How Age and Frequency Impact the Thyroid Cartilages of Professional Singers

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Summary: Objectives/Hypothesis. Young professional singers can easily reach very high pitches. In contrast, older singers often complain that they have to exert substantially more laryngopharyngeal force to reach the same high pitch compared with their earlier years. Various factors such as the property changes of the mucosa and ossification that impact the singing apparatus were suggested as explanations in the literature. The aim of this study was to analyze thyroid deformation—and thereby stiffness indirectly—during singing as a potential reason for this phenomenon.

Study Design. Prospective study.

Methods/design. We examined 44 female professional singers. High-resolution computed tomography scans were performed during singing at the fundamental mean speaking frequency and the first and second octaves above it. Digital Imaging and Communications in Medicine scan data were rendered and visualized 3-dimensionally using MIMICS software. By superimposition of the different 3-dimensional images, different positions of the thyroid were visualized. The distance from the posterior border of the thyroid was measured in all the examinations.

Results. All laryngeal cartilages could be three-dimensionally visualized. The magnitude of the thyroidal deformation significantly depends on pitch and significantly correlates with age ($r^2 = 0.7, P < 0.001$).

Conclusions. The thyroid cartilage is flexible and its formability is especially important during singing. At higher pitches, the cartilage was more deformed. The larynx in older singers showed less thyroid cartilage deformation.

Key Words: Larynx–Singer–Laryngeal cartilage–Deformation–Aging.

INTRODUCTION

Singing at high pitches depends on various parameters. These parameters depend on each other and need to be finely tuned. Some of these parameters are well explored such as subglottal pressure and vocal fold tension. It is certain that subglottal pressure and tension on the vocal folds are two major mechanisms used to control vocal pitch. Furthermore, from physics we know that sound frequency in general depends on both the diameter and the length of the resonating space. It can therefore be assumed that during singing at a higher pitch, the cross section of the vocal tract or the human resonating space equally decreases.

Although young professional singers can easily sing at very high pitches, older singers often complain about having to exert substantially more laryngopharyngeal force to reach high vocal pitches compared with their younger days. Certainly, the properties of the mucosa and humidity are two factors that gain in importance as the singer ages. It, however, remains unclear to what extent deformation of the thyroid plays a role.

Even if deformation of the larynx plays an important role, it could be more and more limited by laryngeal ossification that occurs as singers age. Various studies have assessed laryngeal ossification.

Paulsen et al first mentioned ossification as a likely explanation for the lack of flexibility of the laryngeal cartilages. From the literature, we know that ossification of the larynx starts dorsolaterally and moves ventromedially. Turkmen et al found that ossification in the thyroid starts in the posterior-inferior horn and progresses to the superior horn and central lamina. In the cricoid, it starts posteriorly and moves forward with increasing age. In general, the hyoid bone is the only structure that ossifies in the laryngeal region in persons younger than 20 years.

Using X-ray and light microscopy, Claassen et al postulated a special mode of endochondral ossification. This mode progresses very slowly and in a gender-specific manner. Compared with age-matched women, they found that bone formation in the thyroid was significantly higher in persons aged 41–60 years ($N = 118$, 80 males, 37 females). Using sonography and endoscopy, Bozzato et al found an increase in ossification beginning at the age of 50 years ($N = 101$), which was clearly more progressive in males. There are, however, substantial individual differences in how quickly and when exactly ossification of the larynx occurs. Therefore, individual progression of laryngeal ossification cannot be used for age determination.

Although many studies analyzed laryngeal ossification from various perspectives, none assessed the elasticity of the thyroid. Therefore, the aim of this study was to analyze thyroid deformation, and thereby stiffness (caused by ossification), during singing as a potential explanation for the phenomenon of the increased laryngopharyngeal force required by older singers to reach high pitches.

But what causes the reduction in the compass of a voice? Our hypothesis is that this can be attributed to ossification, which starts around the age of 20 years and significantly increases from age 50 years onward. If the compass in singing remains...
intact despite ossification, the age-related reduction of thyroid deformability could (at least partly) be compensated for by the exertion of greater force.

To be able to reach the desired high frequency, we suspect that singers compensate for the lack of laryngeal elasticity by either adapting their resonance space or creating more tension in their vocal folds. These two mechanisms and others such as moving the occipital bone backward and upward can, of course, go hand in hand.

**STUDY POPULATION AND METHODS**

**Study population**

We used information and high-resolution computed tomography (HRCT) scans of 44 female professional singers recruited in the context of a larger study on laryngeal findings in professional singers. Of the 44 singers, 22 were sopranos and 22 were altos. All were active either as soloists or members of a professional vocal ensemble. The mean age of the sopranos was 44 (range 29–69; median 42) years and 39 (range 28–62; median 38) years for the altos. None of the singers reported having had any voice-related problems in the past 5 years. All singers said that they used two different mechanisms for pitch control: one for the lower range (M1) and one for the upper range (M2). The singers were asked at which pitch they usually changed from M1 to M2 and to state the highest note that they could sing in M1. Exclusion criteria were pregnancy (because they had to undergo HRCT), a “Singing Voice Handicap Index” of more than 17 points, or hidden laryngeal pathologies revealed by videolaryngostroboscopy. The study was approved by the Medical Ethics Committee of Zurich (Switzerland). The statistical analysis was performed with MATLAB and Statistics and Machine Learning Toolbox R2016b. The paired sample t test, Wilcoxon signed rank test, and Bonferroni correction for multiple comparisons were applied where appropriate. We report tests as significant if both comparisons of the mean and median are simultaneously significant and report P values for the less stringent test. For the graphical representation, we relied on the medians with their bootstrapped 95% confidence intervals by 2000-fold resampling, revealing better statistical asymmetries.

**HRCT imaging**

All singers were asked to sing an open Italian /a/ (as in “Caro mio ben” by Giuseppe Giordani) at a comfortable mezzo-forte level and keep this level in mind. In absolute figures, this level varied between 75 and 85 dB. We preferred to let each singer determine the loudness for which she was comfortable rather than prescribing a fixed loudness. We assumed that this approach would facilitate maintaining the same loudness throughout the examination. Each singer then underwent three HRCT scans. To assess the extent of the lower range (M1), all singers sang the highest possible /a/ in the lower range. They also sang at the same /a/ frequency when they switched into the upper range (M2). All singers sang the /a/ at the same loudness as their mean speaking fundamental frequency (F0, calculated using DIVAS software, XION Medical, Berlin, Germany), one octave (F1) and finally two octaves (F2) above F0 separately. All singers were able to sing at F0, F1, and F2. Scans were begun 2 cm below the glottis, with the first scan going up to the hard palate and the second and third scans only as far as the superior horn of the thyroid. The first scan went higher because it was also used for further studies.

A clinical multi-slice computed tomography scanner (Siemens Definition AS 64, Siemens Healthcare, Erlangen, Germany) was used with the following settings: slice thickness 1 mm, pitch 0.8, increment 1 mm, rotation time 1 second, and maximal voltage and tube current 120 kVp and 150 mA (total radiation dosage for all three scans: 2.1 mSv). The scans were performed using a high-resolution technique with the participant in the supine position. The image acquisition time was 10–12 seconds for the first scan and 7–9 seconds each for the second and third scans. No singer experienced any difficulty maintaining a steady tone for this time.

**Post-process imaging**

The Digital Imaging and Communications in Medicine data were post-processed with the segmentation software MIMICS (Version 14.0, Materialise, Leuven, Belgium). The HRCT scans were first segmented into cartilages, then transformed into 3-dimensional models. To analyze the motion of the laryngeal cartilage during singing, we superimposed the 3-dimensionally rendered and reconstructed HRCT scans of the three acquisitions obtained at F0, F1, and F2. To determine how elastic the larynges are, we measured the distance between the posterior border of the thyroid cartilage on four defined landmarks at F0, F1, and F2: level 1 was the cricothyroid joint (CTJ); level 2 was the vocal folds; level 3 was the laryngeal prominence; and level 4 was the upper end of the superior horn of the thyroid cartilage (Figure 1). Because the upper horns are the highest landmark of the thyroid, they experience the greatest movement from any medial-lateral larynx deformation. Therefore,
the narrowing of the thyroid is greatest at the upper horns. Hence, the superior horns of the thyroid cartilage were chosen over the other three landmarks (prominentia laryngis, vocal folds, and CTJ) as an indicator of deformability.

RESULTS

Visualization and 3-dimensional rendering of the laryngeal cartilages
The first step of the HRCT analysis was to segment all laryngeal cartilage skeletons. In all scans, the laryngeal cartilages could be visualized and rendered in three dimensions. The corresponding 3-dimensional images obtained at F0, F1, and F2 could be superposed (Figure 1 and Figure 2).

Voice assessment
The mean F0 of all singers was 199 Hz (range 165–238 Hz, standard deviation [SD] 18.31 Hz); the mean F1 was 397 Hz (range 330–475 Hz, SD 36.36 Hz); and the mean at F2 was 597 Hz (range 495–713 Hz, SD 54.76 Hz). As noted, all singers were able to sing at F0, F1, and F2. To validate clustering the data for the three frequencies, we simultaneously compared the medians and means of F0–F2. The three frequencies separated well, and the medians and means monotonically increased at a significance of $P < 10^{-8}$ (Figures 3 and 4).

Laryngeal deformability in comparison of frequency
In a first step, we analyzed the laryngeal deformability in comparison with frequency in groups, sopranos, and altos. There was no statistically significant difference. Therefore, we pooled both groups together. At level 4 (upper end of the superior horns of the thyroid), the mean distance was 35.98 mm (range 27.14–44.98 mm, SD 3.80 mm) at F0, 35.11 mm (range 25.13–44.81 mm, SD 4.26 mm) at F1, and 30.83 mm (range 22.00–42.56 mm, SD 3.98 mm) at F2. A comparison of the means and medians showed a statistically significant monotonic decrease with increasing frequency at a significance level of $P < 0.0303$ (Figures 2).

At level 3 (laryngeal prominence), the mean distance was 35.68 mm (range 29.51–42.46 mm, SD 2.60 mm) at F0, 35.25 mm (range 29.57–43.12 mm, SD 2.82 mm) at F1, and 32.37 mm (range 25.36–39.59 mm, SD 3.46 mm) at F2.

At level 2 (vocal folds), the mean distance was 32.23 mm (range 26.27–36.65 mm, SD 2.38 mm) at F0, 32.39 (range 28.11–38.91 mm, SD 2.36 mm) at F1, and 30.59 mm (range 25.13–37.02 mm, SD 2.58 mm) at F2.

The comparison of the means and medians at levels 2 and 3 showed a slightly different picture. There was no significant change between the distances measured for F0 and F1, but the distance at F2 was significantly lower at a level of $P < 10^{-6}$ for level 3 and $P < 10^{-4}$ for level 2, respectively.

At level 1 (CTJ), the mean distance was 23.85 mm (range 20.89–26.65 mm, SD 1.47 mm) at F0, 24.39 mm (range 20.95–27.64 mm, SD 1.69 mm) at F1, and 24.07 mm (range 20.54–27.40 mm, SD 1.68 mm) at F2 (Figure 2).

The comparison of the means and medians showed that there was a significant increase in the distances measured between F0 and F1, and a significant decrease to F2 at a level of $P < 0.01$, but the distances at F0 and F2 were not statistically different.

Laryngeal deformability comparison by age
As shown in Figure 5 by linear regression analysis, there is a strong correlation ($R^2 = 0.7$, $P < 10^{-12}$) between age and relative change from distance at F0 to distance at F2 at level 4, that

FIGURE 2. Left: comparison (rear view) of larynges of a 29- and 61-year-old singer at F0. Right: superposed thyroids F2 (blue) over F0 (beige). The thyroid of the 29-year-old singer (top) shows greater deformability compared with the 61-year-old’s thyroid (bottom). For better comparability, the cricoid was depicted as stable and non-tilted.

FIGURE 3. Distance (mm) between the superior horns of the thyroid cartilage (y-axis) versus frequency (Hz) (x-axis). After doubling the frequency from F0 to F1, the mean distance between the tips of the upper horns decreases by 0.9 mm. After doubling the frequency from F1 to F2, however, the mean distance decreases substantially by 4.3 mm.

FIGURE 4. Superior Horn Position vs. Frequency

Mean
95%-CI
Distance
Frequency

f(F₀) < f(F₁) < f(F₂), $p<10^{-8}$
d(F₀) > d(F₁) > d(F₂), $p<0.0303$
is, the relative change decreases with age. We present only the pooled alts and sopranos data in the figure because a comparison between the group individual linear regressions did not reveal neither a statistical difference by means of a t test for the slopes \((P = 0.37)\) nor by means of the cocor test \((P = 0.32)\) for the Pearson correlation coefficient \(R\). We then further pooled the data into groups to see whether this trend would show up statistically significant difference for the group statistics particularly for the data of 33 singers aged below 47 that we pooled in equal-sized groups G1 \((<37\text{ years}, 16\text{ singers }[\text{mean } 31.7\text{ years, SD } 2.6])\) and G2 \((>37\text{ and }<47\text{ years}, 17\text{ singers }[\text{mean } 41.5\text{ years, SD } 2.8])\) yielding to two groups having roughly their age mean 10 years apart. As can be seen in the figure, we have a gap of subjects around this age, and the relative change data have a natural trend to floor at 0\% with increasing age. So the remaining subjects constitute group G3 \((\geq 47\text{ years}, 11\text{ singers }[\text{mean } 55.7\text{ years, SD } 6.8])\). These three groups differed significantly in terms of laryngeal flexibility. Whereas G3 experienced a mean relative change in reduction of the distance between the upper horns of only 5\%, G2 achieved 15\% and G1 up to 20.1\%. The monotonic decreases in the relative change from group G1–G2, and group G2–G3 are highly significant \((P < 0.001)\).

Also, here we analyzed first the laryngeal deformability in the comparison of age group sopranos and alts. There was no significant difference, too. Therefore, we pooled both groups together. We could divide the singers into three significantly different age groups: G1 \((<37\text{ years}, 16\text{ singers }[\text{mean } 31.7\text{ years, SD } 2.6])\); G2 \((>37\text{ and }<47\text{ years}, 17\text{ singers }[\text{mean } 41.5\text{ years, SD } 2.8])\); and G3 \((\geq 47\text{ years}, 11\text{ singers }[\text{mean } 55.7\text{ years, SD } 6.8])\). These three groups differed significantly in terms of laryngeal flexibility. Whereas G3 experienced a mean relative change in reduction of the distance between the upper horns of only 5\%, G2 achieved 15\% and G1 up to 20.1\%. Figure 5 shows that there is a strong correlation \((R^2 = 0.7, P < 10^{-12})\) between age and relative change from distance at F0 to distance at F3 at level 4, that is, the relative change decreases with age. The monotonic decrease in the relative change from group G1–G2 is highly significant \((P < 0.001)\).

**DISCUSSION**

As for a musical instrument, a generator, a sound source, and a resonator are needed by the human voice to create a sound. Pitch can be influenced by various parameters. Whereas one changes the pitch of a wind instrument by acting on flaps and valves, the pitch of the human voice depends on subglottal pressure,1 vocal fold tension, and both elongation and shape changes in the resonant space.22627 Vocal frequency can be adjusted through both shortening of the resonant cavity and reduction of its diameter.2627 Reductions in diameter, however, require a deformable wall. The diameter reduction is achieved by muscular contraction of the pharynx. To our knowledge, the extent to which the thyroid is deformed during singing has not been assessed. Therefore, this is the first study to describe this phenomenon.

Based on our data, we show that the thyroid undergoes deformation as soon as the singer sings at higher pitches. The phenomenon that vocal pitch can be adjusted through changes in the resonant space can be compared with wind instruments.28 The smaller the resonant space of an instrument, the higher its sound, and vice versa.26 This comparison is helpful for understanding certain aspects of the human voice. Through the ability of the human voice to create sounds in the resonance space, however, it is a lot more diverse and sophisticated. To resonate,29 the larynx must be both flexible and have a certain deformability. We, therefore, in line with Ishikii et al, who also stated that the human voice depends on certain properties of the tissue such as pliability or mobility of the vocal folds to create certain voice characteristics. Therefore, our results enable us to postulate that a flexible thyroid cartilage is beneficial for singers to control their singing voice. We are aware that the effect of laryngeal elasticity could be overestimated. Instruments, however, cannot be compared 1:1 with the human voice. We, therefore, will not follow-up on this idea.

Yan et al showed that sopranos have significantly shorter oral length and total vocal tract length, smaller oral volume, and total vocal tract volume than mezzo-sopranos. They found no significant difference in pharyngeal length and pharyngeal volume of female singers.27 We can assume that the resonant space is shortened or elongated by lowering and elevating the larynx, and that its diameter is decreased and increased by contracting and relaxing the M. constrictor pharyngis (MCP). Because this muscle inserts at the thyroid, it is obvious that the contraction also leads to a thyroid deformation. Thereby, it is likely that singers can reduce the diameter of the resonance space.

Our results show that the thyroid cartilage is slightly deformed during singing from F0 to F1 (first octave), whereas it is substantially deformed from F1 to F2 (second octave) (Figure 2). These observations are in line with our observation3 that the first octave is primarily achieved by contracting the cricothyroid muscle. Within the second octave, subglottal pressure conditions increase, and the thyroarytenoid muscle is mainly active.5 According to Bernoulli’s law, the wall of the resonance space must be stabilized or stiffened during that phase given the increased speed of the airflow through the vocal folds. At the same time, further deformation can be observed, most likely through increased pharyngeal muscle contraction (M. constrictor pharyngis inferior [MCPI]). We have furthermore observed that during singing from F0 to F1, a slight widening of the distance from the lowest part of the thyroid cartilage (inferior horns) can be observed mainly in the lower section of the thyroid (Figure 4). This can be explained by a smooth contraction of the upper part of the MCPI, leading to a deformation of the upper part of the thyroid cartilage. This mechanism leads to a leverage effect in the lower part of the thyroid, resulting in a slight widening of the lowest part of the thyroid cartilage (inferior horn of the thyroid). It is only in the second phase (F1–F2) that the entire MCPI contracts, also resulting in deformation of the lower part of the thyroid cartilage. Our results not only show that the deformability is greatest at the height of the upper horns, but that there is a significant reduction in the deformability with increasing age. Our results show a high correlation \((r^2 = 0.7)\) of the relative changes \((\%)\) of the superior horn with age (Figure 5).

In general, we can state that as the singer ages, the thyroid allows for less deformation. This observation can certainly be attributed to increasing calcification and ossification. A handful
of studies\textsuperscript{3,5–11} have investigated this age-related process. From these studies, we know that laryngeal ossification starts dorsolaterally and moves ventromedially. From age 50 years, it is more progressed in male larynges,\textsuperscript{13} and Paulsen et al\textsuperscript{4} first mentioned ossification as a likely explanation for the lack of laryngeal flexibility. Vocal aging, especially in women, has been widely investigated in the literature.\textsuperscript{30} Anatomic and physiological changes, perceptual changes, and loss of vocal range characterize the aging voice. It is also well known that changes in the muscles, connective tissues, and gland cartilages contribute to these changes.\textsuperscript{31}

Therefore, these results support our hypothesis that a flexible larynx is beneficial for a well-functioning singing voice. Or, in other words, a stiff thyroid cartilage can hinder the fine-coordination of the vocal tract in controlling the singing voice. The singer therefore has to compensate by using other muscular techniques when singing at high pitches, which can lead to uncomfortable sensations in the throat.

\begin{figure}
\centering
\includegraphics[width=\textwidth]{figure4}
\caption{Results of the four levels (thyroid upper horn, laryngeal prominence, vocal folds, and CTJ) at F0 (blue), F1 (red), and F2 (yellow).
Levels 4 and 3: a slight decrease in the laryngeal edge distance is visible from F0 to F1, and a substantial decrease from F1 to F2. Level 2: an increase is visible from F0 to F1 and a substantial decrease from F1 to F2. Level 1: a greater increase from F0 to F1 is visible, with just a slight decrease from F1 to F2.}
\end{figure}

\begin{figure}
\centering
\includegraphics[width=\textwidth]{figure5}
\caption{Correlation between the relative changes (%) of the superior horn of the thyroid cartilage and the age of the singers from F0 to F2. The singers were divided into three age groups (green <37 years, blue between 37 and 47 years, and red \geq 47 years). There is a strong correlation ($r^2 = 0.7$) between age and relative change. The older the singer, the less deformation of the thyroid was possible.}
\end{figure}
Summarizing, the larynx is deformed during singing. The deformability of the larynx is significantly reduced during aging. This could explain why singers often complain about having to make a greater effort to reach higher pitches while singing. Besides reduced laryngeal deformability, however, certainly other parameters are also responsible for this phenomenon.

Our study has some limitations. Because it was done in the framework of a larger study, we examined larynges of female professional singers only. Whether our conclusions can also be applied to male singers must first be proven in further studies. In conclusion, we show that the thyroid cartilage is a flexible organ, which is especially important during singing. The higher the pitch, the greater the deformation of the thyroid. The older the larynx, the less the thyroid cartilage is deformed. Therefore, we postulate that a flexible thyroid cartilage is beneficial for controlling the singing voice.

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