

The Effect Of Hyoid Movement On Vocal Fold And False Vocal Fold Configurations In Excised Human Larynges

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SUMMARY: Objectives. While it is generally understood that extrinsic muscle activation may change larynx position and orientation, its effect on vocal fold configuration, particularly the medial surface shape, remains largely unknown. In this study, the effects of hyoid bone movement on the true and false vocal fold configurations were investigated in excised human larynges.

Method. For each larynx, a window was opened on one thyroid lamina to provide a medial view of the true vocal fold and false vocal fold. The larynx was mounted vertically with the cricoid cartilage fixed. The hyoid bone position was systematically modified along both the superior and anterior-posterior directions. The three-dimensional shape of the vocal fold medial surface and false vocal fold was reconstructed at different hyoid positions.

Results. Upward hyoid movement lifted the false vocal fold, increasing both the vertical width and depth of the ventricle. Upward hyoid movement also decreased vocal fold length and tilted the vocal fold upward and backward (except for anterior-superior hyoid movement), and increased glottal width (except for posterior-superior hyoid movement). The effect of upward hyoid movement on vocal fold vertical thickness was insignificant and larynx-specific.

Conclusions. These results suggest that extralaryngeal manipulations have more consistent effects on vocal fold length and tilting than the shape of the vocal fold medial surface, which appears to be largely controlled by intrinsic laryngeal muscles.

Key Words: Hyoid bone–Extrinsic laryngeal muscles–Vocal fold length–Vocal fold thickness–Medial surface–Vocal fold tilting.

INTRODUCTION

Muscular adjustments of vocal fold position, geometry, and mechanical properties are essential to the voice production of varying voice qualities.¹ It is generally understood that such vocal fold adjustments are achieved mainly by actions of the intrinsic laryngeal muscles that modify the relative position of the thyroid, cricoid, and arytenoid cartilages, to which the vocal folds are attached. However, this cartilage framework of the larynx is stabilized by extrinsic laryngeal muscles, which connect the larynx to other structures in the neck and are responsible for the control of vertical position and orientation of the larynx within the neck. It is reasonable to speculate that extrinsic laryngeal muscles, when adjusting the vertical larynx position and orientation, may also change the relative position of the laryngeal cartilages, and thus impact vocal fold geometry, position, and mechanical properties. The goal of this experimental study was to better understand the effect of varying vertical larynx position and orientation on vocal fold configuration, including vocal fold length, thickness, and glottal gap, in excised human larynges. This is an important first step toward better understanding the role of

extrinsic muscles in voice quality control, which has many important applications in vocal pedagogy and clinical voice care.

In humans, the larynx tends to move upward with increasing fundamental frequency of phonation, particularly in untrained speakers.² It is generally assumed that raising the larynx is achieved through actions of the extrinsic muscles that also increase vocal fold length and thus fundamental frequency of voice production.^{3–6} Interestingly, classical singers tend to keep their larynx below its resting position.⁷ Sonninen showed that a major difference between the so-called open and covered modes of singing is in the vertical larynx position.³ A low larynx position appears to be an essential component of the Western classical voice training, whereas a high larynx position is used more often in musical theater voices.

While changes in vertical larynx position modify the length and thus resonance properties of the vocal tract, they may also impact the voice source. Sundberg & Gauffin⁸ showed that the voice source produced with a raised larynx was similar to that of pressed phonation, and the voice source produced with a lowered larynx was similar to that of flow phonation, indicating that changes in larynx position are often associated with changes in vocal fold adduction. Sundberg and Askenfelt⁹ further showed that a decrease in the amplitude of the source spectrum fundamental, often due to a higher degree of laryngeal adduction activity, was essential to the perception of a larynx rise.

Clinically, a habitually elevated larynx position is sometimes associated with hyperfunctional voice,^{10,11} a condition that often presents with increased vocal effort

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and fatigue and may lead to mass lesions of the vocal folds. One treatment option is to lower the larynx by using the yawn-sigh technique.¹² Sonninen and colleagues^{3,4} suggested that the vocal folds are longer, tenser, and thicker at a high larynx position during the so-called 'open voice' singing and are more likely to result in vocal fold injury and development of nodules. He also suggested that lowering the larynx position (as in the covered singing) may counteract this overloading and avoid vocal fold injury.

Despite the clinical and pedagogical importance, experimental investigation of the effect of vertical larynx position on vocal fold configuration has been limited, largely due to difficulties in imaging vocal fold configuration in humans. An exception is the excised larynx study by Vilkman and Karma.¹³ They showed that upward displacement of the hyoid bone abducted the false vocal folds, enlarged the ventricle, heightened the epiglottis, and slightly abducted the vocal folds. However, changes in vocal fold length and medial surface vertical thickness were not measured due to limited access to the medial surface in a full-larynx experimental setup.

The goal of this study was to investigate the effect of varying vertical larynx position on vocal fold length, thickness, and glottal gap, which are important parameters that determine the fundamental frequency and voice quality.^{1,14,15} Due to difficulties in stimulating individual extrinsic larynx muscles and observing changes in vocal fold configuration in humans, an excised human larynx model was used in this study. While the excised larynx model does not allow extrinsic muscle stimulation, it provides insight into changes in vocal fold configuration due to passive external forces applied to the laryngeal framework, an important step toward understanding changes in vocal fold configuration in the presence of extrinsic and intrinsic laryngeal muscle activity.

In this study, the vertical larynx position was varied by pulling the hyoid bone in three directions: superior, anterior-superior, and posterior-superior, while the cricoid cartilage was fixed in place. These three movement directions were included in order to raise the larynx as well as tilt the thyroid cartilage either forward or backward. Ink points were marked on the medial surface of the true vocal fold and false vocal fold, which allowed us to track their positions as the hyoid bone was pulled. Based on current literature on voice source change with larynx position,^{4,8,16} we hypothesized that lifting the hyoid bone vertically increases the hyoid-thyroid distance, which would open up the ventricle, decrease vocal fold vertical thickness, and slightly abduct the vocal folds. Since upward hyoid movement applies an upward force to the thyroid cartilage, we hypothesize that it decreases vocal fold length. We also hypothesize that an anterior-superior movement of the hyoid bone tilts the thyroid cartilage and the vocal fold forward, thus reducing the degree of vocal fold shortening, whereas a posterior-superior hyoid movement tilts the vocal folds backward and increases vocal fold shortening. Since vocal fold length is often assumed to be negatively

related to vocal fold thickness, we hypothesize that vocal fold vertical thickness decreases more with an anterior-superior hyoid movement and less with a posterior-superior hyoid movement.

METHOD

Experimental setup

Three human larynges (all males, age 74, 42, and 58 for larynx numbers 1–3, respectively), together with the hyoid and the hyoid-thyroid connective tissues, were harvested from autopsy at the Department of Pathology, University of California, Los Angeles, and were stored at -80°C . Written consent from organ donors' next of kin was obtained prior to autopsy. The night before the experiment, the larynges slowly thawed in a fridge at -4°C , and on the day of the experiment, they were taken out of the fridge and thawed to room temperature while regularly sprayed with phosphate buffered saline solution. For each larynx, the extralaryngeal musculature was removed to expose the thyroid cartilage. A window was made on the left side of the thyroid cartilage, and the left vocal fold and left false fold were removed in order to provide a medial view to the right vocal fold and right false fold. Care was taken to keep the structural integrity of the thyroid cartilage so that the thyroid cartilage was upright by itself at rest and moved upward as a whole without obvious tilting laterally when the hyoid bone was pulled. Therefore, the posterior part of the thyroid cartilage was kept, and both the superior horn and the cricothyroid joint were left intact, preventing the thyroid cartilage from collapsing. Anteriorly, as much of the cartilage as possible was removed, while avoiding touching the right-side tissues. The hyoid bone and the connecting thyrohyoid tissues were kept. To allow three-dimensional reconstruction of the vocal fold medial surface, ink points were placed on the medial surface of the true vocal fold and false vocal fold as well as within the ventricle. There were, on average, about 3–4 ink points on the vocal fold medial surface. Further increasing the number of ink points was difficult due to the small size of the medial surface and our attempt to maintain vocal fold surface integrity.

The larynx was mounted on a tracheal tube with the cricoid cartilage fixed using screws (Figure 1). Sutures were attached to the hyoid bone such that they could be attached to a motor to move the hyoid bone vertically. An anterior suture loop was attached to the anterior part of the hyoid bone, and a posterior suture loop was placed connecting the posterior left and right ends of the hyoid bone. These two sutures were then connected together through a top suture loop, which was connected to a step motor through a pulley. The connection between the three sutures was adjusted so that the anterior and posterior sutures became tensioned at the same time when the top suture was pulled by the step motor. For superior movement of the hyoid, the pulley was placed directly above the hyoid so that the top suture applied a vertical force to the hyoid bone. For

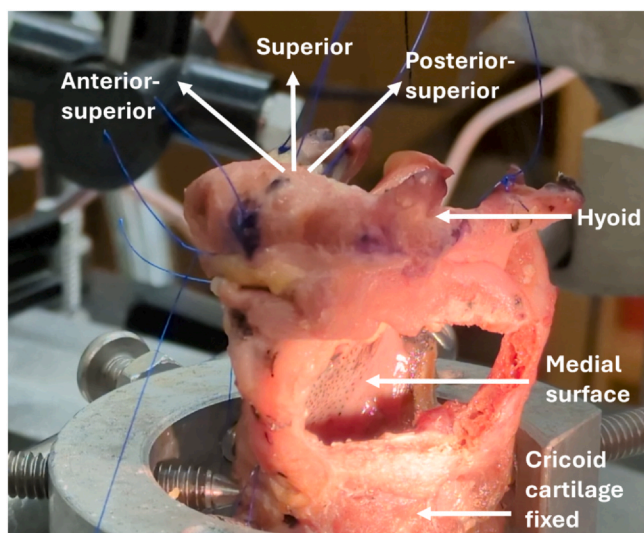


FIGURE 1. Experimental setup showing the mounting of the larynx, the thyroid window providing optical access to the vocal fold, and the sutures attached to the hyoid bone for hyoid vertical movement.

conditions with anterior-superior hyoid movement, the pulley was moved anteriorly so that the top suture was pulled with an anterior angle of 45° with respect to the vertical axis. Similarly, for posterior-superior hyoid movement, the pulley was moved posteriorly so that the top suture was pulled with a posterior angle of 45° with respect to the vertical axis.

For each of the three pulley positions, the top suture and the hyoid were pulled by the step motor for 3 cm in a step of 1 mm. After each step, the medial surface of the vocal

fold and false vocal fold was imaged through the thyroid window from two angles using two high-speed cameras: one pointing at the medial surface from the anterior side and the other pointing from the posterior side. These two views were then used to reconstruct the medial surface of the vocal fold, false vocal fold, as well as the ventricle when it became visible. The reconstruction procedure was described in detail in Schlegel et al.¹⁷ Briefly, for each larynx, a calibration plate with known three-dimensional coordinates was placed at the location that would have been occupied by the removed vocal fold. The two camera views of the calibration plate, together with the known three-dimensional pattern of the calibration plate, were used to derive a mapping function that determines three-dimensional coordinates from the corresponding two-dimensional coordinates in the two camera views.

Data analysis

For each larynx and each hyoid movement direction, two anchor ink points, one located anteriorly and the other posteriorly on the membranous vocal fold medial surface, that were visible in both camera views before and after the hyoid movement were identified. The Euclidean distance between these two anchor points in three dimensions was calculated as a measure of vocal fold length (Figure 2). Because the anterior commissure and the vocal process often were not visible in both camera views, the vocal fold length thus calculated was smaller than the typical values measured from humans. Because of this, in this study we focused on the relative changes in vocal fold length rather than the absolute values of vocal fold length.

A vocal fold angle in the sagittal plane was measured as the angle formed by the line connecting the two anchor

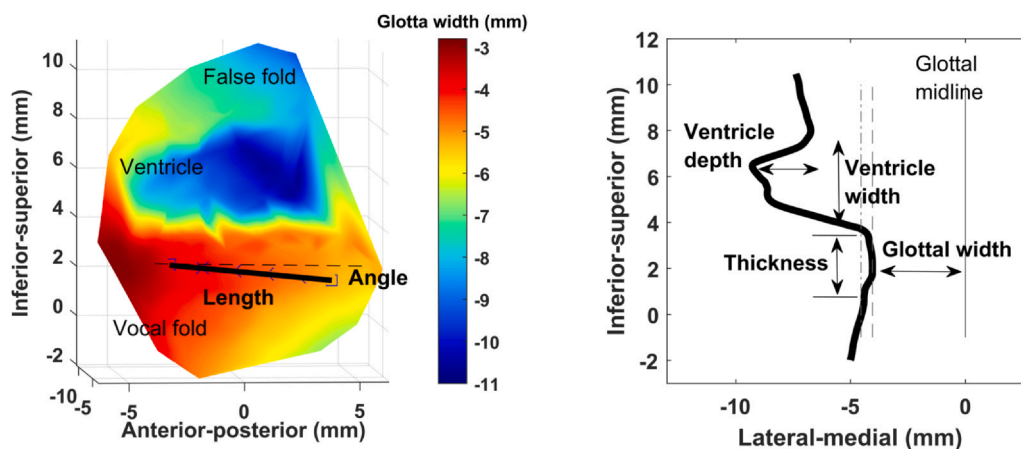


FIGURE 2. Definition of vocal fold length, angle, thickness, glottal width, ventricle depth, and ventricle width. The left panel shows the medial view of a reconstructed larynx, showing the false fold at the top, ventricle in the middle, and vocal fold at the bottom. Vocal fold length was defined as the distance between two ink points (squares) that were visible in both camera views before and after hyoid movement. Vocal fold angle was defined as the angle between the line (solid thick line) connecting the two ink points and a horizontal line (dashed thin line). Vocal fold thickness and glottal width were evaluated at four evenly spaced locations (crosses in the left panel of the figure) along vocal fold length between the two ink points. A typical example is shown in the right panel, which shows the medial contour of the larynx in the coronal plane. Vocal fold thickness was calculated as the vertical span of the portion of the medial surface within a threshold distance (dash-dotted line) from the most medial point of the medial surface (dashed line), as described in Ref. 18. More details in text.

points and a horizontal line (Figure 2). Changes in this vocal fold angle before and after hyoid movement were used to measure vocal fold tilting. The vocal fold angle was defined to be positive when the anterior anchor point was at a higher position than the posterior anchor point. Thus, an increase in the vocal fold angle indicates an upward and backward vocal fold tilting, whereas a decrease in vocal fold angle indicates a downward and forward tilting.

The vocal fold medial surface shape before and after hyoid movement was visually compared. To compensate for vocal fold tilting and facilitate comparison, the re-constructed medial surface was first rotated in the sagittal plane so that the two anchor points were at the same height. Four vertical slices of the medial surface were then taken at locations at 20%, 40%, 60%, and 80% along the distance between the two anchor points. For each slice, an effective vertical thickness was calculated as the vertical span of the portion of the medial surface within a threshold distance from the most medial point of the medial surface (Figure 2), as described in Lehoux and Zhang.¹⁸ By using different values of the threshold distance, this method is designed to quantify the vertical thickness of the most medial portion of the medial surface that provides effective constriction to the glottal airflow and thus has a meaningful impact on voice production. In this study, two values of the threshold distance of 0.1 and 0.5 mm were used.

The glottal width was defined as the minimal distance between the vocal fold medial surface and glottal centerline (Figure 2). Since the glottal centerline was difficult to identify in our hemilarynx setup, it was defined to be the sagittal plane occupied by the calibration plate during the calibration process. While this definition was arbitrary, it was considered acceptable as our data analysis focused on changes in glottal width rather than its absolute values. Similar to vocal fold thickness, the gap width was calculated at four locations at 20%, 40%, 60%, and 80% along the distance between the two anchor points. The medial-lateral depth of the ventricle was calculated as the distance along the medial-lateral direction between the most lateral point (the bottom) and the entrance to the ventricle (Figure 2). The vertical width of the ventricle opening was measured as the maximum vertical span of the ventricle opening.

Linear mixed effect models were developed to relate changes in ventricle depth, ventricle width, vocal fold length, vocal fold angle, vocal fold thickness, and glottal width to the three hyoid movement directions: Measure $\sim 1 + AS + S + PS + APLocation + (1 | \text{Larynx})$. Each model included the fixed effects of hyoid movement directions (AS: anterior-superior; S: superior; PS: posterior-superior) with respect to the baseline condition without hyoid movement. For vocal fold thickness and glottal width, each model also included a fixed effect for the anterior-posterior location (APLocation) on the vocal fold medial surface where the corresponding measure was calculated. Each model also included a random effect for the individual larynx used.

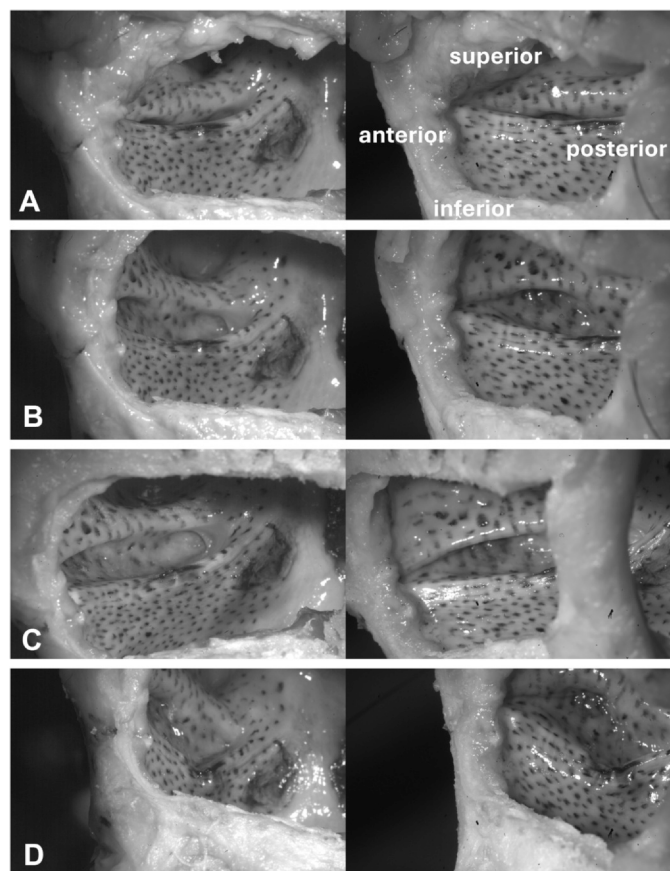


FIGURE 3. The two camera views of the medial surface of the vocal fold, false vocal fold, and the ventricle in between in larynx 1. (A). Larynx at rest without any hyoid movement; (B) after superior hyoid movement; (C) after anterior-superior hyoid movement; (D) after posterior-superior hyoid movement.

RESULTS

General observations

Figure 3 shows the two medial views of the vocal fold and false vocal fold in larynx #1 at rest and after the hyoid bone was pulled along the three directions. At rest (Figure 3a), the false vocal fold was almost sitting on top of the true fold, with the ventricle completely collapsed and hidden from the medial view. Superior hyoid movement lifted the false fold, exposing and enlarging the ventricle (Figure 3b). In contrast, the true vocal fold did not exhibit much movement until the false fold was sufficiently lifted (see Supplementary Material Videos S1–S3). The vocal fold medial surface shape did not appear to change much. Figure 3c shows the medial view after anterior-superior movement of the hyoid. As in the case of superior hyoid movement, anterior-superior hyoid movement lifted the false fold, opening the ventricle. The vocal fold also tilted forward and downward. Similarly, the change in medial surface shape was small. For posterior-superior hyoid movement (Figure 3d), similar observations can be made: the false fold was lifted up, opening the ventricle. The vocal fold was tilted upward and backward, with noticeable vocal fold shortening. There appears to be some change in vocal fold medial surface shape.

The following is the Supplementary material related to this article. Video S1: Medial view of the true and false vocal fold during superior hyoid movement.

The following is the Supplementary material related to this article. Video S2: Medial view of the true and false vocal fold during anterior-superior hyoid movement.

The following is the Supplementary material related to this article. Video S3: Medial view of the true and false vocal fold during posterior-superior hyoid movement.

Supplementary material related to this article can be found online at [10.1016/j.jvoice.2026.09.012](https://doi.org/10.1016/j.jvoice.2026.09.012)

Figure 4 shows the reconstructed coronal slices of the vocal fold medial surface before and after hyoid movement in larynx 1. To facilitate comparison of the medial surface shape, the slices were translated within the coronal plane to match the most medial point of the medial surface before and after hyoid movement. Figure 4 shows that the general medial surface contour was comparable before and after hyoid movement, although some subtle differences can be observed. Note that the slice contour was obtained by interpolation of the reconstructed three-dimensional coordinates of the ink points. The spatial resolution was thus limited by the small number of ink points available for specific conditions. For some conditions, the movement of the thyroid cartilage blocked part of the medial surface after hyoid movement, resulting in a significantly reduced vertical span of the visible medial surface after hyoid movement, as for example in the case of posterior-superior

hyoid movement in Figure 3d. This made a quantitative comparison difficult, as further described below.

Quantitative evaluations

Figure 5 shows the ventricle depth and width for different hyoid movement conditions. Across all three larynges, hyoid movement in all three directions consistently increased the ventricle depth and width, both of which are statistically significant (Tables A1 and A2 in appendix). This is consistent with the observation in Figure 3 and the multimedia videos. While not shown, upward hyoid movement also abducted the false vocal fold.

Figure 6 shows changes in vocal fold length, vocal fold angle, glottal width, and vocal fold vertical thickness. In general, hyoid movement decreased vocal fold length, with the vocal fold shortened by as large as about 15%. The observed vocal fold shortening was statistically significant for superior and posterior-superior hyoid movement but was only marginally significant for anterior-superior hyoid movement (Table A3 in appendix).

Hyoid movement also led to increased vocal fold angle, indicating an upward and backward tilting of the vocal folds. Similar to that for vocal fold shortening, the increase in vocal fold angle was statistically significant for superior and posterior-superior hyoid movement. For anterior-posterior hyoid movement, the vocal fold angle decreased, indicating a forward and downward vocal fold tilting, but this effect was only marginally significant ($P = 0.066$) (Table A4 in appendix).

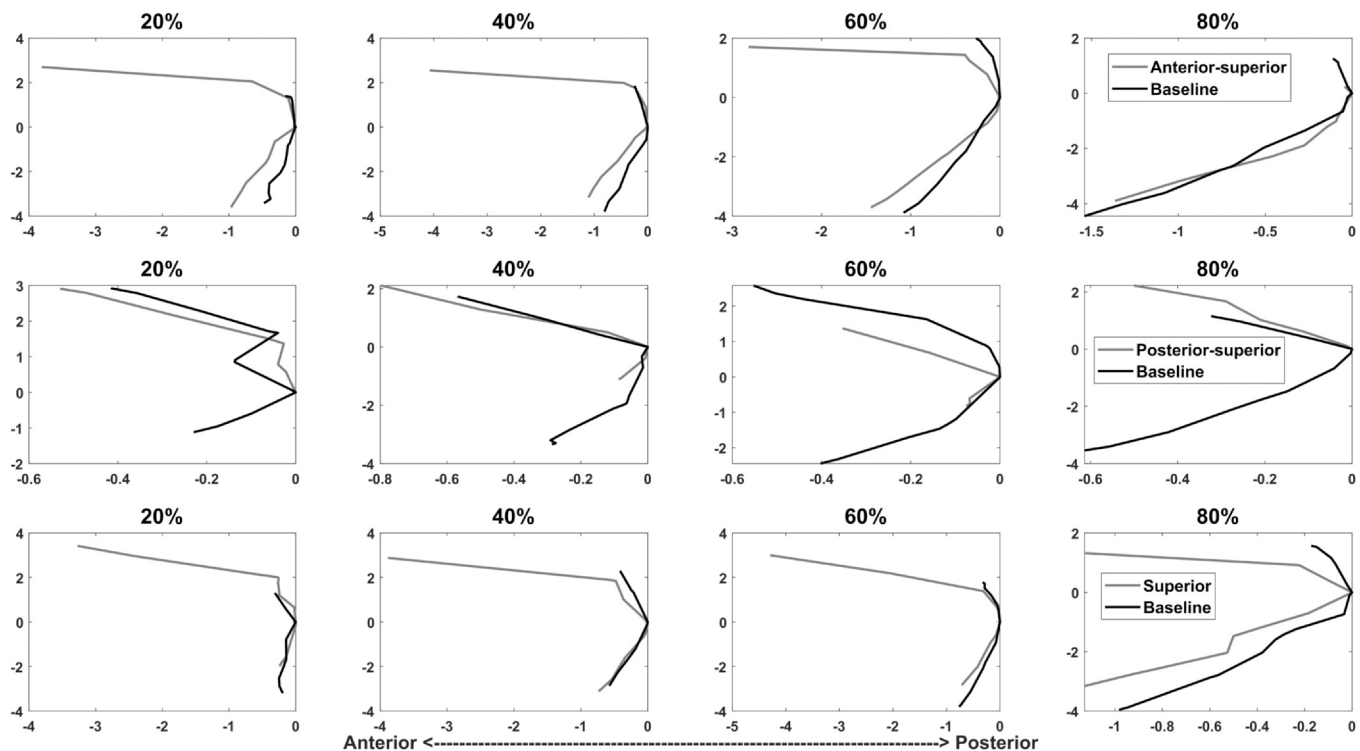


FIGURE 4. The reconstructed coronal slides of the vocal fold medial surface before (black lines) and after (gray lines) anterior-superior (top), posterior-superior (middle), and superior (bottom) hyoid movement at four locations along the AP direction on the vocal fold in Larynx 1. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

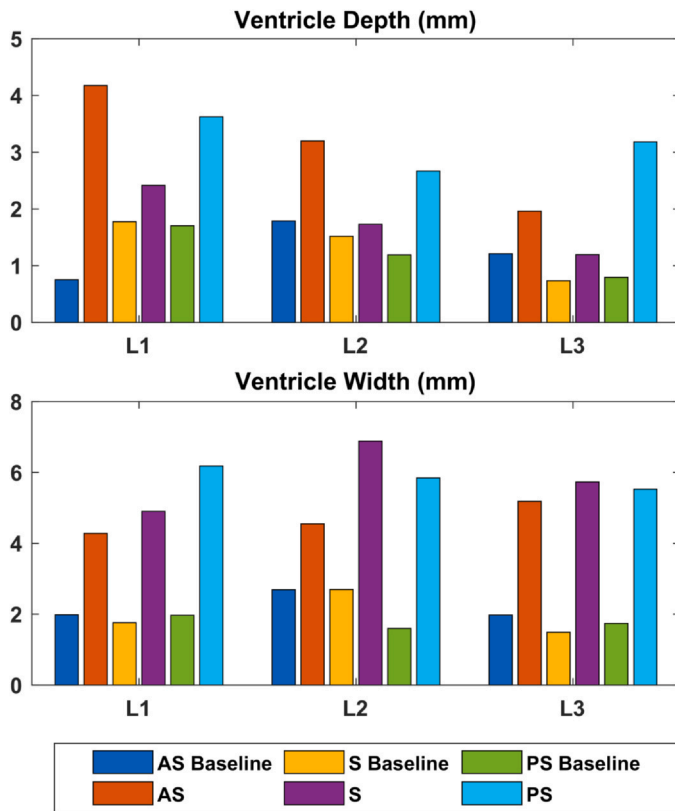


FIGURE 5. Ventricle depth and width at different hyoid conditions for the three larynges (L1, L2, and L3). AS: anterior-superior hyoid movement; S: superior hyoid movement; PS: posterior-superior hyoid movement.

The glottal width increased, indicating vocal fold abduction, for anterior-posterior and superior hyoid movement. This effect was statistically significant. For posterior-superior hyoid movement, a decreasing trend was observed for the glottal width, indicating vocal fold adduction, although this trend was not statistically significant ($P = 0.658$) (Table A5 in appendix). There was a significant effect of AP location, with the glottal width increasing toward the posterior end of the vocal fold, which was consistent with the cadaver position of the vocal fold.

For vocal fold vertical thickness measured using a threshold distance of 0.1 mm, no significant effect of hyoid movement was observed (Table A6 in appendix). The R^2 value for this model was 0.13, indicating large variability. This suggests that hyoid movement in general was not able to induce consistent changes in the most medial, flow-constricting portion of the medial surface. There was a significant effect of AP location, with the vertical thickness decreasing toward the posterior end of the vocal fold, which was consistent with the general anatomical trend of the vocal fold geometry. When the vocal fold vertical thickness was calculated using a threshold distance of 0.5 mm (thus covering a larger vertical span), a significant effect was observed for posterior-superior hyoid movement ($P < 0.001$) (Table A7 in appendix), with the thickness decreasing with posterior-superior hyoid movement. However, this reduction in thickness was likely due to the movement of the thyroid cartilage blocking the medial view in Larynx 1 and 2, thus reducing thickness post hyoid movement, an example of which is shown in Figure 3d.

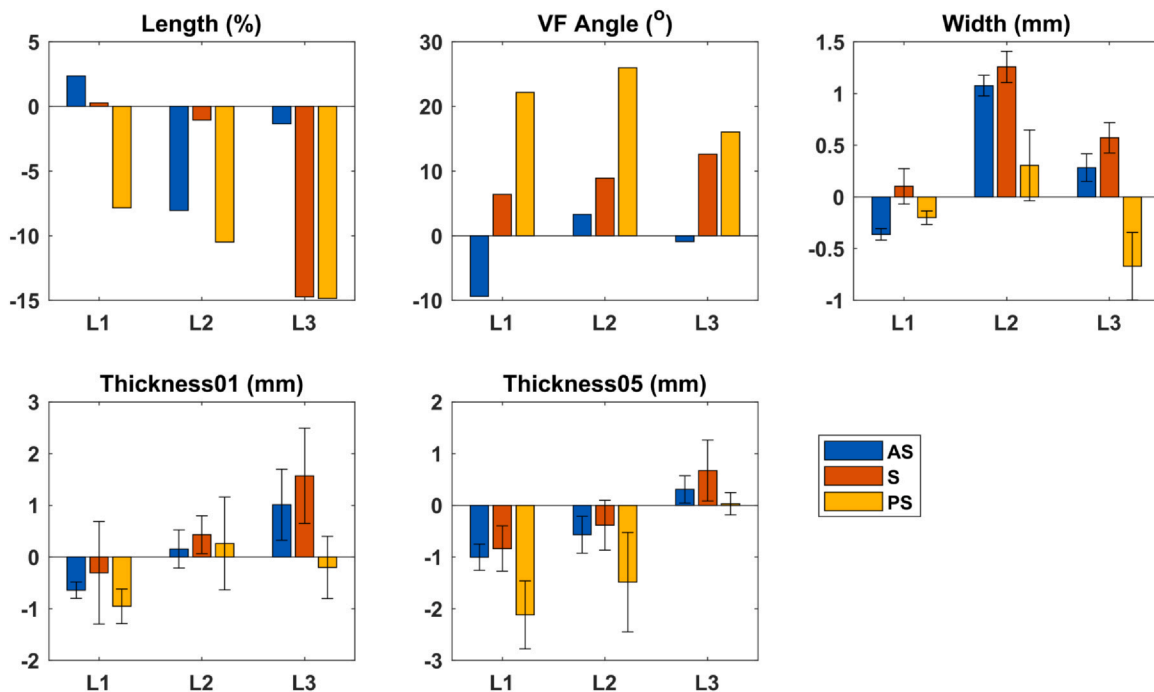


FIGURE 6. Changes in vocal fold length, vocal fold angle, glottal width, and vocal fold vertical thicknesses calculated using two threshold distances (Thickness01: 0.1 mm; Thickness05: 0.5 mm) at different hyoid conditions for the three larynges (L1, L2, and L3). AS: anterior-superior hyoid movement; S: superior hyoid movement; PS: posterior-superior hyoid movement.

TABLE 1.
Statistically Significant Effects of Hyoid Movement. An increase in Vocal Fold Angle Indicates an Upward and Backward Tilting of the Vocal Fold

	AS	S	PS
Ventricle depth	↑	↑	↑
Ventricle opening width	↑	↑	↑
VF length		↓	↓
VF angle		↑	↑
Glottal width	↑	↑	
Thickness (0.1 mm)			
Thickness (0.5 mm)			↓

DISCUSSION AND CONCLUSIONS

Table 1 summarizes the main observations of this study. Upward hyoid movement in this study lifted the false vocal fold, increasing both the opening width and depth of the ventricle. Upward hyoid movement also had a significant effect on vocal fold length, vocal fold angle (vocal fold tilting), and glottal width. In general, upward hyoid movement decreased vocal fold length and tilted the vocal fold upward and backward (except for anterior-superior movement), and increased glottal width (except for posterior-superior movement). These are consistent with the observations reported in Vilkman and Karma¹³ and Sundberg.¹⁹ Our study also showed that the effect of upward hyoid movement on vocal fold vertical thickness was statistically insignificant and larynx-specific, particularly when the thickness was calculated based on the most constricting portion of the medial surface using a threshold distance of 0.1 mm.

Since the cricoid cartilage was fixed in our experiment, the observed changes in vocal fold and false vocal fold configurations in Table 1 should be interpreted as the changes that would happen when an external force is applied to the thyroid cartilage. In our study, this external force was applied due to hyoid movement. The upward hyoid movement increased the distance between the hyoid and thyroid cartilages, which likely applied an upward force to the thyroid cartilage through the connective tissues. This may have caused the thyroid cartilage to rotate upward and backward about the cricothyroid joint, which decreased vocal fold length and increased vocal fold angle. On the other hand, anterior hyoid movement may move the thyroid forward and may tilt the vocal folds forward and downward, counteracting the effect of upward hyoid movement. This explains why changes in vocal fold length and angle were insignificant with anterior-superior hyoid movement (Table 1), and changes in vocal fold length and angle were the largest with posterior-superior hyoid movement (Figure 6; Tables 1, A3, and A4).

In humans, lowering the larynx position often increases the hyoid-thyroid distance, which likely applies a similar upward force to the thyroid cartilage. According to the results from our study, this may shorten the vocal folds, tilt the vocal folds upward and backward, and slightly increase the glottal width. Similarly, our results suggest that a high larynx position with reduced hyoid-thyroid distance may increase vocal fold length

and slightly decrease the glottal width. This may explain why speakers often raise the larynx for high pitch and lower the larynx for low pitch.^{2,7} The increased glottal width with increasing hyoid-thyroid distance also may explain why the voice source produced with a lowered larynx was similar to that of a flow phonation.⁸ However, note that this relationship between larynx height and vocal fold length was observed in the absence of extrinsic muscle activation in this study. Extrinsic muscle activation may change the relationship between external forces applied to the thyroid cartilage and changes in hyoid-thyroid distance, which may, in turn, affect the relationship between laryngeal height and vocal fold length.

An important finding of this study is that no significant effect of hyoid movement on vocal fold medial surface thickness was observed. While this insignificant result could be due to methodology limitations (eg, small number of ink points which may have resulted in large interpolation errors in medial surface shape, medial surface reconstruction errors comparable with the subtle changes in medial surface shape, and reduced medial view due to thyroid movement) and the small sample size, it was consistent with the qualitative observation of small medial surface shape change in the multimedia videos. This seems to suggest that external forces applied to the thyroid cartilage can lead to consistent changes in vocal fold length and tilting but not in medial surface thickness, and that thickness is more consistently controlled by intrinsic muscles. Indeed, in our study the relationship between length and thickness changes was not significant. Thus, while vocal fold shortening may lead to expansion in the cross-sectional area of the vocal folds due to volume conservation, this alone does not necessarily lead to a noticeable change in vocal fold thickness. This is consistent with the finding of a recent numerical study,²⁰ that a noticeable increase in vocal fold thickness occurs only when thyroarytenoid muscle activation causes the inferior medial surface to bulge medially more than the superior medial surface, due to a horizontal rotation of the arytenoid cartilage.²⁰

The lack of a significant relationship between length and thickness change in our study also suggests that vocal fold length, tilting, and thickness can be relatively independently controlled in humans. When vocal fold length and thickness are regulated solely through the actions of the intrinsic muscles, they are often negatively correlated, with vocal fold shortening often accompanied by an increase in thickness.^{21,22} However, by coordinating the intrinsic and extrinsic muscles, vocal fold thickness may be regulated without simultaneous vocal fold length change. For example, vocal fold thickness can be increased by activation of the thyroarytenoid muscles, while the extrinsic muscles can be activated to compensate for the shortening effect of the thyroarytenoid muscle. This relatively independent control of length and thickness makes it possible to independently control fundamental frequency and voice quality, at least to some degree, which is essential to the singing voice.

Previous studies showed that a raised larynx is often associated with increased vocal fold adduction and pressed

phonation,⁸ as often observed in hyperfunctional voices^{10,11} and is considered increased risk of vocal fold injury.³ Our results showed that adjustments in the hyoid-thyroid distance alone did not always produce significant changes in vocal fold thickness, an important parameter controlling the glottal closure pattern and voice quality.¹⁵ This suggests that changes in the voice source with larynx position, as observed in Sundberg and Gauffin,⁸ may be due to coactivation of the extrinsic and intrinsic muscles. Specifically, extrinsic muscle activities that raise the larynx are likely accompanied by increased activity of the thyroarytenoid muscle, due to some kind of neural coupling between these muscles, which is worth further investigation.

It is important to note the limitations of the excised larynx setup. First, as mentioned earlier, the hyoid movement in our experiment was achieved through mechanical manipulation rather than extrinsic laryngeal muscle activation as in humans, which applies additional active forces to the thyroid cartilage that were not present in our excised larynges. This may change the relationships between external forces applied to the thyroid cartilage and changes in hyoid-thyroid distance, as well as between laryngeal height and changes in vocal fold configuration. For example, when the thyrohyoid muscle is engaged to approximate the hyoid and thyroid cartilages in humans, it applies an anterior-superior muscular force to the thyroid cartilage, whereas no such force was present in our experiment when the hyoid was at a position closest to the thyroid cartilage. Similarly, while the condition with the largest hyoid-thyroid distance in our experiment appears to replicate a lowered larynx position, the mechanical conditions involved were likely different from those in a larynx lowered by sternothyroid

muscle activation. Second, the vocal folds were at cadaveric position in our experiment without vocal fold adduction or elongation. In live humans, intrinsic muscle activation likely imposes additional constraints on the movement of the thyroid and arytenoid cartilages, which may impact the results of this study. Finally, the cricoid cartilage was fixed in this study, whereas in humans it may move due to actions of pharyngeal constrictor muscles or the effect of tracheal pull. How these limitations impact the results of this study will be addressed in future studies.

Data Availability

The data that support the findings of this study are available from the author upon reasonable request.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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AUTHOR DECLARATIONS

The author has no conflicts to disclose.

Appendix

TABLE A1.

Linear Mixed Effects Model for Ventricle Depth. $\text{Depth} \sim 1 + \text{AS} + \text{S} + \text{PS} + (1 \mid \text{Larynx})$. $R^2=0.79$

	Estimate	SE	tStat	DF	P-value	Lower	Upper
AS	1.837	0.289	6.358	14	< 0.001	1.217	2.456
S	0.506	0.289	1.751	14	0.102	-0.114	1.125
PS	1.885	0.289	6.525	14	< 0.001	1.265	2.504

TABLE A2.

Linear Mixed Effects Model for Ventricle Opening Width. $\text{Ventricle Width} \sim 1 + \text{AS} + \text{S} + \text{PS} + (1 \mid \text{Larynx})$. $R^2=0.92$

	Estimate	SE	tStat	DF	P-value	Lower	Upper
AS	2.680	0.320	8.383	14	< 0.001	1.995	3.366
S	3.847	0.320	12.030	14	< 0.001	3.161	4.532
PS	3.858	0.320	12.066	14	< 0.001	3.172	4.544

TABLE A3.Linear Mixed Effects Model for Vocal Fold Length. Length $\sim 1 + AS + S + PS + (1 | \text{Larynx})$. $R^2=0.87$

	Estimate	SE	tStat	DF	P-value	Lower	Upper
AS	-0.105	0.213	-0.493	14	0.630	-0.561	0.351
S	-0.482	0.213	-2.269	14	0.040	-0.938	-0.026
PS	-1.091	0.213	-5.134	14	< 0.001	-1.547	-0.635

TABLE A4.Linear Mixed Effects Model for Vocal Fold Angle. Angle $\sim 1 + AS + S + PS + (1 | \text{Larynx})$. $R^2=0.94$

	Estimate	SE	tStat	DF	P-value	Lower	Upper
AS	-4.492	2.250	-1.997	14	0.066	-9.318	0.333
S	9.643	2.250	4.286	14	< 0.001	4.818	14.468
PS	23.227	2.250	10.324	14	< 0.001	18.401	28.052

TABLE A5.Linear Mixed Effects Model for Glottal Width. Width $\sim 1 + AS + S + PS + APLocation + (1 | \text{Larynx})$. $R^2=0.62$

	Estimate	SE	tStat	DF	P-value	Lower	Upper
AS	0.316	0.157	2.011	67	0.048	0.002	0.630
S	0.542	0.157	3.451	67	< 0.001	0.229	0.856
PS	-0.070	0.157	-0.445	67	0.658	-0.384	0.244
APLocation	0.358	0.050	7.197	67	< 0.001	0.258	0.457

TABLE A6.Linear Mixed Effects Model for Vocal Fold Thickness Measured Using A Threshold Distance of 0.1 mm. Thickness01 $\sim 1 + AS + S + PS + APLocation + (1 | \text{Larynx})$. $R^2=0.13$

	Estimate	SE	tStat	DF	P-value	Lower	Upper
AS	0.292	0.199	1.468	67	0.147	-0.105	0.689
S	0.356	0.199	1.790	67	0.078	-0.041	0.753
PS	-0.204	0.199	-1.028	67	0.308	-0.601	0.192
APLocation	-0.181	0.063	-2.871	67	0.0055	-0.306	-0.055

TABLE A7.Linear Mixed Effects Model for Vocal Fold Thickness Measured Using A Threshold Distance of 0.5 mm. Thickness05 $\sim 1 + AS + S + PS + APLocation + (1 | \text{Larynx})$. $R^2=0.49$

	Estimate	SE	tStat	DF	P-value	Lower	Upper
AS	-0.375	0.204	-1.841	67	0.070	-0.783	0.032
S	-0.343	0.204	-1.681	67	0.097	-0.750	0.064
PS	-1.078	0.204	-5.287	67	< 0.001	-1.485	-0.671
APLocation	-0.116	0.064	-1.797	67	0.077	-0.245	0.013

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