seen in professional singers, can potentially predispose to gastroesophageal reflux, a recognized clinical problem in this group (2).

For this reason, the present study was undertaken to determine and characterize the effect of phonation on the intraluminal pressures in the upper gastrointestinal tract and to test the hypothesis that during phonation the UES develops a significantly higher pressure compared with the esophageal body, lower esophageal sphincter (LES), and stomach.

MATERIALS AND METHODS

Subjects

We studied 17 healthy volunteers (7 male, 10 female), aged 18–40 yr, by concurrent high-resolution manometry and voice recording during phonation and, in a subset of subjects, concurrent high-resolution manometry, voice recording, and videofluoroscopy. Studies were approved by the Human Research Review Committee of the Medical College of Wisconsin, and subjects gave informed written consent before their studies. Subjects were excluded if they had any supraesophageal symptoms, including those attributable to gastroesophageal reflux disease; ear, nose, and throat disorders; if they smoked; or if they were on any medication that could affect esophageal motility. All subjects were evaluated with transnasal unsedated esophagogastroduodenoscopy (T-EGD) (24) to exclude any volunteer with esophagitis.

Recording Techniques

Pressure recordings. To measure the intraluminal UES, esophageal, LES, and gastric pressures during rest and phonation, we used a solid-state manometric assembly (ManoScan 360; Sierra Scientific Instruments, Los Angeles, CA) with 36 fully circumferential sensors spaced 1 cm apart (4.2 mm outer diameter). This device uses novel pressure-transduction technology (TactArray; Sierra Scientific Instruments). Each sensor detects pressure over a length of 2.5 mm in each of 12 radially placed elements. Then the individual pressures from these elements were averaged, allowing all sensors to be circumfer-
ential detectors. These sensors are capable of recording pressure transients in excess of 6,000 mmHg/s. Pressure calibration at 0–300 mmHg was done before each study by using the automatic external calibration system of the catheter assembly. The data recordings were viewed by using a synergistic combination of topographic contour display and conventional line traces. The novel concept of eSleeve available with this system allowed us to obtain maximum sphincteric pressure during analysis irrespective of its movements. This modality incorporates perfusion-sleeve device advantages while using solid-state technology.

**Voice recording.** To measure phonation parameters, we recorded live audio during phonation through a microphone using Audacity software v.1.2.6 (http://audacity.sourceforge.net). Voices were recorded at 44 kHz and were saved in 32-bit (floating-point) samples. The files were exported in .wav format to Pratt software for frequency and amplitude analysis. Voice analysis was performed by using Praat software v.4.5.15 (created by Paul Boersma and David Weenink, Institute of Phonetic Sciences, University of Amsterdam, Amsterdam, The Netherlands).

**Videofluoroscopic recordings.** Preliminary studies (20) have shown the UES pressure (UESP) increase during phonation to be significantly greater compared with the rest of the upper gastrointestinal tract. To determine whether this pressure increase was induced by posterior movement of the larynx resulting in compression of the manometric catheter between the larynx and spine giving rise to spurious pressure increase, we recorded the hyoid and laryngeal movement by concurrent videofluoroscopy. In eight subjects, videofluoroscopic recordings were obtained at 90 keV with a 9-inch image intensifier and appropriate collimation so that an image was obtained of the posterior mouth, larynx, and pharyngoesophageal region. Fluoroscopic images were saved on a Super VHS videocassette recorder (Panasonic AG-1960; Proline, Tokyo, Japan), which recorded 30 video frames (or 60 fields) per second for subsequent analysis.

**Study Protocol**

Subjects were studied in an upright sitting position after a 4-h fast, and their nasal cavities were anesthetized with 2% topical lidocaine (Xylocaine; Astra Pharmaceuticals, Westborough, MA) applied by a cotton-tipped applicator just before insertion of the manometric probe. The solid-state catheter was passed transnasally and positioned such that, at rest, the two most proximal manometric sensors were in the pharynx. With this positioning, a minimum of three to four sensors were in the UES high-pressure zone during resting, and the remaining sites were in the esophageal body, LES, and stomach. Positioning of the catheter was verified by using a synergistic combination of topographic contour display and conventional line traces before the beginning of the voice recording. By this arrangement, intraluminal UESP could be measured simultaneously with the esophagus, LES, and stomach even if the catheter moved orad during phonation. All pressure measurements were referenced to atmospheric pressure. Subjects were positioned upright in a chair, facing a microphone positioned 8–10 inches from the lower border of the mandible. Subjects were instructed to hold their heads in a neutral position. They were then given 10 min to acclimate to the catheter and to study the environment before study recordings began.

High and low pitch “a” (as in car) and “ç” (as in key) vowels were tested, and each event was recorded for 10 s. Each phonation event was repeated three times. It was decided not to test rising and falling “glissandos” (23) because preliminary work showed that subjects had difficulty performing this task reproducibly. The manometric and voice recordings were synchronized by means of markers demarcating start and end of each phonation task as well as by simultaneously starting the recording of both manometric and voice-recording devices.

Eight of the seventeen subjects (2 male, 6 female) from this group underwent concurrent videofluoroscopy along with high-resolution manometry and voice recording for determination of hyoid and laryngeal movement during phonation. For videofluoroscopy, subjects were positioned upright in a chair lateral to the image intensifier. The subjects were instructed to hold their heads in a neutral position. The same protocol as mentioned above was followed, and each vowel was tested three times for each pitch. The manometric, voice, and videofluoroscopic recordings were synchronized by using an external timer, which encoded time digitally in hundredths of a second on the video screen (event marker) as well as by starting all the recording devices simultaneously as described earlier.

**Pressure Recording by Water-Perfused UES Sleeve Device**

To verify the high-resolution solid-state eSleeve finding, the effect of phonation on UES was further studied in six subjects by a conventional water-perfused UES sleeve manometric device (Dentsleeve; Mui Scientific, Mississauga, ON, Canada), using a pneumohydraulic capillary infusion system with external pressure transducers (Arndorfer Specialties, Glendale, WI). The pressure transducers were connected to a computerized manometric recording system (Medical Measurement Systems, Enschede, The Netherlands), which also had an event marker to demarcate the start and end of phonation.

**Hyoid and Laryngeal Movements**

The computer analysis system used for analyzing the videofluoroscopic data consisted of an external digitizer (External Super-Multi DVD ReWriter, model GSA-169D; LG Electronics, Seoul, Korea) and VirtualDub v.1.6.16, a video capture/processing utility for 32-bit Windows platforms that captures 30 video frames (or 60 fields) per second. All video radiographic data were analyzed with ImageJ v.1.36b, a public-domain Java image-processing program.

Using these techniques, for each vowel we determined the spatial location of 1) anterior-inferior border of the hyoid bone and 2) the most anterior superior point in the larynx. The spatial location of these sites was noted as Cartesian coordinate (x, y) pairs relative to a coordinate axis wherein the positive y direction was inferior and parallel to the spine. The positive x direction was posterior and perpendicular to the spine. (Note, this is the axis and sign convention of the ImageJ analysis software.) Hyoid and laryngeal movements were measured relative to their prevocalization resting positions. Superior and inferior movement were defined as excursions parallel to an imaginary line connecting the anterior and inferior corners of C3 and C4 vertebrae bodies seen in the lateral radiographic view. Anterior and posterior movements were measured perpendicular to the above-defined superior/inferior direction.

**Data Analysis**

**High-resolution manometry.** Manometric recordings were analyzed to determine the concurrent intraluminal pressures just before as well as during phonation in the 1) UES, 2) distal esophagus (8 cm above LES), 3) LES, and 4) stomach. Pressure measurements were made at the same time points for all sites. Manometric data analysis was done using ManoView software (Sierra Scientific Instruments). The data recordings were viewed by using a synergistic combination of topographic contour display and conventional line traces. The contour plot display has sensor channels located along the vertical axis and time along the horizontal axis (Fig. 1C). Pressures are encoded in color. The novel concept of eSleeve as used in this system allows us to obtain maximum sphincter pressure during analysis, incorporating perfusion sleeve device advantages while using solid-state technology. The recording shows a signal equal to the maximal pressure along the boundaries of the eSleeve and has the advantage that the full data set is available so that the axial pressure distribution may be visualized. In addition, the boundaries of the eSleeve can be adjusted...
during analysis to position it at the appropriate location according to the anatomy. During analysis, data were first corrected for the thermal sensitivity of the pressure elements by using the thermal compensation function of ManoView as previously described (9).

Phonation. Pratt voice files were opened as long sound files, and pitch and amplitude (intensity) were viewed as line plots (Fig. 2B and Fig. 5B). Amplitude and pitch for each synchronized time point of highest UESP were obtained by placing the cursor at the correct time point of the long sound file.

### Statistical Analysis

Spearman rank correlation was used to test the relationship between different vowels, different pitches, and pressures at different sites. This was also confirmed by analysis of covariance. The comparison of UESP to other sites including distal esophagus, LES, and stomach for each vowel during low and high pitches was done by repeated-measures ANOVA. Comparison of pressures before and during vowels in UES, distal esophagus, LES, and stomach was also done by using repeated-measures ANOVA. Values in the tables and figures were reported as means ± SE for normally distributed data. In the instances when data were not normally distributed, box and whisker plots of medicine and range were used for data reporting. To define the sex-based effect on UES, a subgroup analysis was performed. All statistical analysis was performed by using SigmaStat/Software (Jendel, San Rafael, CA). A type-I error probability <0.05 was considered significant unless stated otherwise. Type-I error significance levels were adjusted for multiple-comparison testing.

### RESULTS

All subjects tolerated the procedure well. There were no reports of pain or discomfort with phonation. Voice analysis demonstrated that all subjects were able to produce high- and low-pitch sounds of the same vowel, and the pressures achieved were significantly higher than those in the esophagus, LES, and stomach (*P < 0.005).
low-pitch vowels with significantly different frequencies (252 ± 12 and 260 ± 10 Hz compared with 149 ± 7 and 141 ± 7 Hz for high-pitch ā and e compared with low-pitch ā and e, respectively; $P < 0.001$).

Phonation-Induced Pressure Changes Within the Upper Gastrointestinal Tract

Analysis of manometric recordings showed that phonation was associated with different magnitude of increase in intraluminal pressure in the stomach, LES, esophagus, and UES (Fig. 1, A and C). This phenomenon was observed for both high- and low-pitch ā and ē. Comparison of the phonation-induced pressure changes within the upper gastrointestinal tract showed that the pressure change in UESP was significantly higher compared with the esophageal body, LES, and stomach ($P < 0.005$; Fig. 2) for both ā and ē in high and low pitches. In contrast, phonation-induced intraluminal pressure changes in the stomach, LES, and esophagus were not statistically different (Fig. 2). Comparison of the effect of pitch on phonation-induced upper gastrointestinal tract pressure increases did not show any significant difference.

For all the studied pitches irrespective of the recording device, the median UESP during phonation was significantly higher compared with median UESP immediately before start of phonation ($P < 0.005$) as well as with the median of the 10-min resting baseline pressure for all studied pitches ($P < 0.005$; Fig. 3). There was no significant difference in UESP increases during phonation with different pitches. Spearman Rank correlation coefficient showed that there was no significant relationship between magnitude of UESP change and pitch as well as amplitude of the voice. In addition, no significant relationship was found between phonation-induced UESP change and pressure changes at distal esophagus, LES, and stomach.

Confirmation of Results of High-Resolution eSleeve Manometry With Conventional Water-Perfused UES Sleeve Device

An example of the effect of phonation on UESP by using a traditional UES water-perfused sleeve is shown in Fig. 4, A and B (13).

![Fig. 3. Comparison of immediate prephonation and resting baseline UESP to UES phonation pressures. Median UESP during phonation of different vowels was significantly higher than median UES immediately before phonation and resting/baseline UESP values (*$P < 0.005$).](image1)

![Fig. 4. Example of concurrent manometric (A) and voice recording (B) during high-pitch ā using a conventional water-perfused UES sleeve device and Praat voice-analysis software programs, respectively. During phonation, UESP (blue line) increased from prephonation level. This gradual rise is due to properties of water-perfused sleeve device, which makes this device respond slowly to increases in pressure but instantaneously to decreases in pressure (37). There was a significant increase in mean UESP during phonation compared with mean UESP immediately before phonation, reproducing our findings using solid-state manometry device (C). *$P < 0.005$.](image2)

![Mean UESP during phonation was compared with mean UESP before phonation by using a water-perfused UES sleeve device. Findings were similar to those using UES eSleeve. The mean UESP increase during phonation for high- and low-pitch ā and ē were significantly higher compared with those before phonation ($P < 0.005$; Fig. 4C).](image3)
**Table 1. Average laryngeal and hyoid movement associated with vocalization**

<table>
<thead>
<tr>
<th>UES Pressure, mmHg</th>
<th>Laryngeal Movement, mm</th>
<th>Hyoid Movement, mm</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before</td>
<td>During</td>
</tr>
<tr>
<td>HP å</td>
<td>67 ± 9</td>
<td>147 ± 48*</td>
</tr>
<tr>
<td>LP å</td>
<td>63 ± 7</td>
<td>129 ± 29*</td>
</tr>
<tr>
<td>HP e</td>
<td>64 ± 9</td>
<td>101 ± 28*</td>
</tr>
<tr>
<td>LP e</td>
<td>62 ± 5</td>
<td>83 ± 10*</td>
</tr>
</tbody>
</table>

Values are means ± SE for 8 subjects who had concurrent videofluoroscopy, high-resolution manometry, and voice recording. HP, high pitch; LP, low pitch. *P < 0.05 before vs. during phonation.

Laryngeal Movement During Phonation

Slow-motion and frame-by-frame analysis of the video recordings showed that the laryngeal movement during phonation was minimal and consistently <1 cm in the superior and anterior directions during both vowels and in all pitches. None of the phonations were associated with posterior movement of the larynx (Table 1). An example of digitized videofluoroscopic images used in analysis of laryngeal and hyoid movement during phonation is shown in Fig. 5.

As seen in Table 1, high-pitch and low-pitch vowels were associated with a significant UESP increase (P < 0.05) compared with those immediately before phonation, but none of these pressure increases was accompanied by any posterior laryngeal movement that could have caused compression of the catheter resulting in spurious pressure increase.

Effect of Sex on Phonation-Related UES Contraction

Subgroup analysis of the effect of phonation on UESP and pitch was performed among males and females for all the vowels. Again, the pitch of each vowel (Hz) and change over prephonation pressure of UES during phonation (mmHg) was calculated for each sex. Both high- and low-pitch vowels had significantly lower frequencies in males compared with females (P < 0.02; Fig. 6A). Median UESP increase during phonation was significantly higher in males than females (P < 0.03; Fig. 6B) when comparing global pressure increases across vowel sounds and pitches. Comparison of specific vowel sounds and pitches using multiple, signed-rank testing showed significant sex-based effects for all UESP differences for all the vowels except low å.

DISCUSSION

In this study, the effect of phonation on the intraluminal pressures of the esophagus and its sphincters was characterized by using concurrent manometry, voice recording, and videofluoroscopy in young, healthy volunteers. The study findings indicate that during phonation there develops a significantly higher pressure increase within the UES compared with the esophagus, LES, and stomach. In contrast, study findings indicate that phonation induces similar pressure increases within the stomach, LES, and esophagus.

Although the phonation-induced intrathoracic and intra-abdominal pressure increases have been described previously, the observed disproportionately higher UESP increase during phonation has not been previously reported. Two mechanisms could be envisaged for this disproportionately higher pressure increase seen in the UES compared with the rest of the gastrointestinal tract during phonation: 1) mechanical squeeze of the sphincter by surrounding structures such as posterior movement of the larynx pressing the sphincter against the spine, and 2) neuromuscular reflex mechanisms resulting in UES muscle contraction. Using concurrent videofluoroscopy during phonation, we evaluated the excursion of the laryngeal apparatus and documented the absence of its posterior movement, ruling out the first possibility. This finding supports the notion of a contractile UES response to phonation. Earlier...
studies have suggested the contribution of three muscle groups in generation of intraluminal UESP, i.e., inferior pharyngeal constrictor, cricopharyngeus muscle, and the most proximal part of the esophagus (1, 4, 15). These studies (15) have also documented participation of different components of the UES in different functions. Therefore, the observed phonation-induced pressure increase in this study does not determine the specific contributions of muscular components of the UES. Future electromyographic studies are needed to further characterize the contributions of various muscle components of the UES.

In videofluoroscopic images, absolute differences in distance measurements of the hyoid and larynx for between-subject comparisons of excursion can potentially be misleading, because of the possibility of physical scale variability; that is, larger subjects may have the capability of larger hyoid or laryngeal excursion and vice versa. To address this issue, we normalized hyoid and laryngeal excursion by calculating the relative movement of the hyoid and larynx for each subject. For each subject, the resting position of hyoid and larynx just before phonation was subtracted from the values during mid-phonation. These measurements showed that during phonation laryngeal and hyoid excursion were anterosuperior rather than being posterior. This finding is in concordance with previous studies that report that nonsingers or untrained subjects tend to raise vertical positions of the larynx as vocal frequency increases (27). The absence of posterior movement of the larynx during phonation eliminates the possibility of UESP increase caused by compression of the UES between the larynx and the spine.

Previous studies using manometry and fluoroscopy had shown that anterior movement of the larynx generated by the anterior motion of the hyoid caused by the contraction of the suprahypopharynx muscles can result in UESP drop and sphincter opening (12). This observation is confirmed by our findings. The anterior movement of the larynx and hyoid bone during vocalization reported in our studies could induce a drop in UESP had the described UES contraction not occurred. There was a significant UESP increase during phonation compared with immediately before phonation and baseline resting UESP. This finding suggests a possible protective role for the described phonation-induced UES contraction in case gastrointestinal reflux events were to occur during singing or phonation.

Our finding of voice-induced UESP increase corroborates an earlier report in two subjects using a single recording balloon (17). This study showed correlation of the voice pitch with UESP increase. However, in this study the effect of phonation on intraluminal pressure of the rest of the gastrointestinal tract was not investigated; therefore, the unique response of the UES to phonation, which is different from its general effect on the upper gastrointestinal tract, could not have been determined. In addition, the present study using a state-of-the-art multisite recording technique did not corroborate the correlation of pitch with the UESP increase described in the aforementioned study. This discrepancy could be due to the nonspecific nature of the recording technique used in this previous study. The recording balloon could have also recorded from areas other than the UES.

In this study, we further characterized the UES response to phonation by defining the effect of sex on this response. Study findings indicate that there exists a significant difference between males and females in the UESP increase during phonation. This difference exists despite the fact that males in this study produced significantly lower pitch compared with females. Considering the fact that our study did not show any correlation between the pitch and UESP increase and the prior contradicting report (17) of correlation of higher UESP increase with higher pitches, our observed higher UESP increases associated with lower pitch phonation in males eliminates the possibility that this sex-based difference could be due to differences in pitch and supports the notion of existence of a neuromuscular mechanism for this difference. However, given the small number of subjects in each sex group, our findings await confirmation by future studies.

Similarly, we did not find any correlation between gastric, LES, and distal esophageal pressures with that of UESP increase during phonation, suggesting the existence of different mechanisms for the observed pressure increases in the UES and the rest of the upper gastrointestinal tract. These findings suggest that although the UESP increase seems to be generated by the contraction of the sphincter muscle, the pressure in-
increases in the esophagus, LES, and stomach seem to be secondary to pressure transmission from the surrounding structures, such as those induced by contraction of the thoracic and abdominal wall muscles.

It is reasonable to expect that the constant presence of the catheter may affect normal phonation, even though the subject does not perceive any discomfort or pain. Recording the baseline phonation characteristics before intubation and then comparing these characteristics with those measured in the presence of the catheter would provide insight into this effect; however, this test was not performed because the principle interest in this study was not focused on the effect of the catheter on phonation properties and, rather, on the UES pressure changes during phonation.

In summary, phonation induces a significant increase in UESP. This UESP increase is significantly higher than that of the stomach, esophagus, and LES, indicating the existence of a pharyngo-UES contractile reflex. This work was supported in part by National Institute of Diabetes and Digestive and Kidney Diseases Grants R01-DK-25731 and 1-P01-DK-68061-D1A1.

REFERENCES