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# Control of the glottal configuration in ex vivo human models: quantitative anatomy for clinical and experimental practices

Aude Lagier<sup>1,2,3</sup>, Daphné Guenoun<sup>1</sup>, Thierry Legou<sup>2</sup>, Robert Espesser<sup>2</sup>,  
Antoine Giovanni<sup>2,3</sup>, Pierre Champsaur<sup>1</sup>

## Abstract

*Introduction* The objective of this paper was to identify the determining factors of the glottal prephonatory configuration from the point of view of the resulting muscular actions (i.e., arytenoids adduction, membranous vocal fold adduction, and tension).

*Materials and methods* 21 human non-embalmed excised larynges (12 females and 9 males) were studied. Experiment A (11 larynges) studied four conditions of adduction of the vocal folds and arytenoids. Experiment B (10 larynges) studied the effect of cricothyroid approximation on the vocal fold length and the cricothyroid angle.

*Results* Experiment A: The mean glottal area significantly decreased from 41.2 mm<sup>2</sup> mean with no adduction, to 10.2 mm<sup>2</sup> mean with arytenoid adduction, to 9.2 mm<sup>2</sup> with membranous vocal fold adduction, and down to 1.1 mm<sup>2</sup> with the combination of arytenoid and membranous adduction. The effect of the task was statistically significant. Experiment B: The length of vocal folds increased from 13.61 mm median to 14.48 mm median, and the cricothyroid angle decreased of 10.05 median along with cricothyroid approximation.

*Discussion* The results of experiment A emphasize the sub-division of adductor intrinsic muscles in arytenoids adductors (i.e., LCA and IA), and membranous vocal fold adductor (i.e., TA). The results of experiment B quantify the effect of cricothyroid approximation on the vocal folds length. The implications of these results can be useful in both clinical practice and experimental studies.

**Keywords** Larynx · Glottis · Vocal folds adduction · Phonation control · Voice

## Introduction

The human larynx is a complex structure of several cartilages mobilized by small muscles called intrinsic laryngeal muscles. The movements of the vocal folds mainly result of the mobilization of a set of two complex joints (i.e., the cricoarytenoid joints), under the action of the intrinsic laryngeal muscles. Prades et al. studied the biomechanics of the cricoarytenoid joint and showed that the adduction of the vocal process (to close the glottis) combines anterior tilt, superomedial shift, and a lesser degree of internal rotation of the cricoarytenoid joint [17]. Understanding the role of the intrinsic muscles in vocal folds movements is of interest in physiological and clinical fields. Currently, three groups of intrinsic laryngeal muscles are defined: adductors for vocal fold closing, abductors for vocal fold opening, and lengtheners for increasing vocal folds length. The latter group also induces vocal fold tension, especially when they contract simultaneously with muscles that shorten the vocal folds [16]. Determining the role of each laryngeal muscle is possible in excised larynges [4], and the muscular actions are consistent with the cricoarytenoid joint degrees of freedom: the anterior–posterior translation

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✉ Aude Lagier  
aude.lagier@univ-amu.fr

<sup>1</sup> Laboratoire d'Anatomie, Faculté de médecine de Marseille, Aix-Marseille Université, 27 Bd Jean Moulin, 13385 Marseille Cedex 05, France

<sup>2</sup> Laboratoire Parole et Langage, UMR CNRS-Aix-Marseille Université, 7309 Aix-en-Provence, France

<sup>3</sup> Service d'ORL et Chirurgie Cervico-Faciale, CHU La Conception, AP-HM, Marseille, France

is the largest movement; medial–lateral and vertical glidings are less important [4]. During phonation, EMG studies showed that all adductor muscles (thyroarytenoid—TA, lateral cricoarytenoid—LCA, arytenoid—IA) are active. Cricothyroid—CT and TA tend to co-contract during pitch elevation, most likely stiffening the vocal fold due to their opposite actions of lengthening and shortening the vocal fold [16, 20]. In summary, studies on excised larynges trying to imitate the isolated action of each intrinsic muscle may not accurately predict the biomechanical effect of intact, normal human intrinsic muscles, because the co-contraction of the different muscles may lead to different resultant movements.

From the point of view of the resultant movement, the prephonatory glottal configuration is the key point. The prephonatory glottal configuration is determined by the degree of adduction of the folds and by the viscoelasticity of the vocal folds tissues. The three viscoelastic-related physical properties are mass, stiffness, and viscosity [13]. Adduction and viscoelasticity of the vocal folds are very important in the quality of the voice, in the control of the fundamental frequency, and in the relationship between fundamental frequency and subglottal pressure [2, 13]. Improving the prephonatory glottal configuration is the main objective in the surgery of unilateral laryngeal paralysis: the better the adduction, the better the voice quality [10].

The objective of this paper was to identify the determining factors of the glottal prephonatory configuration from the point of view of the resulting muscular actions (i.e., arytenoids adduction, membranous vocal fold adduction, and tension) instead of the action of each intrinsic muscle.

## Materials and methods

### Larynges

Twenty-one human larynges (12 females and 9 males) were harvested from fresh cadavers, within 24–48 h after death, according to French law. At the time of death, the age of the persons ranged from 71 to 92 years. No history of voice disorders, thoracic or cervical cancer or surgery was known for any subject, and no abnormalities were observed on the larynges. Larynges were rapidly frozen in 0.9 % saline. They were thawed overnight in saline solution and cleaned with removal of the extraneous tissues and supraglottic structures (epiglottis and vestibular folds) before the experiment, according to the literature [1].

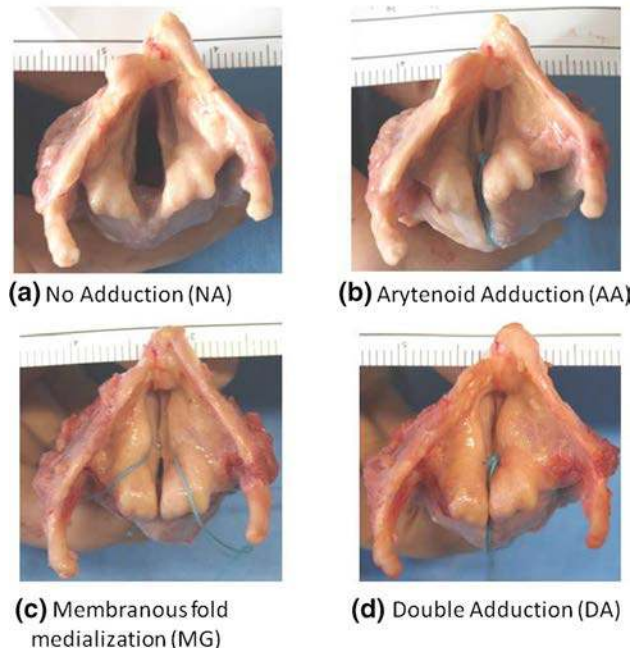
## Experiments

### Experiment A: adduction

Eleven larynges (7 females and 4 males) were tested in the first part of the experiment, dedicated to the adduction.

Each larynx was tested in the four following conditions in a random order (Fig. 1):

- No adduction (NA) was the spontaneous position of the vocal folds
- Arytenoid adduction (AA): the action of adductor muscles of the arytenoid process (i.e., lateral cricoarytenoid and inter-arytenoid muscles) was reproduced: the maximal adduction was obtained with a stitch between the vocal processes of the arytenoid cartilages.
- Vocal fold medialization with Montgomery implant (MG): the action of adductor muscles of the membranous vocal fold (i.e., thyroarytenoid muscle) was reproduced according to type I thyroplasty [11, 12] bilaterally performed with two montgomery implants [15] inserted through bilateral windows into the thyroid ala.
- Double adduction (DA): combination of arytenoid adduction and Montgomery implants.



**Fig. 1** Superior view of the larynx in experiment A: four conditions of adduction

## Experiment B: vocal fold lengthening

Ten larynges (5 females and 5 males) were tested in the second part, testing the lengthening of vocal folds. In this experiment, all the larynges were set in double adduction.

The resultant action of the tensor muscles (i.e., cricothyroid muscles) was reproduced with cricothyroid approximation. A wire was attached on the midline, from the arch of the cricoid cartilage to the inferior edge of the thyroid cartilage (Fig. 2). The wire was connected to a micrometer and the approximation stepped 1 mm to 1 mm.

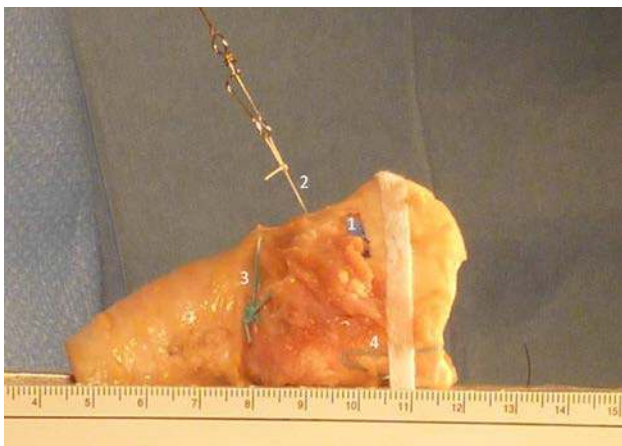
A wire was placed on the external aspect of the cricoid and another one on the thyroid cartilage as markers for measures and comparison of cricothyroid angle when cricoid and thyroid approximated. Due to the variability of the insertions of these two wires, only the variations of the cricothyroid angle were measured.

The length of the vocal folds was measured from the end of the vocal process to the anterior commissure for each level of cricothyroid approximation.

### Measures

Two cameras were placed orthogonally around the larynx. The first one (Canon® 600D, lens EFS 18–135 mm) was in the axis of the larynx, at 50-cm distance to take pictures of the superior aspect of the vocal folds. The second one (Panasonic® Lumix F535) was on the left side of the larynx, at 50-cm distance to take pictures of the CT angle.

The open-access software Image J (<http://rsb.info.nih.gov/ij/>) was used for the measurement of glottal area in experiment A, and of the vocal fold length and cricothyroid angle in experiment B was performed using. Each picture was taken with a measurer to calibrate the pixels/millimeter ratio.



**Fig. 2** Left lateral view of the larynx set for experiment B. (1) Montgomery implant, (2) wire responsible for cricothyroid approximation, (3) cricoid wire for CT angle measurement, and (4) thyroid wire for CT angle measurement

In experiment A, the mean of three measures of the glottal area was calculated for each larynx in the four conditions. In experiment B, the mean of three measures of CT angle and of vocal folds length was calculated for each larynx in the 25 conditions.

### Statistical analysis

The descriptive statistics are expressed as median, minimum, and maximum.

The analyses were conducted using linear mixed models to account for the grouping structure (by larynx) of the data. Package lme4 [3] was used in R software [18].  $P$  values were computed using a parametric bootstrap procedure [7] and noted as  $P_{boot}$ ; the significance of statistical threshold was set at 0.05.

## Results

### Experiment A: adduction

The mean glottal area significantly decreased from 41.2 mm<sup>2</sup> mean with no adduction, to 10.2 mm<sup>2</sup> mean with arytenoid adduction, to 9.2 mm<sup>2</sup> with membranous vocal fold adduction, and down to 1.1 mm<sup>2</sup> with the combination of arytenoid and membranous adduction.

The dependent variable was the log-transformed glottal area. The log-transformation enhanced the normality of the distribution of the residuals of the model. The predictors were the condition (NA, AA, MG, and DA), the sex and their interactions. A random intercept was added to account for the variability across the 11 larynges. Forty observations were available (the log-transformation implied that four null area were removed from the analysis). The interaction between condition and sex was not significant ( $P_{boot} = 0.92$ ), and it was removed from the final model.

The comparisons of interest needed the estimation of two variants of the model, and consequently, the significance threshold was Bonferroni-corrected at  $P < 0.025$ . The results showed that the area for the AA condition was significantly smaller than for the NA condition ( $\beta = -1.508$ ,  $t = -4.55$ ,  $P_{boot} < 0.001$ ); the median decreased by 22 %; the area for the AA and MG conditions could not be distinguished ( $\beta = -0.275$ ,  $t = -0.83$ ,  $P_{boot} = 0.44$ ); the area for the DA condition was significantly smaller than for the MG condition ( $\beta = -1.412$ ,  $t = -3.75$ ,  $P_{boot} < 0.001$ ); the median decreased by 25 %; the area for the DA condition was also significantly smaller than for the AA condition ( $\beta = -1.686$ ,  $t = -4.49$ ,  $P_{boot} < 0.001$ ); the median decreased by 19 %. As the interaction with sex was not significant, this configuration was the same for both male and female, the area for male

being greater than for female by a factor 2.3 ( $\beta = 0.834$ ,  $t = -3.30$ ,  $P_{\text{boot}} = 0.002$ ). Figure 3 shows the predicted area by the model and the boxplots of the observed length on the logarithm scale.

### Experiment B: lengthening

The length of vocal folds increased along with the cricothyroid approximation from 13.61 mm median (min 8.46 mm; max 16.09 mm) up to 14.48 mm median (min 9.10 mm; max 17.65 mm). The lengthening of the vocal folds was 11.6 % (min 4.9 %; max 27.6 %). The maximum vocal fold length was achieved for a median of 22 mm of CT approximation (min 16 mm, max 25 mm)

Examination of the data suggested that the evolution of the length of the vocal folds would be best modeled with a quadratic function of the CT approximation.

The dependent variable was the vocal folds length. The predictors were the CT approximation (linear and quadratic component), the sex and their interactions. A random intercept and random slopes for each component of the CT approximation were added to account for the variability across the ten larynges. All the random terms were uncorrelated. Two hundred and thirty-four observations were available.

The interaction between sex and CT approximation was not significant (Likelihood ratio test with KR approximation:  $F_{\text{test}} = 0.044$ ,  $df = 2$ ,  $p = 0.95$ ). This interaction was removed from the final model. The results showed that the quadratic term ( $\beta = -0.00033$ ,  $t = -5.5$ ,  $P_{\text{boot}} < 0.001$ ) and the linear term ( $\beta = 0.13$ ,  $t = 6.86$ ,  $P_{\text{boot}} < 0.001$ ) were significant. As expected the VF length was

significantly longer for the males ( $\beta = 4.02$ ,  $t = 4.02$ ,  $P_{\text{boot}} < 0.001$ ). Figure 4 shows the predicted length by the model and the boxplots of the observed lengths.

The cricothyroid angle (CT angle) decreased of  $10.05^\circ$  median (min  $-5.42^\circ$ , max  $-14.25^\circ$ ) along with cricothyroid approximation. The maximum decrease in CT angle was achieved for a median of 22 mm of CT approximation (min 16 mm, max 25 mm).

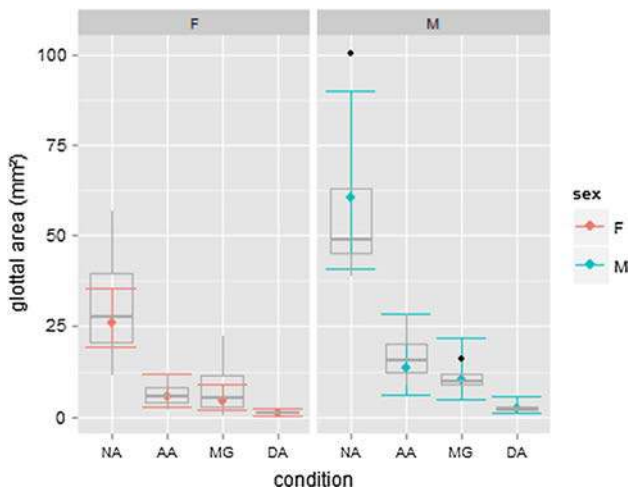
Examination of the data suggested that the evolution of the CT angle would be best modeled with a quadratic function of the CT approximation.

The variable dependent was the CT angle. The predictors were the CT approximation (linear and quadratic component), the sex and their interactions. A random intercept and random slopes for each component of the CT approximation were added to account for the variability across the ten larynges. All the random terms were uncorrelated. Two hundred and forty-six observations were available.

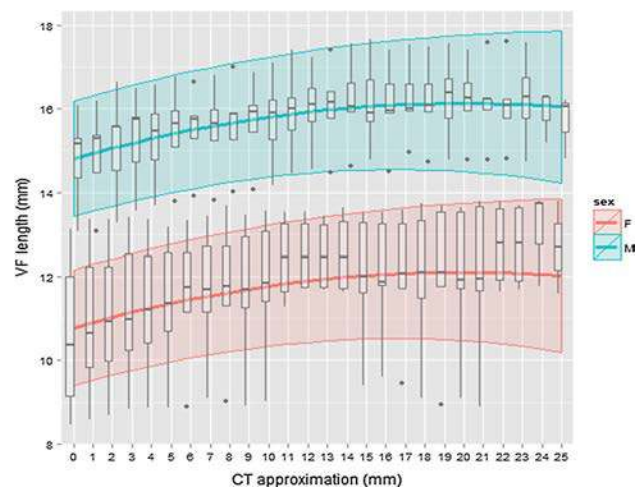
The interaction between sex and CT approximation was not significant ( $P_{\text{boot}} = 0.92$ ). This interaction was removed from the final model. The results showed that the quadratic term ( $\beta = 0.0115$ ,  $t = 4.63$ ,  $P_{\text{boot}} < 0.001$ ) and the linear term ( $\beta = -0.697$ ,  $t = -10.12$ ,  $P_{\text{boot}} < 0.001$ ) were significant. The predictor sex was not significant ( $\beta = -0.912$ ,  $t = -1.41$ ,  $P_{\text{boot}} = 0.52$ ). Figure 5 shows the boxplots of the observed angles and the angles predicted by the model.

### Discussion

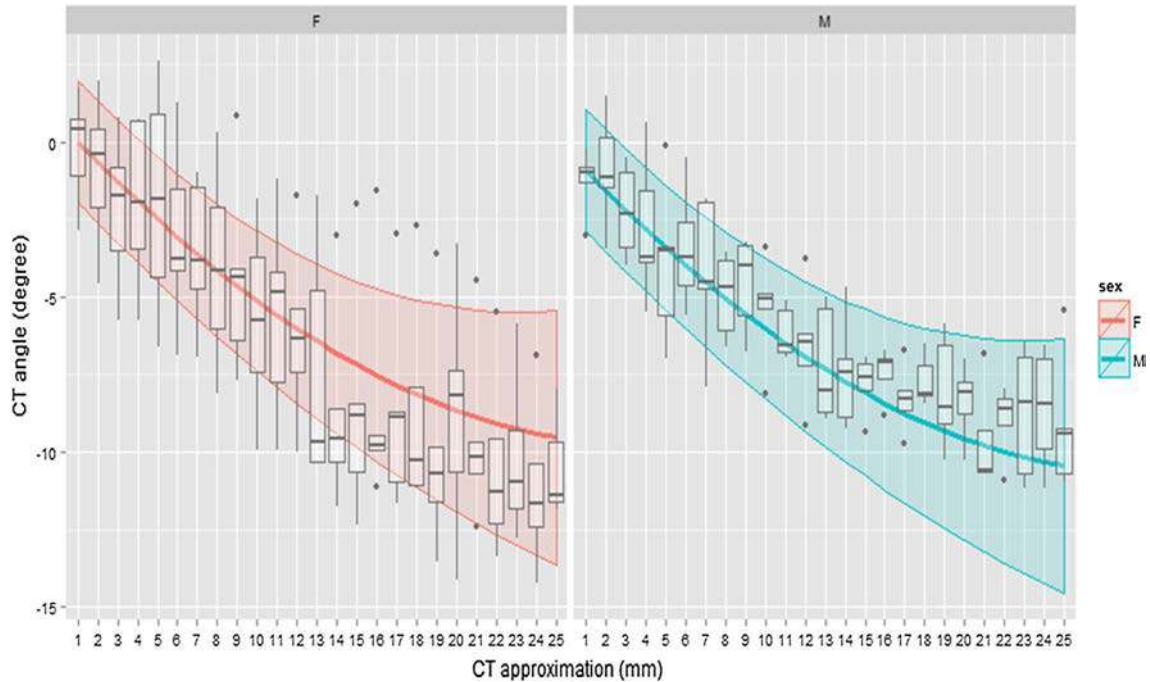
The results of experiment A emphasize the subdivision of the group of adductor intrinsic muscles in arytenoids adductors (i.e., LCA and IA), and membranous vocal fold



**Fig. 3** Glottal area and the four conditions of adduction (NA no adduction, AA arytenoid adduction, MG membranous fold medialization, DA double adduction). The boxplots show the observed data, the colored point the area predicted by the model (with the fixed effects only). The colored lines indicate the 95 % confidence interval (color figure online)



**Fig. 4** Vocal folds length according to the CT approximation and the sex. The boxplots show the observed data, the colored lines the length predicted by the model (with the fixed effects only). The colored bands indicate the 95 % confidence interval (color figure online)



**Fig. 5** CT angle according the CT approximation and the sex. The boxplots show the observed data, the *colored lines* the angle predicted by the model (with the fixed effects only). The *colored bands* indicate the 95 % confidence interval (color figure online)

adductor (i.e., TA). The results showed that the reproduction of the TA adduction (with the Montgomery implant) is necessary to bring the vocal fold its volume back.

These results are interesting for the methodology of physiological studies on excised larynges as they emphasize the consequences of the different techniques of adduction: the reproduction of the actions of both arytenoid and vocal fold adductors (double adduction) leads to the narrower glottal area. This double adduction is also nearer to the reality, as shown in the *in vivo* literature based on electromyographic studies. Electromyographic routine uses some laryngeal gestures to test the validity of proper electrode placement: activation of TA increases during sustained vowel; LCA activation is a burst at onset and offset of phonation but not a sustained activation during prolonged phonation; the CT activation increases during the high end of a pitch glide [9, 16].

This study aimed at creating more physiological prephonatory glottal configuration, but not at achieving the very precise control used in real voice: *in vivo*, glottal configuration may differ with the phonetic context [16], but also with the expressive intention or with the different singing modes [6].

The results of experiment B clearly showed direct action of cricothyroid approximation on the vocal fold length. *In vivo* canine experiments with stimulation of the laryngeal superior nerve found similar results, but the lengthening was much higher in dogs with a maximum range of length change 44.7 % mean [19] vs 11.6 % mean in this study.

The choice of the range of the CT approximation (0–25 mm) was arbitrarily chosen. The parabolic aspect of the curves highlights that this range was probably too large, and the thyroid could not continue its course toward the cricoid, because they were in contact.

The two main limits of the study are the low number of larynges and the inability to reproduce the TA isometric contraction. The number of larynges studied was counter-balanced by the high number of measures and the intra-larynx and inter-larynx reproducibility of responses to the tasks in the two experiments.

The design of the study, based on excised larynges, could not reproduce isometric longitudinal contraction of the TA. According to the cover-body theory of vocal folds vibration [8], physical quantities of the vocal folds, such as length, mass, and stiffness, are the physical quantities that determine the control of fundamental frequency. These quantities depend on the complex co-activations of CT and TA muscles. CT activation is always associated with an increase in fundamental frequency. For given levels of CT activation, the effects of TA activation on F0 are different [14]. Simulations on 3D numerical models of vocal folds show that these co-contractions impact the vocal fold morphology and the stress distribution, and must be one of the major determinants in the effective depth of tissue vibration [5]. However, this component of vocal fold physiology could not be assessed here, and the role of the TA in the control of vocal fold stiffness could not be properly studied.

The results presented above are of clinical relevance: the surgical management of unilateral laryngeal paralysis (excluding re-innervation techniques) can propose injection laryngoplasty, medialization laryngoplasty (with montgomery implant or others), and finally the association of adduction arytenopexy with medialization laryngoplasty as described by Zeitels et al. It is noteworthy that adduction arytenoidopexy is always associated with membranous vocal fold medialization [21], and is logically performed when the vocal improvement of the vocal fold medialization is not sufficient.

The excised larynx is a very convenient tool to teach the classical surgical techniques on the larynx, but also for the tests and comparisons of different techniques of laryngeal surgery [10, 11]. Hoffman et al. compared the different surgical techniques for treatment of unilateral laryngeal paralysis in canine excised larynges, and concluded that the association of medialization laryngoplasty (i.e., with montgomery implants) and arytenoid adduction leads to the greatest improvement in phonatory parameters. The medialization laryngoplasty alone did not create the arytenoid adduction. They also showed that, contrary to injection laryngoplasty, medialization laryngoplasty did not cause detrimental changes to the mucosal wave [10].

In conclusion, the results of this study emphasize that the adductor group of intrinsic laryngeal muscles are functionally splitted in arytenoid and membranous vocal fold adductors. This has clinical implications in the choice of the surgical technique for unilateral laryngeal paralysis, but also for the methodology of experiments in vocal physiology on excised larynges. The action of the cricothyroid muscle on the vocal fold length was confirmed, but some significant difference in the range of the lengthening has been highlighted in comparison to animal studies. The main limit of the study was the inability to reproduce the TA longitudinal isometric contraction and, therefore, the inability to reproduce the whole control mechanisms of fundamental frequency.

#### Compliance with ethical standards

**Conflict of interest** The authors declare that there is no conflict of interest of any kind.

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