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Ex vivo investigation of the effect of the transverse arytenoid ligament on abduction of the arytenoid cartilage when performing equine laryngoplasty

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Abstract

AIMS: To investigate the effect of the transverse arytenoid ligament (TAL) on abduction of the arytenoid cartilage when performing laryngoplasty.

METHODS: Modified prosthetic laryngoplasty was performed on right and left sides of 13 cadaver larynges. Increasing force was sequentially applied to the left arytenoid cartilage at 3 N intervals from 0–24 N, when the force on the right arytenoid cartilage was either 0 or 24 N, before and after TAL transection. Digital photographs of the rostral aspect of the larynx were used to determine the left arytenoid abduction angles for these given force combinations and results compared before and after TAL transection. Longitudinal and transverse sections of the TAL from seven other equine larynges were also examined histologically.

RESULTS: Increasing force on the left arytenoid cartilage from 0–24 N produced a progressive increase in the angle of the left arytenoid cartilage ($p<0.001$) and increasing force on the right right arytenoid cartilage from 0–24 N reduced the angle of the left arytenoid cartilage ($p<0.001$). Following transection of the TAL the mean angle of the left arytenoid increased from 36.7 (95% CI=30.5–42.8)° to 38.4 (95% CI=32.3–44.5)°. Histological examination showed that the TAL was not a discrete ligament between the arytenoid cartilages but was formed by the convergence of the ligament and the left and right arytenoideus transversus muscles.

CONCLUSIONS: Transection of the TAL in *ex vivo* equine larynges enabled greater abduction of the left arytenoid cartilage for a given force. These results indicate that TAL transection in conjunction with prosthetic laryngoplasty may have value, but the efficacy and safety of TAL transection under load *in vivo*, and in horses clinically affected with recurrent laryngeal neuropathy must be evaluated.

KEY WORDS: *Equine, larynx, laryngoplasty, transverse arytenoid ligament, transection.*

F_{max} Force needed to maximally abduct the left or right arytenoid

TAL Transverse arytenoid ligament

Introduction

Prosthetic laryngoplasty, combined with ventriculocordectomy, is the treatment of choice for horses with severe recurrent laryngeal neuropathy (Witte *et al.* 2009). In brief, prosthetic laryngoplasty involves the placement of a suture to mimic the force of the affected (predominately left) cricoarytenoideus dorsalis muscle. This results in abduction of the arytenoid cartilage and enlargement of the airway for horses with recurrent laryngeal neuropathy. To this end a suture is placed through the caudal and dorsal aspect of the cricoid cartilage and continued through the ipsilateral muscular process of the arytenoid cartilage. A loop is then formed and tightened to the desired degree of arytenoid abduction. The success rate of the combined procedure is estimated to be 68–82% (Kidd and Slone 2002; Witte *et al.* 2009; Raffetto *et al.* 2015). Studies of horses undergoing prosthetic laryngoplasty for treatment for recurrent laryngeal neuropathy have reported loss of abduction of the arytenoid cartilage as a substantial post-operative complication of the procedure (Goulden and Anderson 1982; Dixon *et al.* 2003; Kraus *et al.* 2003). In one study re-examining 198 horses following prosthetic laryngoplasty, the median abduction grade was seen to reduce from Grade 2 to Grade 3 within 7 days. Loss of abduction in this study necessitated a second surgery to replace the prosthesis in 5% of horses, and loss of abduction between 7 days and 6 weeks of surgery necessitated a second surgery to replace the prosthesis in a further 5% of horses (Dixon *et al.* 2003). The mechanism by which abduction is lost, sometimes resulting in complete failure of laryngoplasty, is not fully understood, but is most likely the result of the prosthesis cutting through the muscular process of the arytenoid cartilage (Baker 1983; Dean *et al.* 1990; Herde *et al.* 2001) or the cricoid cartilage (Brandenberger *et al.* 2017). One theory to explain why this occurs is that the force applied to abduct the left arytenoid cartilage influences the stability of the construct, and therefore the more force that is needed to abduct the cartilage the higher is the risk of postoperative loss of abduction (Schumacher *et al.* 2000; Kelly *et al.* 2008; Perkins *et al.* 2011). Another theory is that repetitive loads on either the suture construct itself or through the right arytenoid cartilage, such as induced by swallowing and coughing, result in gradual loosening and failure of the prosthesis (Dean *et al.* 1990; Herde *et al.* 2001; Dixon *et al.* 2003).

We postulate that loss of abduction, regardless of the reason why the prosthesis cuts through the muscular process, could be influenced by the transverse arytenoid ligament (TAL), a fibrous band extending between the dorsomedial angles of the arytenoid cartilages (Figure 1), linking the two arytenoid cartilages (Hare 1975; Schummer *et al.* 1979). The TAL is connected to the arytenoideus transversus, a paired muscle that occupies the dorsal surface of the arytenoid cartilages and inserts onto the muscular process and the ridge that extends from it rostrally (Schummer *et al.* 1979; Dyce *et al.* 2002; Rhee *et al.* 2009). The presence of this interarytenoid connection prevents complete autonomous movement of each arytenoid cartilage (Lozier and Pope 1992).

Laryngeal paralysis is a common cause of obstruction of the upper portion of the airway of dogs (O Brien 1975), and affected dogs are most commonly treated by lateralising the arytenoid cartilage in combination with transection of the sesamoid band that connects the left and right arytenoid cartilages (Hedlund *et al.* 1992; White 1995). Transection of this connection is purported to change the force needed to open the paralyzed arytenoid cartilage and to decrease the post-operative cyclic stresses imposed by the contralateral arytenoid cartilage (Hedlund *et al.* 1992; White 1995). Although laterisation in dogs is a different technique to prosthetic laryngoplasty in horses some parallels could be drawn. The standard technique of prosthetic laryngoplasty performed in horses does not include transection of the TAL (Fulton 2012). The exact role of this anatomical structure of horses is unknown. We sought to investigate its histological morphology and biomechanical influence on laryngeal function to determine if transecting this ligament at the time of prosthetic laryngoplasty might decrease the likelihood of loss of abduction of the arytenoid cartilage after prosthetic laryngoplasty.
The aim of this study was to investigate the effect of the TAL on abduction of the arytenoid cartilage when performing laryngoplasty. The hypothesis was that transecting the TAL would result in increased left arytenoid cartilage abduction when the same forces were applied to the left and right arytenoid cartilages, and that increasing force on the right arytenoid cartilage would result in decreased left arytenoid cartilage abduction.

Materials and methods

Specimens

Twenty, grossly normal larynges were harvested from the cadavers of 7–15-year-old horses (mean 11.3 (SD.3.0) years) greater than 1.63 m tall. Extrinsic muscles were removed, taking care not to damage the intrinsic muscles, ligaments, and joints. Both cricoarytenoideus dorsalis muscles were also removed.

Mechanical testing

Thirteen larynges were preserved in a 2% solution of 2-phenoxyethanol at 4°C to maintain tissue pliability. Experiments were performed within 1 week of harvest, and the larynges were acclimatised to room temperature (23°C) prior to testing. Each larynx was positoned horizontally on a retort stand, with a clamp fixed to the the dorsal aspect of the cricoid cartilage. A 0.5-cm diameter plastic bolt was inserted ventrally through the cricotracheal ligament and secured ventrally to a wooden board which was also held by the retort stand. A coloured pin was placed mid-corniculate process as a marker.

Both arytenoid cartilages were abducted, using a modified technique of prosthetic laryngoplasty (Fulton 2012), with first a 7-metric polyester suture placed in the muscular process of the arytenoid cartilage. A large hole was created, using a 0.25-cm diameter drill-bit, in the cricoid cartilage lateral to the median ridge and at the junction of the caudal and middle third of the cricoid lamina (Figure 1), which was large enough to minimise friction on the suture material (Perkins *et al.* 2011). The ends of each suture were passed through the hole in the cricoid lamina, tied together to make a loop, and each loop was attached to a portable force indicator (Mecmesin, Horsham, UK). Force was applied manually to the sutures as required, then fixed using artery forceps.

The force required to maximally abduct each arytenoid cartilage (F_{max}) was determined prior to transection of the TAL. F_{max} was defined as the force at which more tension on the suture failed to cause the arytenoid cartilage to continue abducting. The mean F_{max} of the 13 larynges was 24 N. Tension on an arytenoid cartilage was recorded as 0 N when no force was applied to abduct the cartilage. The force on the left arytenoid cartilage was then increased in increments of 3 N up to 24 N, while the force on the right arytenoid cartilage was maintained first at 0 N and then 24 N. Photographs were taken of the rostral aspect of the larynx at each combination of force with a digital camera placed at a standard distance (60 cm) from the most rostral aspect of the epiglottis.

After transecting the TAL of each larynx transversely on the midline using Metzenbaum scissors, the process was repeated using the same combinations of the increments of force applied to the left and right arytenoid cartilages.

Left and right arytenoid angles were measured by a single observer (ZW), using a software package to assess digital images (UTHSCSA Image Tool, Version3.0 Final; University of Texas, Houston, TX, USA). A line was initially drawn from the dorsal to ventral aspects of the rima glottidis. A third of this length was then continued dorsally above the larynx. Tangents were then drawn to touch the abaxial margins of the corniculate processes and the angles these lines made with the dorsoventral line recorded (Figure 2).

To assess the repeatability of the measurements and determine the intra-observer variation, photographs from five larynges that had undergone a standard left-and right-sided prosthetic laryngoplasty were chosen at random. The left arytenoid angle was measured four times from the photographs of these larynges when 0 N and 24 N were applied to the right arytenoid cartilage, and CV calculated.

Histological examination

The TAL was examined histologically to determine its morphology and to identify potential risks of transecting it *in vivo*. Seven larynges were preserved in a solution of 10% formalin the day they were harvested and stored in the solution for 2 weeks before longitudinal and transverse sections of the TAL and associated structures were cut from them. The sections were processed for histological examination and stained with H&E using routine techniques. Serial sections were processed until good quality sections of the TAL were identified between the two arytenoideus transversus muscles and continued until it could no longer be seen. Morphology and location of different tissue types were then documented.

Statistical analyses

A mixed-effects model was fitted to the data for the measurements of the left arytenoid abduction angle, with the larynx as a random effect. Fixed effects included whether the TAL was intact or transected, force on the right arytenoid cartilage, and force on the left arytenoid cartilage. Forces on the left and right arytenoid cartilages were treated as categorical variables to allow for a potential, non-linear effect. Interaction terms were initially included for status of the TAL and force on the left arytenoid cartilage, condition of the TAL and force on the right arytenoid cartilage, and for force on left arytenoid cartilage and right arytenoid cartilage. Statistical analyses were performed using the statistical software JMP (SAS Institute, Cary, NC, USA).

Results

Mechanical testing

The CV for the intra-observer variation in measurement of the left arytenoid angle was 0.9%.

The final mixed effects model for the left arytenoid angle included the main effects of status of the TAL (i.e. intact or transected) ($p<0.001$), force on the left arytenoid cartilage ($p<0.001$), and force on the right arytenoid cartilage ($p<0.001$). The interaction terms were not included in the final model (>0.9). Overall model fit was good (adjusted $R^2=0.91$).

Controlling for other factors in the model, the mean angle of the left arytenoid was lower with the TAL intact (36.7 (95% CI=30.5–42.8)°) than after transection (38.4 (95% CI=32.3–44.5)°)), with an estimated mean difference of 1.75 (95% CI=1.11–2.38)°.

Increasing force on the left arytenoid cartilage produced a progressive increase in the angle of the left arytenoid cartilage and increasing force on the right right arytenoid cartilage reduced the angle of the left arytenoid cartilage (Figure 3). When the TAL was intact and the force applied to the left cartilage was 24 N, the mean angle of the left arytenoid was reduced from 47.6 (95% CI=41.4–53.8)° to 42.8 (95% CI=36.6–48.9)°, when the force on the right cartilage was increased from 0 to 24 N. Following transection of the TAL and when the force applied to the left cartilage was 24 N, the mean angle of the left arytenoid was reduced from 48.6 (95% CI=42.4–54.8)° to 44.7 (95% CI=38.5–50.9)°, when the force on the right cartilage was increased from 0 to 24 N.

Histological examination of the TAL and associated structures

The TAL was seen grossly to extend between the two arytenoid cartilages and was widest axially where the left and right arytenoideus transversus muscles converged (Figure 4). Histologically the ligament was closely intertwined with oblique muscle fibres of the left and right arytenoideus transversus muscles (Figure 5). Dense collagenous tissue was most apparent on the rostral and caudal aspect of the symphysis of the ligament. Small nerve fibres and blood vessels were seen in the adipose tissue located dorsal and ventral to the ligament. The ligament was covered ventrally with thick mucosa composed of stratified squamous epithelium at its rostral aspect and pseudostratified columnar epithelium at its caudal aspect. Lymphoid aggregates, sometimes forming follicular nodules, were occasionally apparent within the superficial layers of the lamina propria of the mucosa, and numerous submucosal mucus glands were identified.

Discussion

The effect of transection of the TAL seen in this study was that, with the same forces applied to the left and right arytenoid cartilages, transection of the TAL resulted in an increased angle of abduction of the left arytenoid cartilage. In addition, with incremental increases in force on the left arytenoid cartilage, abduction of the left arytenoid cartilage increased. Conversely with increased force applied to the right arytenoid cartilage, a decrease in the left arytenoid angle was seen. Less force, therefore, would be needed to achieve the same abduction in the left arytenoid after TAL transection. Failure of prosthetic laryngoplasty in horses has been associated with the amount of tension applied on the prosthesis; with less force used to abduct the left arytenoid cartilage being associated with reduced loss of abduction post-operatively (Schumacher *et al.* 2000; Dixon *et al.* 2003).

The arytenoid cartilages normally adduct completely during swallowing, which occurs approximately 1,000 times a day in the average horse (Witte *et al.* 2010) After swallowing, they briefly maximally abduct (Dixon *et al.* 2003). The force applied to the prosthesis during swallowing, in horses that have undergone prosthetic laryngoplasty, may be up to 84% of the single-cycle failure force (Witte *et al.* 2010). Therefore, a one cause of loss of abduction of the left arytenoid cartilage after prosthetic laryngoplasty may be the repetitive medioventral force applied to the surgically-abducted arytenoid cartilage immediately after swallowing (Dixon *et al.* 2003). Our *ex vivo* study demonstrated that increasing the force on the right arytenoid cartilage from 0 to 24 N resulted in a decrease in the abduction of the left arytenoid cartilage. We hypothesise, therefore, that the axial force placed on the left arytenoid cartilage during maximal abduction of the right arytenoid cartilage after swallowing could be a mechanism whereby the surgically-abducted left arytenoid cartilage loses abduction in the immediate post-operative period.

The TAL was shown during histological examination not to be a discrete ligament between the arytenoid cartilages but to be formed by the convergence of the ligament and the left and right arytenoideus transversus muscles. The arytenoideus transversus muscle of horses with recurrent laryngeal neuropathy is one of the more severely affected muscles (Lopez-Plana *et al.* 1993; Rhee *et al.* 2009), and therefore this ligament may be easier to identify in horses with severe muscle atrophy caused by recurrent laryngeal neuropathy that are undergoing prosthetic laryngoplasty than in horses with normal laryngeal function. The TAL lies in close proximity to the laryngeal mucosa, and transecting the TAL during lateralisation of the canine arytenoid cartilage is associated with a risk of penetrating the laryngopharyngeal mucosa (Payne *et al.* 1990), which increases the risk of infection around the surgical implant. Transecting the inter-arytenoid connection in canine larynges has been described as technically difficult and time-consuming (Lozier and Pope 1992). During histological examination of the TAL of the equine cadaver larynges, some moderately-sized blood vessels and some nerve fibres were identified in the adipose tissue surrounding the ligament. Studies are required to assess the best surgical approach to the TAL during prosthetic laryngoplasty, the likelihood of substantial bleeding occurring with TAL transection, the potential for destabilisation of laryngeal integrity and the likelihood of TAL transection resulting in detrimental effects on laryngeal function if nerves associated with the TAL are inadvertently transected.

This *ex vivo* study demonstrated that as force on the right arytenoid cartilage increased, abduction of the left arytenoid cartilage decreased. Abduction of the right arytenoid cartilage during inhalation and immediately after swallowing might, therefore, be a mechanism by which the left arytenoid cartilage loses abduction in the immediate post-operative period. We also found that TAL transection enabled greater abduction of the left arytenoid cartilage for a given force. Although the results of this study indicate that TAL transection in conjunction with prosthetic laryngoplasty may have value, the efficacy and safety of TAL transection under load *in vivo* and in horses clinically affected with recurrent laryngeal neuropathy must be evaluated. Nevertheless given the anatomy of the TAL and its mechanical impact on arytenoid abduction with and without right side abduction, further study of the function of the TAL may lead to modification of prosthetic laryngoplasty and have relevance to arytenoid subluxation.

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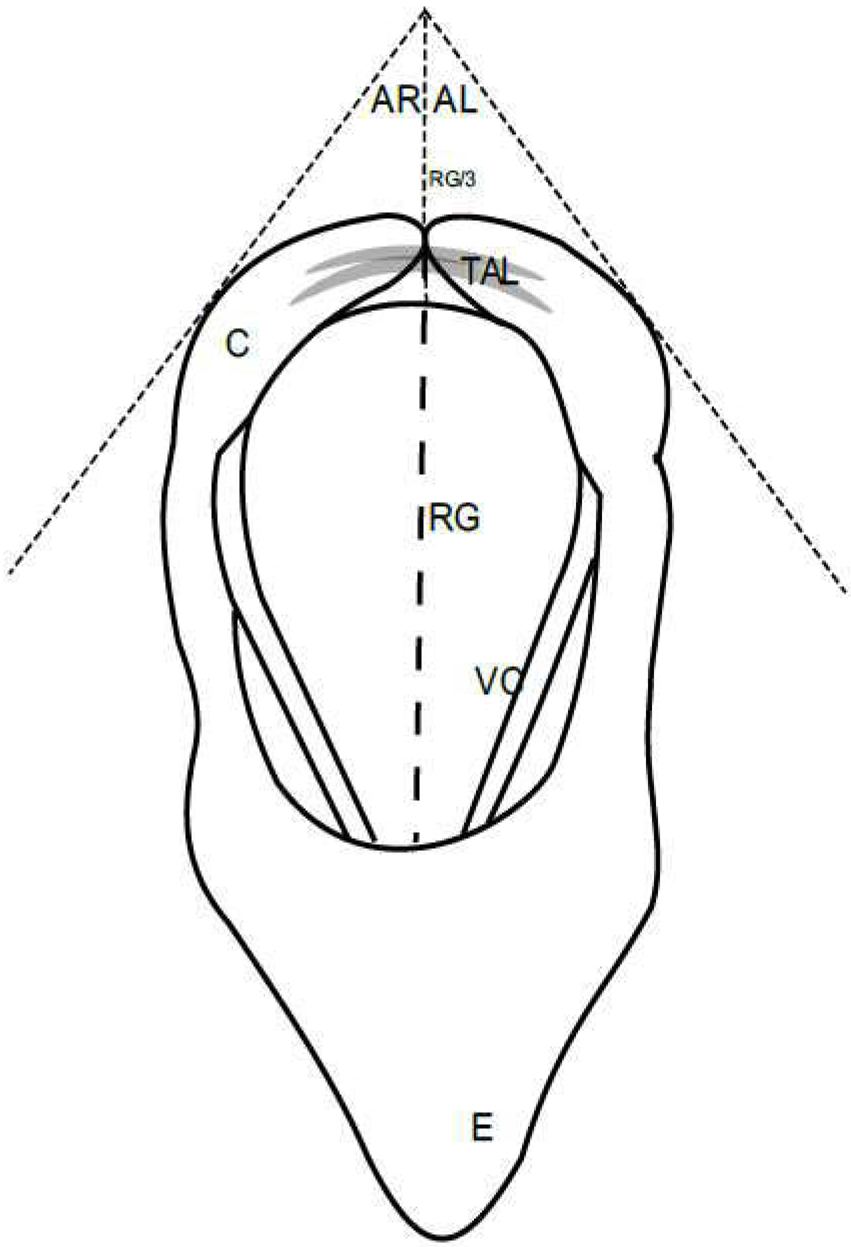
Figure 1. Diagram of the dorsal view of the equine larynx including bilateral modified prosthetic laryngoplasty showing thyroid cartilage (T) and transverse arytenoid ligament (TAL). Placement of sutures through the cricoid cartilage (Cr) into the muscular process of the arytenoid (MPA) is demonstrated including the attachment to the force tranducers (FT).

Figure 2: Diagram of the frontal view of the equine larynx showing the epiglottis (E), vocal cord (VC), transverse arytenoid ligament (TAL), corniculate process of arytenoid (C), dorsoventral line through rima glottidis (RG), left arytenoid angle (AL) and right arytenoid angle (AR).

Figure 3: Mean (±SE) left arytenoid angle of 13 equine cadaver larynges when 0 N (black and dark grey bars) and 24 N (pale grey and speckled bars) were applied to the right arytenoid cartilage and the force applied to the left arytenoid cartilage was increased at 3N increments from 0 N up to a maximum of 24 N, following prosthetic laryngoplasty before (black and pale grey bars) and after (dark grey and speckled) transverse arytenoid ligament transection.

Figure 4. Dorsal view of an equine larynx (a) before and (b) after transection of the symphysis of the two arytenoideus transversus muscles (TA) showing the anatomical location of the transverse arytenoid ligament (white arrows) and the relationship to the arytenoideus transversus muscle, with the cricoid cartilage (C), cricoarytenoideus dorsalis muscle (CAD), corniculate process of the arytenoid cartilage (CPA) and the muscular process of the arytenoid cartilage (MPA).

Figure 5: Photomicrographs of parasagittal sections of the dorsal aspect of the equine larynx at (a) low and (b) high magnification showing the morphology of the transverse arytenoideus ligament (*) and the arytenoid cartilage (A), artery (ar), cartilage (C), cricoarytenoideus dorsalis muscle (CAD), oesophagus (Oe), arytenoideus transversus muscle (TA), vein (v), (H&E).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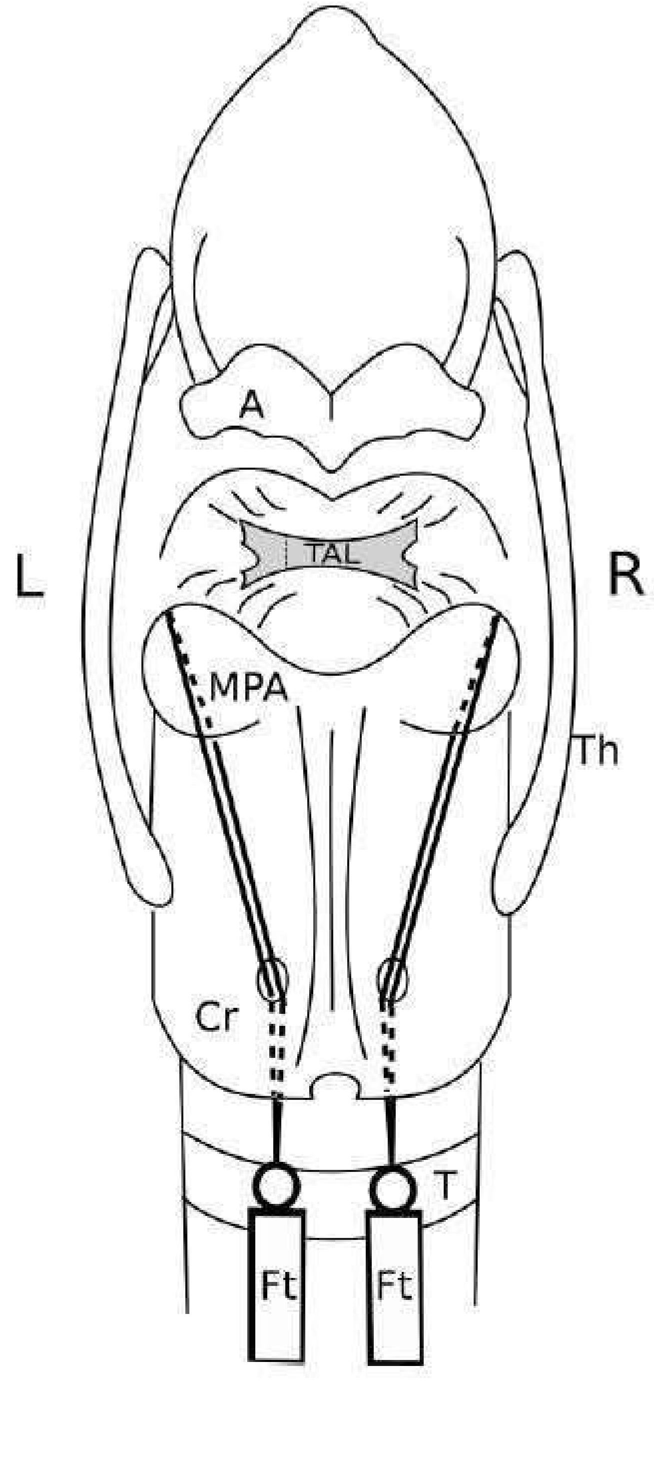
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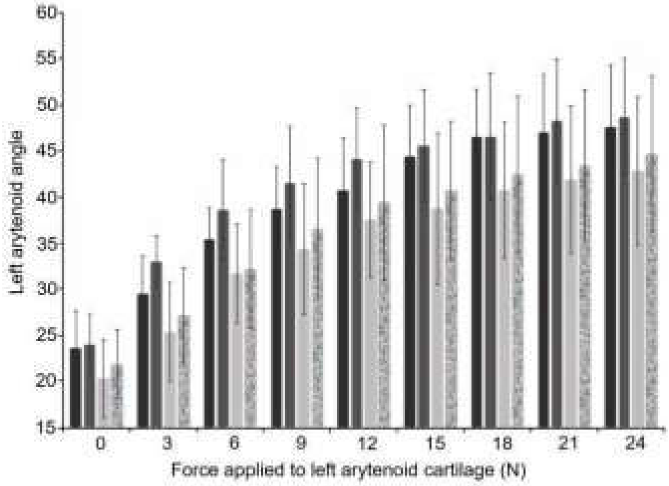

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55
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45
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Left arytenoid angle
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9
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18
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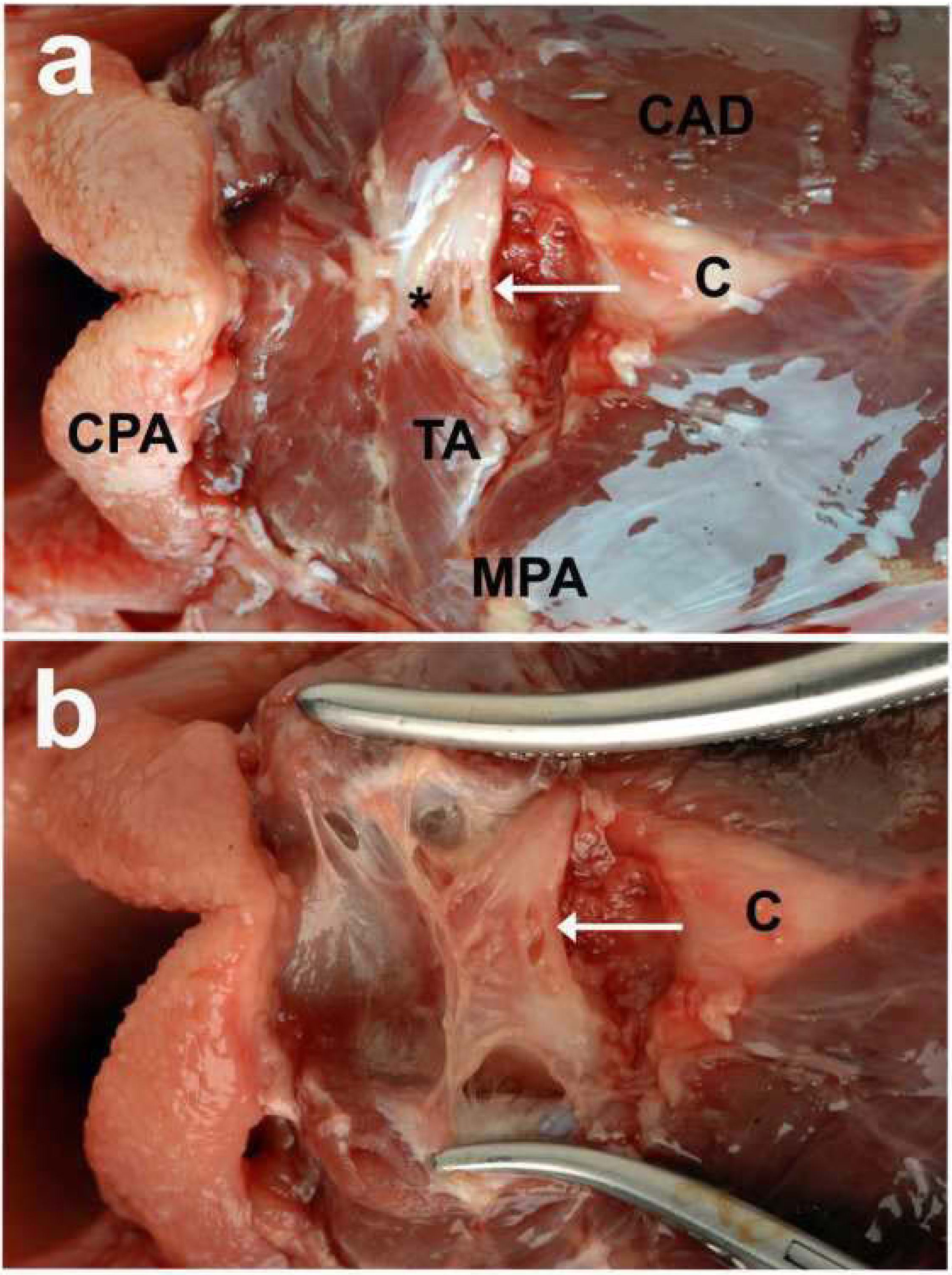
24
Force applied to left arytenoid cartilage (N)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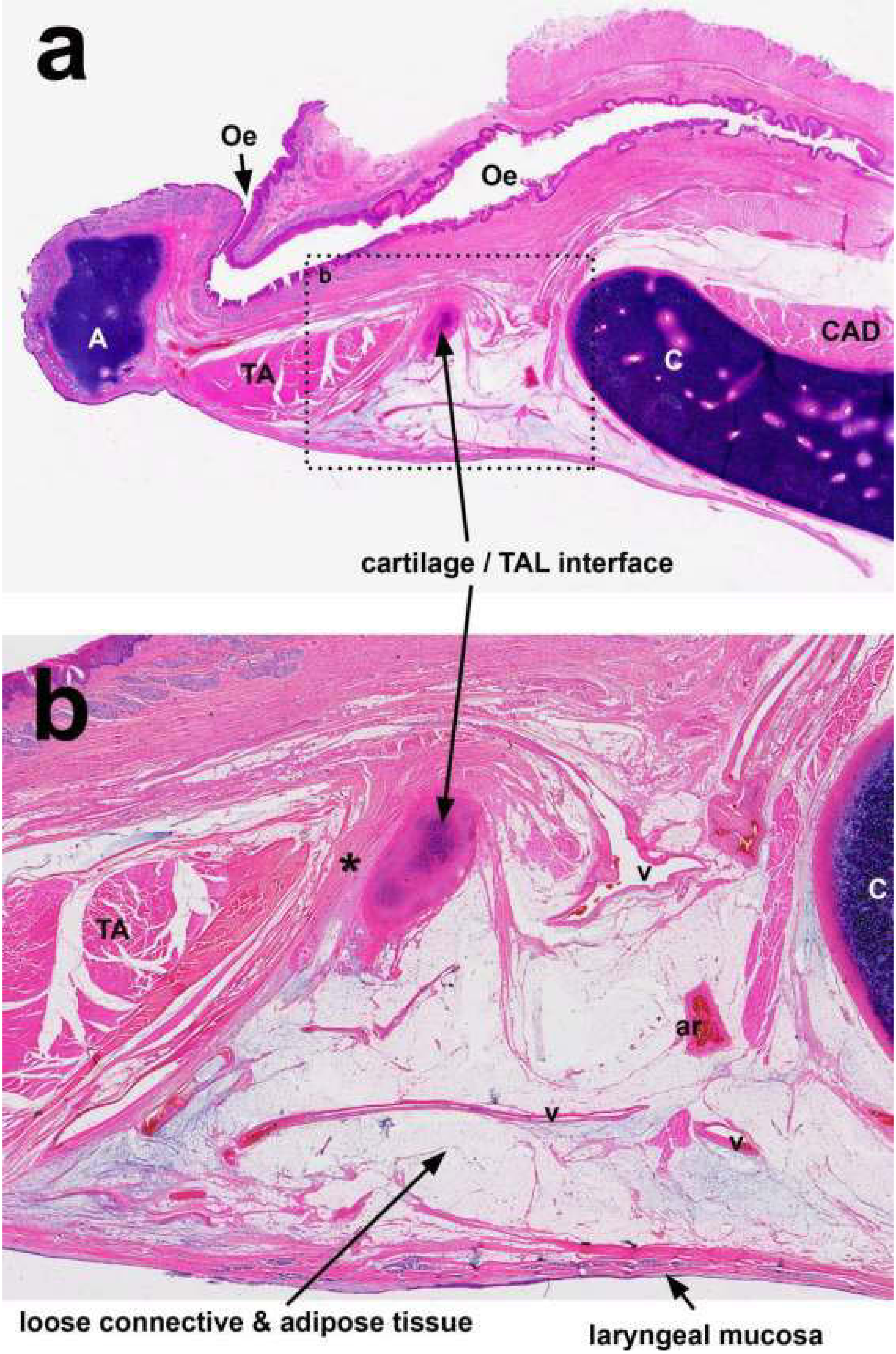
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loose connective & adipose tissue
laryngeal mucosa