Length-Tension Relationship of the Feline Thyroarytenoid Muscle

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Summary: Vocal fold tension during phonation is generated by coordinated contraction of the intrinsic laryngeal muscles. The thyroarytenoid muscle has been found to have increased stiffness at various levels of strain when compared with other intrinsic laryngeal muscles. The objective here is to test the hypothesis that the thyroarytenoid muscle exhibits high passive tension during maximal isometric tetanic force generation, and to test the hypothesis that the thyroarytenoid maintains the ability to generate contractile force at high levels of strain more effectively than other skeletal muscle. The thyroarytenoid muscles (n = 9) and digastric muscle strips (n = 7) were removed from adult random-bred cats. Maximal isometric tension and passive tension at optimum length were measured from each muscle in vitro. Active and passive length-tension curves were constructed for each muscle. The contractile properties of the thyroarytenoid group were compared with those of the digastric muscle group. The thyroarytenoid muscle group required on average 140 mN of passive tension to generate maximal isometric tetanic tension. This represented 39% of the average maximal isometric tetanic tension generated by the muscles. These results were significantly higher than the digastric muscle group, which required on average 28 mN of passive tension (9% of maximal isometric tetanic tension, $p < 0.05$). At 110% of optimum length, the thyroarytenoid muscle maintained 89.8% of maximal isometric tetanic force, whereas the digastric muscle group maintained 67.7% of maximal isometric tetanic force ($p < 0.05$). The thyroarytenoid muscle exhibits higher passive tension when generating maximal isometric tension than the digastric muscle control group. The thyroarytenoid muscle maintains higher levels of active tension at high strain than the digastric muscle control group. We conclude that these findings are related to the ability of the thyroarytenoid muscle to function as a fine tensor of the vocal fold in a high strain environment.

Key Words: Larynx—Thyroarytenoid muscle—Muscle physiology—Feline.

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INTRODUCTION

Sound generated by the larynx during phonation is the result of airflow producing vibration of the vocal fold cover over the vocal fold body.[1] The vocal fold cover consists of mucosal epithelium and lamina propria, whereas the body consists of the vocalis and the thyroarytenoid muscles. The fundamental frequency produced during phonation is determined by the mass, viscosity, and stiffness of the vocal fold cover, which is modulated to a significant degree by complex interaction of the intrinsic laryngeal muscles.[2]

In 1966, Hast hypothesized that active vibration of the vocal fold is produced by subglottic air pressure and modulated by the muscular tonus of the vocal fold.[3] Rubin reported that in order to generate sufficient subglottic pressure for phonation, the vocal folds had to be approximated closely and under significant tension.[4] Furthermore, Arnold reported that in the adducted vocal fold, the cricothyroid muscle is the primary determinant of gross vocal fold tension and that the thyroarytenoid muscle functions isometrically to modulate vocal fold tension on a finer level.[5] It has been hypothesized that the thyroarytenoid muscle has a stiff stress-strain relationship, which may contribute to its role in modulating tension of the vocal fold.[6]

Most skeletal muscle generates maximal active contractile force at lengths where there is nearly zero passive tension.[7,8] The unique properties of the few muscles where high passive tension is exhibited (bladder, external anal sphincter, esophagus) are hypothesized to serve the specialized role of those individual muscles.[9–11]

Based on the ability of the thyroarytenoid muscle to contract in a high-tension environment, we hypothesized that the average passive tension present at maximum isometric contraction would be significantly higher in the thyroarytenoid muscle than muscle that does not function in that type of environment. The digastric muscle served as a control skeletal muscle in this experiment. We also hypothesized that the thyroarytenoid muscle would maintain higher percentage of maximal isometric force than digastric muscle when stretched beyond optimum length.

MATERIALS AND METHODS

Study design

All experiments were performed in accordance with NIH standards, approved by the Ann Arbor VA Medical Center Animal Care and Use Committee. Contractile properties and active and passive length-tension curves were measured for 9 thyroarytenoid muscles and 7 digastric muscle strips as described below. The digastric muscle was chosen as a control muscle because it is a skeletal muscle in a similar location, has cranial nerve innervation, and was accessible for dissection. Data obtained from the thyroarytenoid preparations were compared with those obtained from the digastric preparation.

Animal surgery

Anesthesia was induced in each animal using an intramuscular dose of ketamine hydrochloride (10 mg/kg) and xylazine (2 mg/kg). Videolaryngoscopy was performed to ensure normal vocal fold mobility. Animals were intubated, and anesthesia was maintained using inhaled isoflurane (1.5%). A vertical incision was made in the cervical midline, and the strap muscles were separated. The larynx and anterior bellies of the digastric muscles were exposed.

Thyroarytenoid muscle assessment

A laryngectomy was performed on each animal. The larynx was immediately submerged in a physiologic salt solution (composition in mM: NaCl, 137; $NaHCO_3$, 24; glucose, 11; KCL, 5; $CaCl_2$, 2; $MgSO_4$, 1; NaH_2PO_4, 1; and tubocurarine chlorine, 0.025), which was oxygenated with 95% O_2 and 5% CO_2. Contractile properties assessment for the thyroarytenoid muscles was performed in a manner similar to that previously described by Alipour-Haghighi and Titze.[2,12] Each thyroarytenoid muscle was dissected from the larynx under an operating microscope, carefully isolated on an anterior strip of thyroid cartilage and the intact arytenoid cartilage. Attachments of the lateral cricoarytenoid muscle, posterior cricoarytenoid muscle, and interarytenoid muscle were divided at the arytenoid cartilage. In order to preserve the effect that the vocal fold cover has on the contractile properties of the muscle, the mucosa and lamina propria overlying the thyroarytenoid muscle was maintained adherent. After dissection, one 4.0 nylon suture was tied to the anterior cartilage strip and another to the arytenoid process.

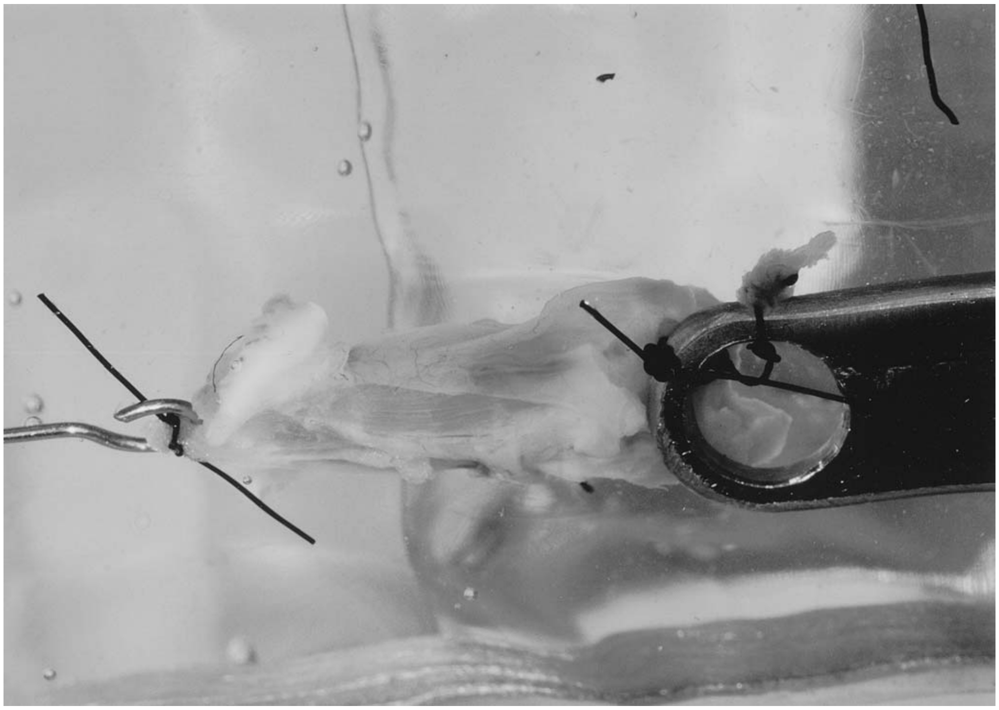

FIGURE 1. Feline thyroarytenoid muscle preparation. The whole muscle is dissected in vitro and attached to a force transducer (hook) at one end and an inflexible post at the other.

Prior to the beginning of experimentation, microdissection of six cadaver feline larynges was performed. During these dissections, attempts were made to separate the vocalis portion of the thyroarytenoid muscle from the lateral portion of the thyroarytenoid muscle. In these animals, it was found that no clear dissection plane could be created without damaging the muscle preparation. Thus, whole muscle thyroarytenoid preparations were used for experimentation.

The thyroarytenoid muscle preparations were immersed in a horizontal bath containing the same buffered physiological salt solution as previously described.[13] The solution was maintained at 25°C. The muscle was attached to an inflexible post at one end and directly to the arm of a calibrated force transducer at the other end using nylon sutures (Figure 1).

Digastric muscle assessment

Prior to euthanasia, a strip of digastric muscle was dissected by incising the muscle parallel to the direction of the muscle fibers and by carefully dissecting out a strip measuring approximately 15 mm long by 3 to 5 mm in diameter. A suture was secured at each end of the muscle strip, and the bundle was removed and immediately placed in the same oxygenated physiologic solution described above. The muscle strip was subsequently immersed in a horizontal bath containing the solution, and it was attached to the contractility apparatus as described for the TA muscle using the attached sutures.

Measurement of contractile properties

The force transducer used to measure contractile properties was calibrated prior to each experiment by hanging known weights (0 g to 50 g) vertically from the transducer and constructing a force-voltage curve. The transducer was set to zero millinewtons of force after immersion into the horizontal bath and prior to tying on each muscle sample. Muscles were stimulated directly by an electrical field generated between two platinum electrodes. The muscles were stimulated with 0.2-ms pulses of supramaximal intensity. Muscle length was adjusted until a single stimulus pulse elicited maximum force during an isometric twitch. The maximal isometric twitch force (P_t), the time to peak tension (contraction time), and corresponding muscle length (L_o) were recorded. For the thyroarytenoid muscles, this length was directly measured from the insertion of the muscle fibers on the anterior thyroid cartilage strip to the insertion of the muscle fibers on the muscular process of the arytenoid cartilage using a micrometer. For the digastric muscles, Lo was measured as the distance between the two tied sutures, using a micrometer. Tetanic force was measured by administering a 300-ms pulse train at increasing frequencies (50 Hz, 80 Hz, 100 Hz, 120 Hz, and 140 Hz) until maximal tetanic isometric force (P_o) was obtained.

Measurement of active and passive length-tension properties

Once Lo and Po were established for each muscle, the muscle length was shortened in 0.3-mm increments. One minute after each length change, the passive tension was recorded. The muscle was then stimulated using identical parameters to those at Po, and active isometric whole muscle tension was recorded for that length. The muscle was progressively shortened a total of 5.1 mm or until active tension was approximately 0.3 Po.

After each muscle was shortened, Lo and Po was found again in the same fashion as described. Typically, the second Po determination was less than the first due to repeated stimulation. This second measurement was used to calculate the percentage of Po for the active tension measurements at lengths greater than Lo. Once Po was determined again, each muscle was lengthened in 0.3-mm increments. One minute after each length change, the passive tension was recorded. The muscle was then stimulated using identical parameters to those at Po, and active isometric whole muscle tension was recorded for that length. The muscle was progressively lengthened until passive tension exceeded active tension. In some cases, muscle disruption occurred prior to this point due to high strain.

Data analysis

The differences in the average passive tension at Po and differences in the percentage of Po maintained at 110% of Lo between the thyroarytenoid muscle group and the digastric muscle group were tested using a paired *t* test. If data failed equal variance testing, the Mann-Whitney rank sum test was performed. Alpha was set at 0.05.

RESULTS

Active tension

Single 0.2-ms pulses applied at supramaximal intensity resulted in twitch contractions in all muscle samples. Repetitive stimulation (50 Hz, 80 Hz, 100 Hz, 120 Hz, and 140 Hz) for 300 ms produced tetanic responses. Representative examples of a twitch and a tetanic response are shown in Figure 2. The properties of the thyroarytenoid muscles and the digastric muscle strips are depicted in Table 1. Nine thyroarytenoid muscles and seven digastric muscle strips were tested. Body mass between the 2 groups was similar. The thyroarytenoid preparations weighed on average more than the digastric muscle strips. However, the thyroarytenoid samples included the arytenoid cartilage, the anterior thyroid cartilage strip, and the mucosa overlying the muscle, which contributed to the overall weight of the sample. These structures were maintained attached to the muscle so that each sample could be fixed at optimum length following experimentation for later study. Removing these structures would have allowed for direct comparison of sample weight and normalization of data to the weight of the samples, but it would have disrupted the muscle fiber bundles. In order to preserve our samples for later study, we elected to normalize our data to maximum isometric tetanic force (Po) and optimum length (Lo) for direct comparison between TA and digastric samples, as described below.

Table 2 shows the contractile properties for each group. The average optimal length (Lo) for the thyroarytenoid muscles was 12.8 ± 0.92 mm. The digastric muscle strips were slightly longer at 17.7 ± 2.9 mm. The average time to peak tension (contraction time) for the digastric group was 36.25 ms. The average time to peak tension for the thyroarytenoid group was shorter at 25.8 ms. Maximal twitch forces were similar between the two groups, with thyroarytenoid and digastric samples generating 47.67 mN and 44.54 mN of force, respectively.

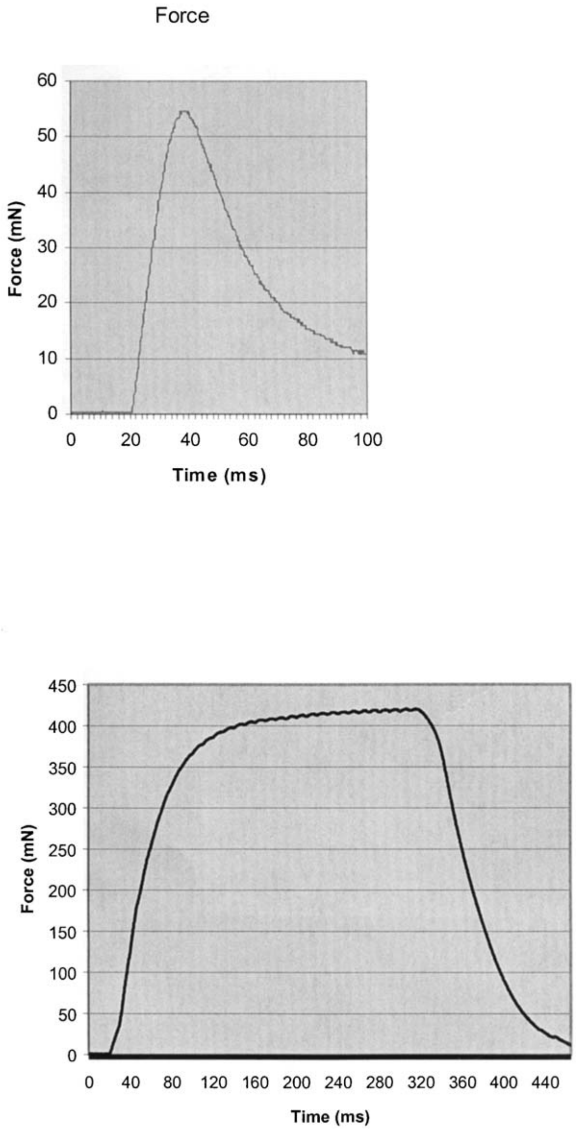

FIGURE 2. Example of a muscle twitch and tetanic contraction. **A**. Twitch contraction. **B**. Tetanic contraction.

The average maximal tetanic whole muscle force for the thyroarytenoid muscles was 362 ± 60 mN. This value was similar for the digastric muscle strips (340 ± 159 mN).

Passive tension

The thyroarytenoid muscles demonstrated significantly higher passive tension at Lo when compared with the digastric muscle strips. The average passive tension in the thyroarytenoid group was 140 ± 52 mN, which equals 39.4 ± 15.2% of maximal tetanic force (Po). The average passive tension in the digastric group was 28 ± 11 mN, amounting to 9.0 ± 3.3% of maximal tetanic force (Po). Both the absolute passive force and the percent of Po differences are statistically significant ($p < 0.05$).

TABLE 1. *Animal and Muscle Properties*

	Thyroarytenoid	Digastric strip
n	9	7
Body mass. (kg)	4.3 (±1.5)	4.2 (±1.7)
Preparation mass. (g)	421* (±71)	202 (±51)
Lo (mm ±SD)	12.8 (±0.92)	17.7 (±2.9)

Notes: Data are presented as means ± standard deviation. *Thyroarytenoid preparation weight is an aggregate weight of the thyroarytenoid muscle, vocal fold mucosa, and cartilaginous attachments.

Length-tension relationship

Active and passive length-tension curves for both the thyroarytenoid muscle and the digastric muscle are plotted in Figure 3. For each length change in the muscle, the active and passive tension values recorded were expressed as a percentage of Lo and Po for that muscle. This allowed for normalized comparison between the thyroarytenoid and digastric groups. In both groups, maximal active tension rises as muscle length approaches Lo. Thereafter, with further increases in length, there is a reduction in the maximal force that the muscles can produce. However, at high levels of strain, the thyroarytenoid muscle is able to maintain more active force than

TABLE 2. *Contractile Properties*

	Thyroarytenoid	Digastric strip
Time to peak tension (contraction time) (ms ± SD)	25.8 (±6.48)	36.25 (±3.95)
Pt (mN ± SD)	47.67 (±15.49)	44.54 (±27.05)
Po (mN ± SD)	362 (±60)	340 (±159)
Passive Tension at Lo (mN ± SD)	140* (±52)	28 (±11)
Passive Tension as % Po (% ± SD)	39.4* (±15.2)	9.0 (±3.3)

Notes: Data are presented as means ± standard deviation. An asterisk indicates values with statistically significant differences ($p < 0.05$).

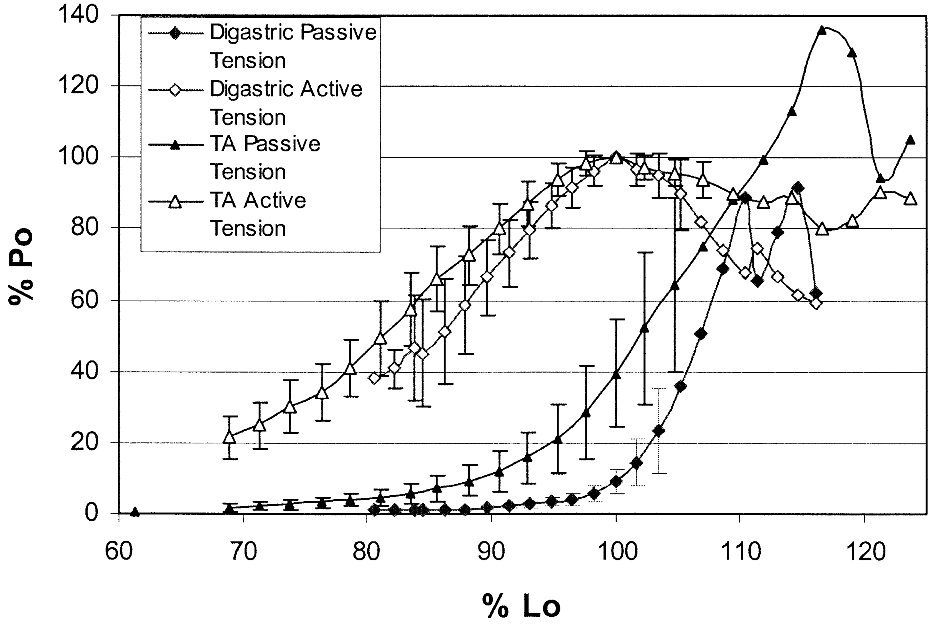

FIGURE 3. Active and passive tension versus muscle length.

the digastric muscle strips. Average isometric force at 110% of Lo was 89.8% of Po (±8.6%) in the thyroarytenoid group and was 67.7% of Po (±15.9%) in the digastric group. The difference is statistically significant ($p < 0.05$).

Similarly, in both groups, there is a nonlinear rise in passive tension as the muscles are lengthened. However, at all lengths, the thyroarytenoid group exhibits higher passive tension than the digastric group. Data points at high levels of muscle stretch with very high passive tension become erratic secondary to muscle tearing and damage.

DISCUSSION

This study confirms some of the known active contractile properties of the feline thyroarytenoid muscle and identifies some unique properties of the thyroarytenoid muscle in various length-tension conditions. The active contractile properties of the feline thyroarytenoid muscle reported in this study are similar to values reported previously. Hirose et al described the maximum isometric titanic whole muscle force to be 39 g (approximately 382 mN).[14] This is similar to our value of 362 mN.

Additionally, time to peak tension for the thyroarytenoid muscle has been reported to be from 21 to 28 ms.[12,14] These results are consistent with our findings of 25.8 ms in the feline thyroarytenoid muscle. Hast reported shorter times to peak tension in both the canine and feline thyroarytenoid muscle of 14 ms and 11 to 13 ms, respectively.[3]

Furthermore, this study demonstrates some unique contractile properties of the feline thyroarytenoid muscle. The hypothesis that the thyroarytenoid muscle requires high passive tension to generate maximal isometric tension appears to be true. The passive tension equals 39.4% of the active tension at optimum length in the thyroarytenoid muscle, but only 9% of the active tension in the digastric muscle. Significant differences in the passive length-tension relationship may exist between thyroarytenoid skeletal muscle and other skeletal muscle. The thyroarytenoid muscle demonstrates high passive tension at Lo and at lengths above Lo. As well, the muscle demonstrates significant passive tension at lengths below Lo. This is different than most skeletal muscle, which displays essentially no passive tension at Lo and at lengths below Lo.[7,8]

Few other skeletal muscles with high passive tension at Lo and steep length-tension relationships have been reported. Krier et al report high passive tension at Lo in the cat external anal sphincter (12.2% of Po).[10] They hypothesize that the high passive tension may help generate resistance to fecal material and may serve in the maintenance of fecal continence.

The second hypothesis that the thyroarytenoid muscle is able to maintain a higher percentage of maximal tetanic force beyond the optimum length is true also. At 110% of Lo, the thyroarytenoid muscle generates on average 89.8% of Po, whereas the digastric muscle generates 67.7% of Po.

The thyroarytenoid muscle, in conjunction with the other intrinsic laryngeal muscles, modulates the tension in the vocal fold cover to vary the fundamental frequency during phonation. Because the thyroarytenoid muscle is the only intrinsic laryngeal muscle that comprises the body of the vocal fold and is adherent to the vocal fold cover, it is likely that the muscle is subjected to a wide range of passive tension generated by the other intrinsic laryngeal muscles during varied pitch production in speech and singing. One can speculate that in order to function, the thyroarytenoid muscle must be able to contract effectively under higher passive tensions than other skeletal muscles, which do not share these unique anatomic and physiologic features. It follows that the thyroarytenoid muscle may have evolved with a different length-tension relationship than other skeletal muscle, in order to serve its role in phonation.

It is not clear what the underlying mechanism is for the high passive tension at Lo and stiffness of the passive length tension curve. In other skeletal, smooth, and cardiac muscle, passive tension has been felt to be related to the degree of connective tissue within the muscle.[9–11] The high passive tension in the thyroarytenoid muscle may be related to the significant connective tissue load that is imparted by the adherent vocal fold mucosa and lamina propria.

In summary, this study demonstrates that the passive tension at Lo and the passive length-tension relationship differ for the thyroarytenoid muscle as compared with other skeletal muscle. As well, the thyroarytenoid muscle can maintain higher force above optimum length than other muscle. This difference may be related to adaptation of the thyroarytenoid muscle to its role as a component of the vocal fold body and as a tensor of the vocal fold.

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