Vocal Fold Adjustment Caused by Phonation Into a Tube: A Double-Case Study Using Computed Tomography

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Summary: Objectives. Phonation into a tube is a widely used method for vocal training and therapy. Previous studies and practical experience show that the phonation becomes easier and louder after such an exercise. The purpose of this study was to find out whether there are systematic changes in the vocal fold adjustment after the exercise.

Methods. Two volunteer subjects (1 male and 1 female) without voice disorders were examined with computed tomography (CT). Both produced a sustained vowel [a:] at comfortable pitch and loudness before and after the tube phonation and a vowel-like phonation into the tube. Computed tomography (CT) scans were obtained before, during, and after the exercise, twice for each condition. The gathered CT images were used for measurements of vertical vocal fold thickness, bulkiness, length, and glottal width.

Results. No prominent trends common to both subjects were found in vocal fold adjustment during and after the phonation into the tube. Variability observed under the same conditions was usually of the same magnitude as the changes before and after the tube phonation.

Conclusions. Changes in vocal tract configuration observed after the resonance tube exercises in previous related studies were more prominent than the changes in vocal fold configuration observed here.

Key Words: Vocal folds–Resonance tube–Computed tomography.

INTRODUCTION

Phonation into a tube is a useful method widely used for vocal training and therapy. It belongs to a wider group of semi-occluded vocal exercises that take advantage of a semi- or full closure of the vocal tract.[1] Other commonly used exercises of this type are tongue trills, nasals, and voiced fricatives.[2] Humming into various small glass tubes started to be used in the beginning of 20th century by Spiess[3] for improving vocal function. Tube phonation has been used, for example, for treatment of hypernasality.[4,5] Method of phonation into glass tubes, so called resonance tubes, has also been used for decades in Finnish voice and speech training and therapy where it has become popular.[6,7] Sovijärvi[8] was at first interested in testing different kinds of glass tubes in the children with hypernasality, but soon he started to use the tubes also with adult singers who had voice problems. According to his observations, phonation into the tubes improved voice quality of the patients with functional phonasthenia, laryngeal paresis, and vocal fold nodules.[6,9] This method has also been used for vocal care and further vocal training in healthy and normophonic subjects using their voice extensively (ie, singers or teachers) because the voice is perceived as being louder and feels to be easier to produce after such an exercise.[7]

Laukkanen et al[10] showed that sound pressure level (SPL) slightly increased after the tube phonation. This was also observed by Vampola et al[11] who calculated SPL values using finite element modeling method. Despite number of studies published about resonance tubes, the exact mechanism of their functioning has not been fully understood. Basically, there are three possibilities of adjustment caused by the exercises, such as (1) change in the voice source (vocal folds), (2) change in the vocal tract (filter), and (3) change caused by the interaction between the voice source and the filter.

To investigate the *changes in the voice source*, Laukkanen[12] analyzed electroglottographic signals of vibrating vocal folds and showed that the quasi-open quotient decreased during and after the exercise, which was possibly related to change in adduction of vocal folds. Speed quotient rose indicating more rapid collisions of the vocal folds. Furthermore, Laukkanen et al[7] studied muscle activities in a single female subject via electromyography (EMG) and found that the ratio of thyroarytenoid (TA) versus cricothyroid muscle activity increased. According to Hirano[13] and Yumoto et al,[14] increased activity of TA muscle makes the vocal fold thicker and bulged. In addition, according to Chhetri et al[15] who investigated neuromuscular mechanisms for modulating glottal posture in canine larynx, TA activation has been shown to close the mid-membranous glottis. So far, however, there has not been any conclusive evidence of specific consistent changes in vocal fold adjustments caused by the semi-occluded voice exercises.

Changes in the vocal tract and size of its cavities caused by the resonance tube exercise were examined by Vampola et al.[16] The study reported that the velum raised and closed the nasopharyngeal port. In addition, cross-sectional areas of vocal tract (nasal cavities excluded) expanded and its total volume became

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considerably larger. Similar results have been reported by Guzman et al.[17]

Interaction between the voice source and the filter has been found to be able to change the vibration properties of the vocal folds solely owing to the changes in the supraglottal tract.[18,19] The use of the resonance tube creates a constriction and this reduction of cross-sectional area increases the vocal tract resistance. Simultaneously, the tube elongates the vocal tract causing the first formant frequency to go down.[20] This increases the inertive reactance of the vocal tract resulting in an increased source-filter interaction.[2] The resonance tube also increases supraglottal pressure that tends to decrease transglottal pressure (ie, the difference between the subglottal pressure and the supraglottic pressure).[1,5] Sufficiently low transglottal pressure makes phonation more economical in terms of preserving the vocal folds from powerful collisions and provides a sensation of maximal outcome achieved with minimal effort.[2,5]

In 1960s, Hollien et al[21–26] published an original methodology for measurement of vocal fold anatomical dimensions *in vivo* using X-ray laminagrams. The contours of the laryngeal tract and vocal folds were outlined in the X-ray images and a system of lines and points was designed to measure the length, thickness, and surface tilting of the vocal folds related to changes in fundamental frequency (F_0). Present study applies the methodology of Hollien et al[21–26] to the modern examination method of computed tomography (CT) imaging (Methods section). The purpose of this study was to find out whether there are systematic changes in the vocal fold adjustment caused by the phonation into a tube. To do that, vocal fold geometry before, during, and after the phonation into the resonance tube was measured and compared.

Based on the studies of Laukkanen et al,[7] who observed increased activity of TA muscle after the tube phonation, the following three hypotheses were formulated and investigated here: (1) the vocal folds are going to be more bulged and thicker, (2) the glottal width is going to decrease, and (3) the length of the vocal folds is not going to change. The first two hypotheses stem from the knowledge on the effect of the TA activity: The TA muscle has been shown to bulge the vocal fold[13] and to shift the vocal fold margin more medially.[13,14] The third hypothesis considers that the phonations before and after the tube phonation are both requested to be produced at comfortable pitch, thus not requiring considerable vocal fold length adjustments.

MATERIALS AND METHODS

Subjects and CT recordings

Two vocally healthy subjects were investigated: subject F—a female, aged 48 years, and co-author A.M.L. and subject M—a male, aged 35 years, and co-author M.A.G. Both subjects had no voice or hearing problems and were experienced in semi-occluded exercises. The subjects were informed about potential risks related to the radiation dose during CT examination and signed a consent form. The recordings were performed at two different occasions using a CT device (Light Speed VCT GE–64 and Toshiba Aquilion). The subjects were placed in supine position. The recording time for subject F was 2 seconds. During this time, 181 images with the resolution of 512 × 512 pixels were collected. The thickness of each slice was 0.625 mm. For subject M, the recording time was 3.36 seconds yielding 510 images with the resolution of 512 × 512 pixels and slice thickness of 0.5 mm. For both subjects, the scanning covered an area from below the larynx up to the bottom of nasal cavities.

Phonatory tasks and CT measurements

The subjects were asked to produce a sustained vowel [a:] at a comfortable pitch and loudness before and after phonating into the resonance tube. The resonance tube training exercise was performed for about 5 minutes into 27-cm long resonance tube (glass) with the inner and outer diameters of 8 and 9 mm. The duration of 5 minutes was previously found long enough for sensing the changes in voice production and causing clear changes in the vocal tract configuration after the exercise.[16,17] To investigate the variability of the glottal configuration during the individual phonations, the CT scanning was performed twice before, twice during, and twice after the phonation into the tube. The freely downloadable software *OsiriX* (version 3.9.4, 32-bit; Osirix, Pixmeo, Switzerland)[27] was used for setting the planes to the desired position and for inserting a calibrated distance line into the image. For further analysis, the gathered images were exported and processed with the *ImageJ* 1.45s software (National Institutes of Health, Maryland, USA), which provided an environment for the final measurements of lengths, thicknesses, and areas, using the calibrated distance line.

To measure the relevant lengths of the vocal folds, it was necessary to set the transverse plane (Figure 1A) to be parallel to the upper surface of both vocal folds (Figure 1B). Then, the transverse plane was shifted to reach the level of glottis (Figure 2) similar to the one as shown by Hirano in a collection

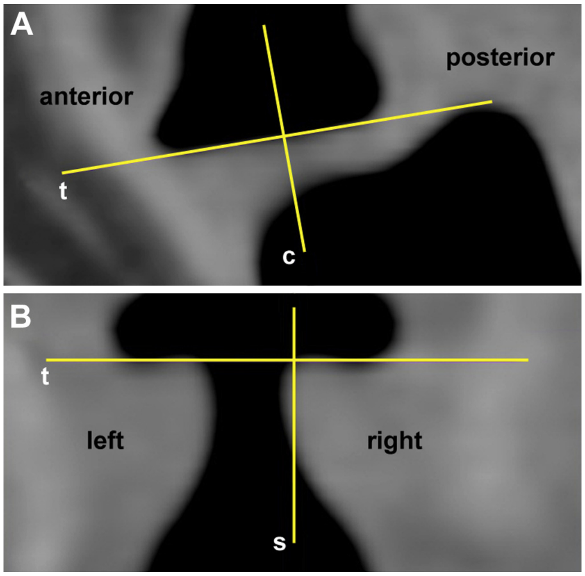

FIGURE 1. The sagittal (**A**) and coronal (**B**) slices demonstrating how the transverse plane was adjusted to be parallel to the upper surface of the vocal folds: t, transverse plane; c, coronal plane; s, sagittal plane.

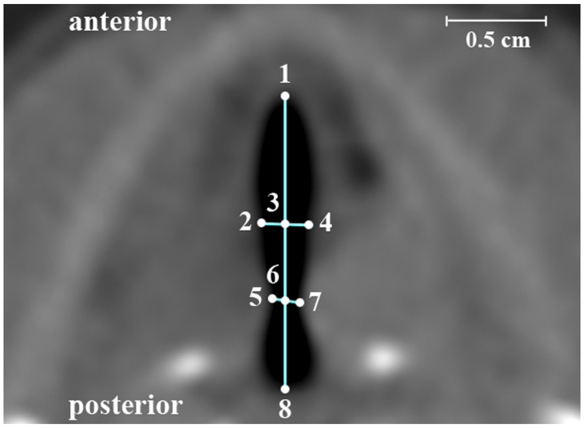

FIGURE 2. The CT transverse plane of the vocal folds and the points marked as one to eight used for glottal measurements.

of histological slices (Hirano,[13] section AH9, page 20). The resulting image was used to measure the distances between the points marked on the Figure 2. Herein, the points 1 and 8 correspond to the anterior and posterior commissures and create an axis, which intersects the apex of the anterior commissure and the middle of the distance between the vocal processes. The medially prominent parts of the arytenoid cartilages were marked as the points 2, 4, 5, and 7. Finally, the points 3 and 6 were recognized as the intersections between the axis of glottis and lines connecting points 2, 4 and 5, 7.

The width of glottis, thicknesses, and areas of the vocal folds were measured in the coronal plane perpendicularly to the vocal fold surface. These measurements were first attempted to be done in the mid-membranous part of the vocal folds, but herein, an unusual cross-sectional shape of the vocal folds was found in subject M. Therefore, the coronal plane was placed more posteriorly at the ratio of 0.86 of a reference vocal fold length, that is, the distance between the anterior apex of the lowest part of the laryngeal ventricle and the vocal processes (Figure 3). At this position, the left vocal fold (LVF) of subject M showed standard shape with a well-defined upper vocal fold surface, allowing the cross-sectional shape measurements to be performed here. The plane adjustments were done in the following way: (1) The transverse plane was set to be parallel to the upper surface of the vocal folds (Figure 1A); (2) The mid-sagittal plane was rotated to intersect the apex of the anterior commissure and the midpoint between vocal processes. This kept the coronal plane perpendicular to the axis of the glottis (Figure 3B, c_1); (3) The coronal plane was moved to the position fulfilling the 0.86 ratio of the reference vocal fold length (Figure 3B, c_2). This coronal plane (Figure 4) was used for measurement of the vocal fold thicknesses at 1- and 2-mm distances (Figure 4; T1 and T2) from the glottis, and of the cross-sectional vocal fold areas (Figure 4; A1 and A2). The process of location of the planes and the corresponding measurements of the vocal fold morphology were performed three times for each of the CT scans. The three measurements were used to determine the *measurement uncertainty* for each single phonation, which was expressed as the *standard error of the mean*. This measurement uncertainty could then be compared with the variability observed during the task repetition (called *repetition variability* further on) and with the changes caused by the resonance tube exercises. The change caused by the exercise was considered significant when it was greater than the repetition variability and measurement uncertainty. Glottal width was measured

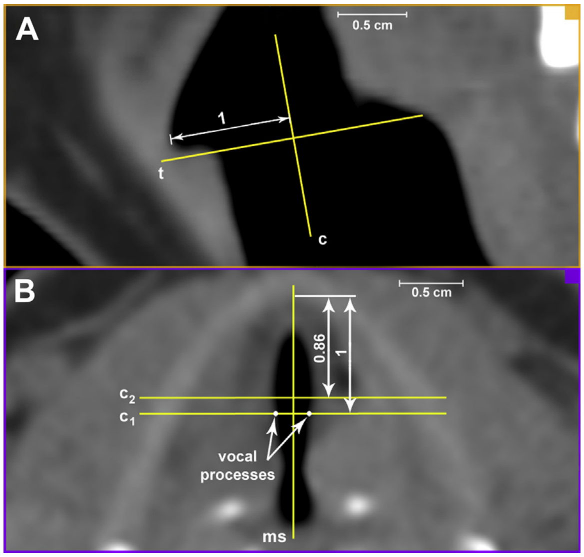

FIGURE 3. The CT mid-sagittal (**A**) and the transverse (**B**) slice demonstrating the adjustment of the coronal plane c. Labeling: c_1, position of the coronal plane at the level of vocal processes; c_2, position of the coronal plane used for the measurement of vocal fold vertical thickness and cross-sectional area; ms, position of the mid-sagittal plane; t, position of the transverse plane. The distance labeled by number 1 indicates the reference vocal fold length, that is, the normalized distance between the anterior apex of the lowest part of the laryngeal ventricle and the vocal processes.

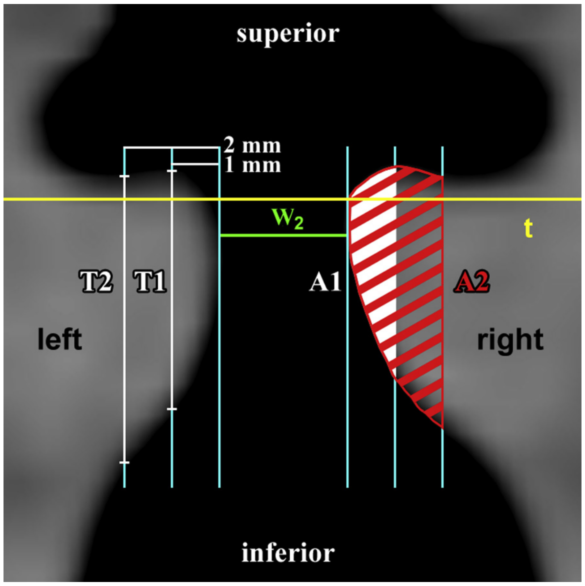

FIGURE 4. Scheme of the CT coronal plane used for the measurement of the vertical thicknesses (T1, T2), areas (A1, A2), and the glottal width w_2; t, transverse plane.

here as a distance between the most medial parts of the vocal folds (Figure 4, w_2). Because of an unusual shape of the upper surface of the right vocal fold (RVF) in subject M, the transverse plane (Figure 4, t) was used as the upper boundary for the measurements of T1, T2, A1, and A2 in this subject.

RESULTS

The results are shown in five graphs (Figures 5–9) showing the changes in the vocal fold adjustment related to the resonance tube exercise. The X-axis shows the states before ("Before"), during ("Tube"), and after ("After") the phonation into a tube. The measured distances (ie, length, vertical thickness) or areas are displayed on the Y-axis. Because each recording of "Before," "Tube," and "After" was made twice, the graphs always contain two mean values (labeled as "maximum" and "minimum") for each of the conditions, which indicate the *repetition variability*. The mean values were obtained from three repeated measurements of the same phonation. The spans of the error bars show the *standard error of the mean* for each of the measurements (based on the three measurement repetitions) revealing the *measurement uncertainty*. The mean values are interconnected horizontally among the different states to illustrate the changes caused by the resonance tube exercise.

Vertical thickness

The vertical thicknesses T1 and T2 of the vocal folds (measured at the depth of 1 and 2 mm) in subject F are shown in Figure 5. For better clarity, the LVF and RVF are shown separately. The results reveal that:

1. The LVF was generally thicker than the RVF before the exercise (4.40–4.61 mm vs 4.03–4.23 mm for T1; 5.53–5.56 mm vs 5.13–5.26 mm for T2, respectively) as well as after the exercise (4.56–4.69 mm vs 4.15–4.29 mm for T1 and 5.71–5.75 mm vs 5.51–5.56 mm for T2).
2. The thickness T2 (at 2-mm depth) was about 1 mm larger than the thickness T1 (at 1-mm depth) for both the vocal folds.
3. The measurement uncertainty (note the error bars indicating the standard error) of the determined mean thickness T1 was on average ±0.05 mm for the LVF and ±0.09 mm for the RVF; for T2, it was ±0.07 and ±0.09 mm, respectively.
4. The repetition variability was on average 0.19 mm, which was mostly larger than the measurement uncertainty (especially LVF in Figure 5). Particularly large repetition variability (0.8 mm) was observed during the tube phonation in RVF.
5. The thickness differences for T1 between "Before" and "After" conditions were smaller than the repetition variability, thus indicating no significant change. The average thickness T2, however, increased from "Before" to "After" by 0.24 mm in LVF and 0.34 mm in RVF. This increase was slightly larger than the repetition variability and the measurement uncertainty, thus indicating significant change.

Figure 6 illustrates the results from the measurement of the vertical thicknesses in the male subject M, in the same way as done in Figure 5. The results show that:

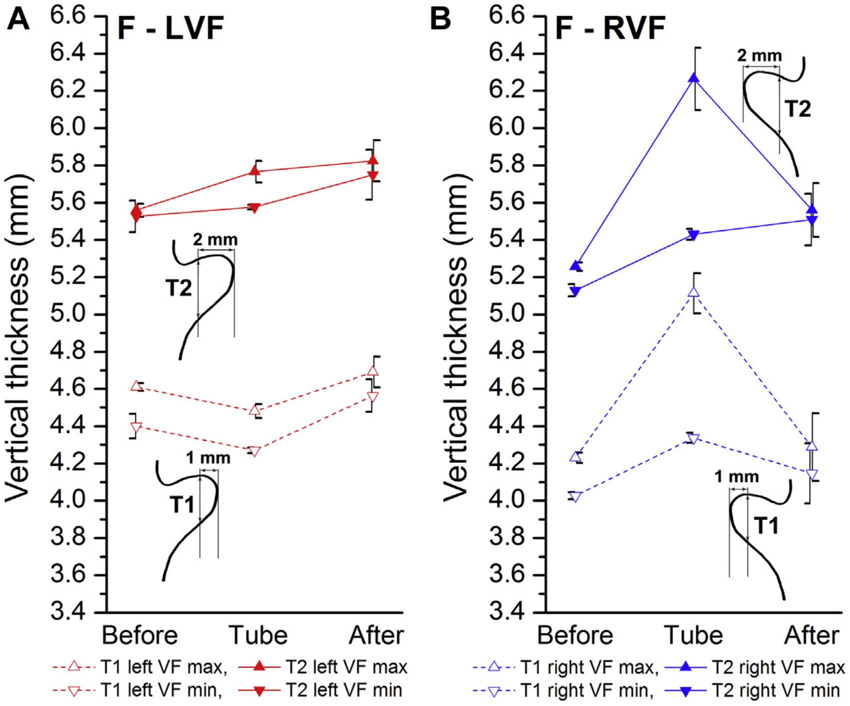

FIGURE 5. Changes in left vocal fold (**A**, LVF) and right vocal fold (**B**, RVF) vertical thickness in subject F measured at 1- and 2-mm depth (T1 and T2).

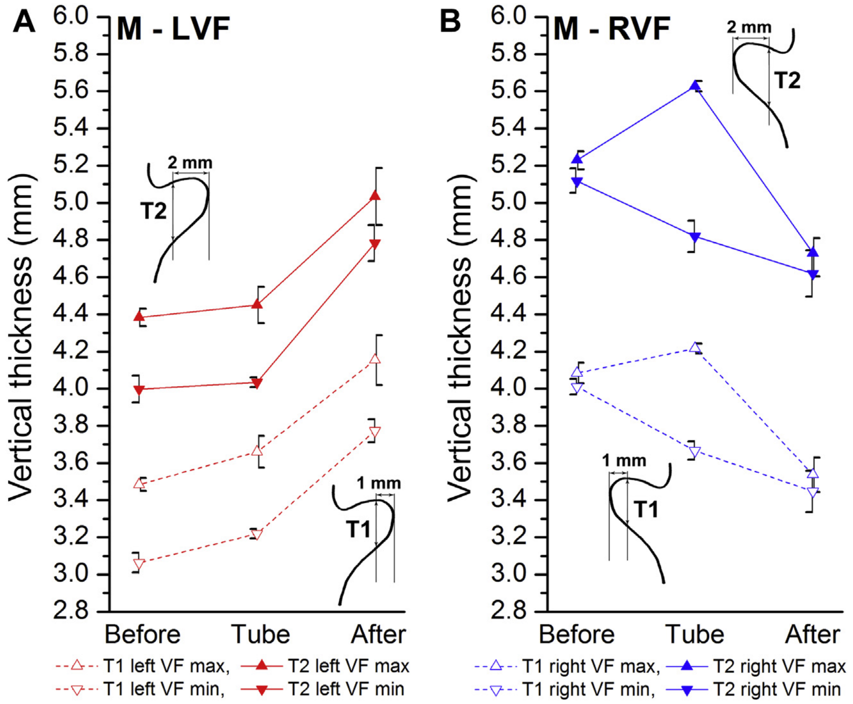

FIGURE 6. Changes in the left and right vocal fold (**A**, LVF; **B**, RVF) vertical thicknesses in subject M measured at 1- and 2-mm depth (T1 and T2).

1. The RVF was markedly thicker than LVF before the exercise (4.01–4.08 mm vs 3.06–3.48 mm for T1 and 5.12–5.23 mm vs 4.00–4.38 mm for T2, respectively) but became slightly thinner after (3.45–3.54 mm vs 3.77–4.15 mm for T1 and 4.62–4.71 mm vs 4.78–5.03 mm for T2, respectively).
2. Similarly to subject F, the thickness T2 (at 2-mm depth) was about 1 mm larger than the thickness T1 (at 1 mm depth) in both vocal folds.
3. The measurement uncertainty was mostly below ±0.1 mm (±0.06 mm for T1 and ±0.08 mm for T2, on average).

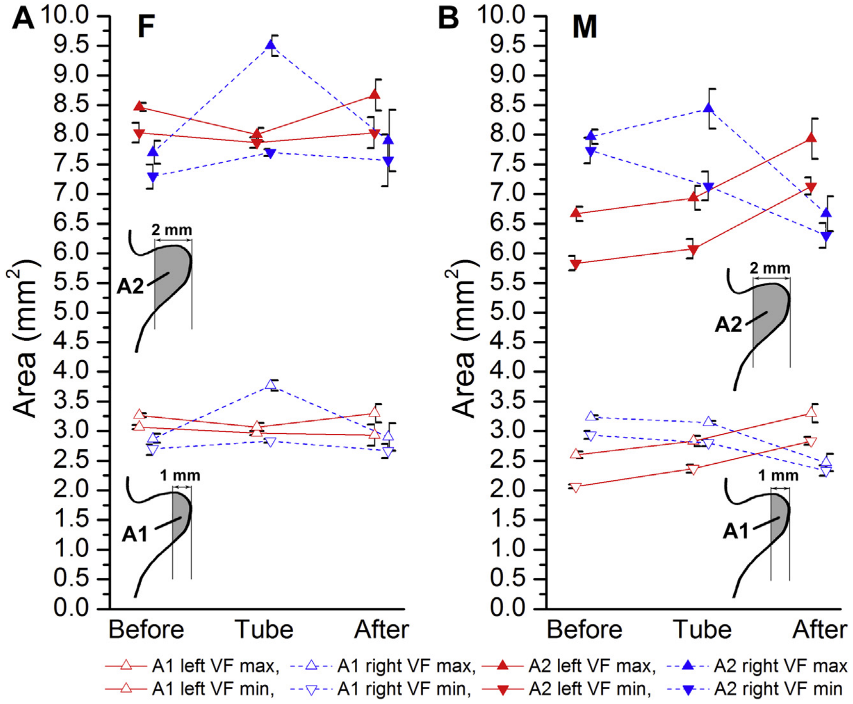

FIGURE 7. Changes in vocal fold cross-sectional areas bounded by the vertical lines at 1- and 2-mm distances for the subjects F (**A**) and M (**B**) and both the vocal folds (left, *solid lines*, right, *dashed lines*).

4. The repetition variability was around 0.4 mm (see particularly the LVF in Figure 6A), which was considerably larger than the measurement uncertainty.
5. The changes of vocal fold thickness caused by the exercise were about 0.6 mm for both T1 and T2. These were found significant because they were larger than the repetition variability and the measurement uncertainty. The changes were, however, not uniform: The LVF became significantly thicker, whereas the RVF became significantly thinner after the exercise than before. As a result, the LVF and RVF became considerably more symmetric after the exercise than before.

Cross-sectional areas

Figure 7 shows the "bulkiness" of the vocal folds measured via cross-sectional areas A1 and A2 (1 and 2 mm from the vocal fold margin) and their changes for both the subjects. Subject F is shown on the left (Figure 7A) and subject M on the right (Figure 7B). The results for subject F show that:

1. The LVF (red) was slightly bulkier than RVF (blue) both before and after the exercise.
2. Area A2 was about 2.7 times larger than A1 for both the vocal folds.
3. The measurement uncertainty was on average about ±0.1 mm^2 for A1 and ±0.21 mm^2 for A2.
4. The repetition variability was on average 0.33 mm^2 for A1 and 0.62 mm^2 for A2, which was larger than the measurement uncertainty.
5. The changes caused by the exercise were on average less than 0.1 mm^2 for A1 and, less than 0.2 mm^2 for A2, which was smaller than the repetition variability and the measurement uncertainty; thus, no significant change in vocal fold bulkiness could be detected for this subject.

The results for subject M (Figure 7B) show that:

1. The LVF (red) was less bulky than RVF (blue) before the exercise, but the opposite became true after the exercise. This is reflected in both the areas A1 and A2.
2. Similarly, as in subject F, the area A2 was about 2.7 times larger than the area A1 for both the vocal folds.
3. The measurement uncertainty was on average about ±0.08 mm^2 for A1 and ±0.21 mm^2 for A2.
4. The repetition variability was on average 0.37 mm^2 for A1 and 0.66 mm^2 for A2, which was larger than the measurement uncertainty.
5. The bulkiness changes caused by the exercise were, on average, 0.7 mm^2 for A1 and 1.3 mm^2 for A2. These changes are larger than the repetition variability as well as than the measurement uncertainty and can therefore be considered significant. However, the RVF and LVF showed changes in opposite directions.

Glottal width

Figure 8 depicts the changes of the glottal widths w_1 and w_2. The width w_1 is the distance measured in the transverse plane between the points 2 and 4 representing medial peaks of the vocal processes (hereafter "cartilaginous glottal width"). The width w_2 is a minimal distance between the vocal folds

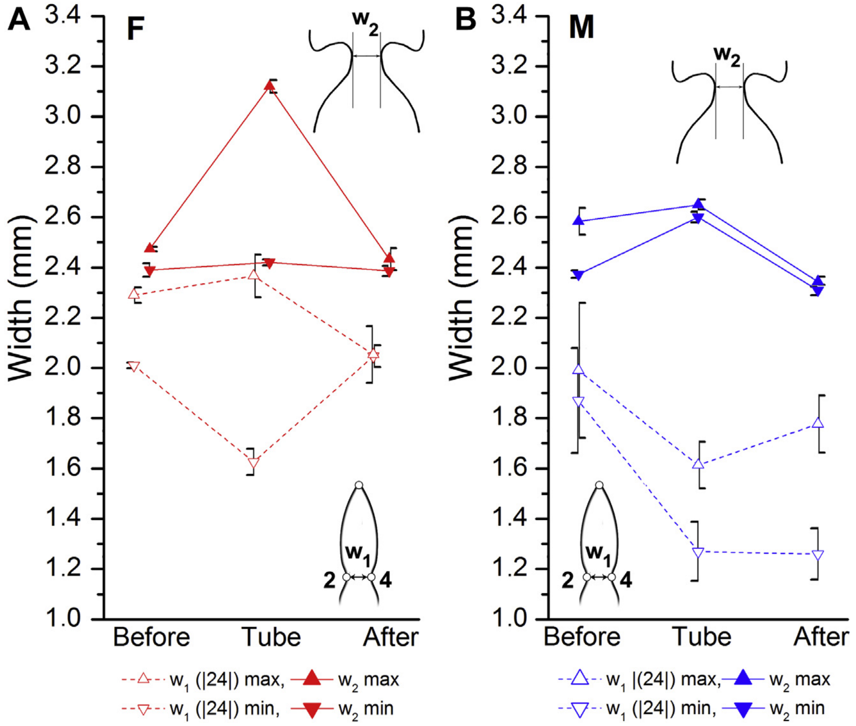

FIGURE 8. Glottal widths w_1 (empty symbols, *dashed lines*) and w_2 (filled symbols, *solid lines*) for subject F (**A**) and subject M (**B**).

measured in the coronal plane in the membranous part of the glottis (hereafter "membranous glottal width").

For the female subject F, Figure 8A shows that:

1. The membranous glottal width w_2 was larger (average size: ~2.54 mm) than the cartilaginous glottal width w_1 (average size: ~2.05 mm) both before and after the exercise.
2. For both glottal widths, the repetition variability changed: it became smaller after the exercise (0.006 mm for w_1 and 0.047 mm for w_2) than before the exercise (0.28 mm for w1 and 0.083 mm for w_2) and it was largest during the exercise (0.74 mm for w_1, 0.70 mm for w_2).
3. For both glottal widths, the repetition variability was larger than the measurement uncertainty (0.056 mm and 0.02 mm on average for w_1 and w_2) before and during the exercise but not after the exercise.
4. The changes from before to after the exercise were smaller than the repetition variability and the measurement uncertainty; thus, no significant change in glottal width was found for neither w_1 nor w_2 in subject F.

For the male subject M, Figure 8B shows that:

1. Similarly to subject F, the membranous glottal width w_2 (average size: 2.48 mm) was larger than the cartilaginous glottal width w_1 (average size: 1.63 mm) both before and after the exercise.
2. The repetition variability differed greatly: it decreased after the exercise for w_2 (0.21 mm before vs 0.03 mm after) but increased for w_1 (0.12 mm before vs 0.52 mm after).
3. The repetition variability of w_2 was larger than the measurement uncertainty (on average ±0.1 mm) before the exercise but not during and after the exercise. The repetition variability of w_1 was smaller than the measurement uncertainty (on average ±0.13 mm) before the exercise but not during and after the exercise.
4. The changes from before to after the exercise were smaller than the repetition variability or the measurement uncertainty; thus, no significant change in glottal width was found for neither w_1 nor w_2 in subject M. A decreasing trend was found for the cartilaginous width w_1 from before to after (1.87–1.99 mm vs 1.26–1.78 mm).

Vocal fold length

Figure 9 shows the changes of the membranous vocal fold lengths measured in both of the subjects as the distances between the anterior commissure (Figure 9, schematic, point 1) and vocal processes (Figure 9, schematic, points 2 and 4) in the transverse plane. The figure shows that:

1. The female vocal fold length (~6.7 mm) was about half the size of the male one (~13.5 mm).
2. In both the subjects, repetition variability (F: 0.23 mm, M: 0.45 mm, on average) was similar in size to the measurement uncertainty (female: ±0.13 mm, male: ±0.29 mm, on average).
3. There were no clear changes from before to after the exercise: the changes were smaller than both the repetition variability and the measurement uncertainty. Nevertheless, the vocal folds tended to be slightly longer (~7.5 mm in female and ~14.1 mm in male) during the exercise than before or after.

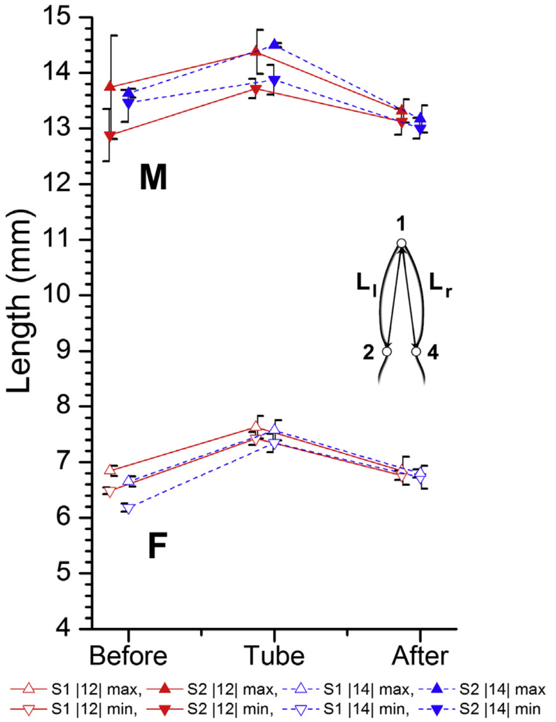

FIGURE 9. The membranous lengths of the left (L_l [|12|]; red symbols, *solid lines*) and right (L_r [|14|]; blue symbols, *dashed lines*) vocal fold. The values for the female subject F are shown as empty and for the male subject M as filled triangles. The membranous length corresponds to the distance between the anterior commissure and the vocal process. (For interpretation of references to color in this figure legend, the reader is referred to the web version of this article.)

Besides of the lengths L_l and L_r, also other vocal fold lengths were measured in the transverse plane (such as |15|, |17|, |18|, |24|, and |57| according to Figure 2), but these did not bring any further information on the vocal fold changes, and therefore are not presented here.

DISCUSSION

The first hypothesis assumed that the vocal folds become more bulged and thicker after the resonance tube exercise. This was assumed because of an increased TA muscle activity reported previously in a single-subject pilot EMG study of Laukkanen et al.[7] Such a change was not uniquely observed here. Although there was some increase of the vertical thickness at the 2-mm depth in subject F (Figure 5), who was identical to the one used in the study of Laukkanen et al,[7] this trend was not found in subject M. Subject M showed an increase of the vertical thickness and vocal fold area of the LVF but a decrease of the RVF (Figures 6 and 7B). Moreover, the measured vocal fold areas did not verifiably increase in subject F after the exercise (Figure 7A). These results therefore did not confirm the vocal folds to be more bulged and thicker after the phonation into a tube.

The second hypothesis expected the glottal width to decrease. This hypothesis was based on the finding that the phonation into a tube causes an increased resistance at lips that could result in an increased effort to produce a sound during the exercise.[5,12] Laukkanen[12] reported increased closed quotient (CQ) during and after the resonance tube exercise, which could be related to an increased adduction leading to a decreased glottal width (w_1) at the vocal processes. However, the results obtained here did not show any clear trend toward a smaller glottal width w_1 in subject F (Figure 8A)—the repetition variability observed before the exercise was much larger than the overall changes stimulated by the exercise. In subject M, there was a tendency toward smaller w_1 after the exercise, but the change was not convincing owing to high repetition variability and higher measurement uncertainty (Figure 8B). In summary, no clear changes of w_1 were observed here, but the measurement uncertainty does not allow the hypothesis to be fully rejected based on our data.

According to Titze[2,28] and Laukkanen et al,[7] the narrow constriction at the lips also increases the mean intraglottal pressure, which reduces an impact stress and tends to separate the vocal folds. This should have been projected in Figure 8, where the membranous glottal width w_2 was expected to increase after the exercise. Our data, however, do not show any such increase for neither of the subjects. On the contrary, there was some tendency for the width w_2 to decrease in subject M, although not significantly because of the repetition variability. Inconsistency of the results can also be found in the literature: Guzman et al[17] reported that CQ decreased after the exercise, which was exactly the opposite of what Laukkanen[12] had showed. Moreover, a lower glottal resistance was observed after exercising in normal voiced subjects, whereas increased resistance was found in a patient with hypofunctional voice quality (eg, Laukkanen et al[10,29]). Various trends in glottal adduction changes therefore appear to be rather usual after semioccluded voice exercises.

The third hypothesis expected no change of the vocal fold length because no change of F_0 was required. Objective measurements done previously confirmed only small changes of F_0 after the tube phonation—4 Hz decrease in subject M[17] and 15 Hz decrease in subject F.[16] Overall, the length measurements seem to be in accordance with literature: the membranous vocal fold length in the female subject F was approximately twice smaller than in the male subject M, as expected owing to the sex differences.[30,31] As hypothesized, no significant change of the vocal fold length was found in neither of the two subjects. Detailed analysis showed that in subject F, the nonsignificance was owing to the repetition variability before the exercise ($\sim$0.5 mm), which was larger than the length changes before and after the exercise. For subject M, the nonsignificance was more owing to the measurement uncertainty: the length changes before and after the exercise were of the same magnitude as the measurement uncertainty on the RVF (±0.3 mm, Figure 9A, blue) and smaller than the measurement uncertainty on the LVF (±1 mm before the exercise, Figure 9A, red). These data suggest that either the vocal fold length did not change considerably from "before" to "after," or the change was so small that it could not be detected with this measurement method. The same measurements nevertheless allowed detecting an unexpected trend of the vocal folds to be longer during the tube phonation exercise than before or after the exercise in both subjects. The reason for this trend remains to be investigated.

Although no clear uniform trends in glottal configuration adjustments were observed here from before to after the tube phonation, acoustic investigations showed changes in voice quality and increased SPLs in the same subjects after the resonance tube phonation exercises. These were already reported in related studies[16,17] and are therefore only briefly summarized here. The SPL increased for both the subjects especially in higher frequency regions (speaker's formant). For subject F, the overall change of SPL caused by change in the vocal tract dimensions was also confirmed with the simulation, which showed 3-dB increase.[16] Because the simulation used the same source signal, it indicated that an important role in the SPL increase is played by the vocal tract.[16]

Indeed, the lack of clear systematic trends in vocal fold adjustment observed here contrasts with the clearly identifiable and much more prominent changes of the vocal tract (ie, expansion of its cavities, closure of the nasopharyngeal port, etc.) visible in the same CT recordings of the same subjects as analyzed here and in their MRI recordings performed on the same occasion. These vocal tract changes were reported in the related studies of Vampola et al,[16] Guzman et al,[17] and Laukkanen et al.[32] It is known that the vocal fold behavior can be considerably influenced by the interaction with the vocal tract.[2,18,19,33] The systematic changes in voice quality and vocal fold vibration reported in these subjects in the previous studies[16,17,32] seem therefore more likely to be caused by vocal tract changes and source-filter interaction effects rather than by the laryngeal muscle adjustments.

As far as the limitations of the study are concerned, the subjects underwent the examinations in a supine position because of the CT equipment requirements. This may have caused some changes in the configuration of the phonatory apparatus.[34] Nevertheless, the subjects subjectively reported that the resonance tube exercises in supine position did not prevent them in achieving voice quality improvements similar to those achieved in the upright position. Furthermore, acoustic comparisons of the phonations in supine and upright position done in a separate study revealed similar changes of voice quality parameters (eg, formants shifts), caused by the tube phonation in both positions.[32] Logically, because supine position was used for all the phonations, similar gravitational influences on the phonatory apparatus can be expected to occur before, during, and after the tube phonation and the changes in glottal configuration after the tube phonation may be expected to be owing to other factors. These findings and considerations indicate that the supine position should not have any critical negative effect and may be used for evaluation of the tube phonation effects when upright position is not possible. Some limitation may be posed here also by the unusual geometry of the RVF in subject M mentioned in the Methods section. However, because structural asymmetry of the larynx is frequently found in normal subjects[35–39] and subject M did not report any vocal problems, the data were kept here.

The CT images of the vocal folds were obtained during sustained phonations with the acquisition time of 2 and 3.36 seconds. This caused the captured vocal fold shape to be averaged during vibration over a large number of vibratory cycles. How much were the resulting vocal fold shapes affected by the averaging process remains unclear and also poses a limitation of this study. However, because the averaging process was the same for all the conditions, it should not have any major effect on the reported comparisons before and after the tube phonations.

Only two subjects were examined in this study. This limitation is owing to radiation hazards, which do not allow examining large number of subjects and relies on volunteers, who are motivated to undergo the examination, such as the two researchers-coauthors of this study. Although examinations of more subjects would be desirable to have a better insight into the vocal fold adjustments caused by the tube phonation, this has not been targeted here for ethical reasons. However, because technological improvements of less-invasive imaging techniques (such as micro-MRI) are progressing (eg, Chen et al[40] and Delyiski & Hillman[41]), hopefully a larger study may become possible in future. A more accurate technique is requisite because the standard CT devices used here were at their limits for such precise measurements.

In conclusion, the results presented here did not show any systematic trends in the adjustment of the vocal folds that occurred after the phonation into a tube and were common to both of the subjects. Importantly, variability observed under the same conditions was mostly larger than the changes before and after the tube phonation. As such, the data indicate that the subjects did not adjust the vocal folds exactly the same way when repeating similar phonations. Such variability was seen here under all the conditions—before, during, and after the tube phonation.

There may be other factors, which were not investigated here, such as the optimum glottal width predicted from modeling studies of voice production.[42,43] These studies suggest that for an optimal voice production, the vocal folds should be neither hyperadducted nor hypoadducted, thus requiring different types of glottal adjustment for people using their vocal folds suboptimally (abduction for pressed voices vs more adduction for breathy voices). The tendency for less variability after the exercise was observed here for the vocal fold length and membranous glottal width (Figures 8 and 9); these adjustments can, however, be expected to become more apparent in subjects with voice disorders who were not targeted here. Further studies may be directed toward investigating whether the variability is smaller after the exercise than before. Although these gross glottal adductory changes are likely to be important in voice disorders,[44–46] our study together with results from other studies indicate that great accuracy of glottal configuration may not be critical: appropriate vocal tract adjustments may improve the voice quality and positively influence also the glottal behavior regardless of its exact configuration.[47,48] However, further studies with more subjects (with and without voice disorders) are needed to clarify the effects of the semi-occluded voice exercises in more detail.

CONCLUSIONS

1. No prominent uniform changes from before to after the exercise were found for vocal fold vertical thickness, bulkiness, glottal width, or vocal fold length.
2. The only significant change before and after the exercise was observed in the vertical thickness T_2 of the female (increase for both vocal folds) and vertical thickness and bulkiness of the male subject (but here the LVF and RVF showed opposite behavior).
3. The changes from before to after the exercise were of the same magnitude or smaller than the repetition variability before the exercises.
4. The measurement uncertainty was mostly smaller or similar to the repetition variability. This indicates that the lack of detection of any systematic changes in vocal fold configuration caused by the exercise is more likely owing to the inherent variability of glottal adjustment than because of the measurement uncertainty of the method used.
5. There was some tendency for smaller repetition variability after the resonance tube exercise for the vocal fold length and membranous glottal width. Study with larger number of subjects and potentially also with more precise measurement technology is needed to see whether the repetition variability decreases after the resonance tube exercises.
6. Changes in vocal tract configuration observed in previous studies after the resonance tube exercises were more prominent than the changes in vocal fold configuration observed here. Vocal tract resonance and vocal fold-vocal tract interaction phenomena therefore seem to play a more dominant role in the resonance tube exercises than the changes in vocal fold configuration.

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