Establishment and Analysis of False Vocal Folds Hypertrophy Model in Excised Canine Larynges

*Yanchao Jiao, †Ruiqing Wang, †Qingkai Zeng, *Xinlin Xu, ‡Yu Zhang, §Bobby Leggon, §Jack Jiang, and *Peiyun Zhuang, *†‡Xiamen, China, and §Madison, Wisconsin

Summary: Objective. This study aimed to investigate the role of false vocal folds (FVFs) medialization in phonation and the acoustic impact of ventricular hypertrophy by establishing an FVF hypertrophy model.
Study Design. A prospective *in vitro* experiment was carried out.
Setting. The study was carried out using a pseudolung platform with high-speed camera in a soundproof room.
Materials and Methods. Control, degree I, and degree II FVFs hypertrophy were simulated in 10 excised larynges via fructose injection of 0.1 mL for degree I and 0.25 mL for degree II. Mean flow rate (MFR), fundamental frequencies (F0), formants, and sound pressure level were measured with a subglottal pressure of 1.5 kPa and 2.5 kPa, respectively.
Results. When the subglottal pressure was controlled at both at 1.5 kPa and at 2.5 kPa, the degree of FVF hypertrophy significantly influenced the distribution of the formants, F0, and MFR in excised canine larynges. Increasing the degree of hypertrophy was associated with a decrease in F0 and an increase in MFR. In degree II FVF hypertrophy models, the sound pressure level and the first formant were significantly higher ($P < 0.05$) than in normal models.
Conclusion. Hypertrophy of the FVFs has a significant influence on the distribution of sound energy and is associated with changes in sound quality.
Key Words: Excised canine larynges–False vocal folds hypertrophy–Voice formant–Laryngeal aerodynamic–Acoustics.

INTRODUCTION

The false vocal folds (FVFs) are structures above the true vocal folds (TVFs), and can be described as wedge-shaped tissues associated with the thickened inferior margins of the quadrangular membrane.[1] During phonation, the average distance between the FVFs is maintained by the laryngeal muscles to prevent unwanted ventricular fold vibration. Additionally, the FVFs appear to modulate the phonation by reducing the glottal flow of every other vocal fold vibratory cycle.[2] Patients with dysphonia often have hypertrophied FVFs, but little is known about the impact of FVFs on phonation. Although the use of excised canine larynges is a well-established model for simulating pathologies of the TVFs, the use of excised canine larynges to simulate FVF vibration has been explored to a much lesser extent.[3–5] However, because the phonatory characteristics of the excised canine larynx are very similar to those of the excised human larynx, we chose a canine model to predict the changes in human phonation.[6]

The geometry of the region surrounding the FVFs during phonation is important to understanding the aerodynamics and acoustics of the voice. Agarwal et al studied the FVFs and reported the shape and size differences between male and female human larynges. The shape and dimensions of this region during phonation were estimated using lamina graphic tracings of the larynx. Statistical analysis showed significantly greater FVF height in men than in women for both experiments, which suggests that our study should take gender into consideration.[7]

Finnegan and Alipour's study indicated that the presence of the epiglottis and the FVFs enhanced the second partial of the acoustic signal, and the absence of the epiglottis and FVFs increased low-frequency noise (between 0 and 300 Hz).[8] Bailly et al analyzed the aerodynamic effects of the FVFs using the Bernoulli equation and concluded that the presence of the FVFs may have supportive effects on the oscillation of the TVFs.[9]

A self-oscillating vocal fold model established by Alipour et al was extended to include the FVFs and was used to study time-dependent pressure and velocity distributions throughout the larynx. This model produced realistic results and demonstrated interactions among phonation variables, including the influence of the FVFs.[10] Alipour also found that glottal resistance increased systematically as the ventricular gap became smaller. Wide ventricular gaps were associated with increases in fundamental frequencies (F0) and decreases in glottal resistance. Sound pressure level (SPL) did not appear to be impacted by the adjustments in ventricular gap used in this research. Ventricular compression may interact with TVF posture and vibration, resulting in predictable changes in aerodynamic, physiological, acoustic, and perceptual measures of phonation. Therefore, narrow ventricular gaps may be associated with disordered phonation.[11]

The degree of ventricular fold hypertrophy has a significant impact on vocal fold function, and thus maintenance of the shape and size is a typical method used for treatment and prevention of ventricular fold hypertrophy.[12] Some patients with dysphonia demonstrate visible evidence of FVF hypertrophy and supraglottal compression. However, there is little research concerning the effect of the FVFs on human phonation. Although it is very difficult to establish a model that completely simulates hypertrophy of the FVFs, more data from canine models

Accepted for publication April 27, 2017.
Support funding: This work was supported by the National Natural Science Foundation of China, NSFC 81371080, China and the Young Talent Project supported by the Provincial Health Office, 2013-ZQN-JC-35.
From the *Department of ENT, Xiamen University Zhongshan Hospital, Xiamen, Fujian, China; †Medical College of Xiamen University, Xiamen, Fujian, China; ‡Key Laboratory of Underwater Acoustic Communication and Marine Information Technology of the Ministry of Education, Xiamen University, Xiamen, Fujian, China; and the §Department of Surgery, Division of Otolaryngology-Head and Neck Surgery, University of Wisconsin School of Medicine and Public Health, Madison, Wisconsin.
Address correspondence and reprint requests to Peiyun Zhuang, Department of ENT, Xiamen University Zhongshan Hospital, Xiamen, Fujian, China. E-mail: peiyun_zhuang@yahoo.com
Journal of Voice, Vol. ■■, No. ■■, pp. ■■-■■
0892-1997

http://dx.doi.org/10.1016/j.jvoice.2017.04.022

need to be collected to investigate the role of the FVFs in phonation and the effects of FVFs hypertrophy on the aerodynamics and acoustics of the excised canine larynx. Based on these findings, we established a model for FVF hypertrophy in excised canine larynges. F0, SPL, the formants, and mean flow rate (MFR) were collected and analyzed.

MATERIALS AND METHODS

Establishment of FVFs hypertrophy model of excised canine larynges

Acquisition of excised canine larynges

Ten male excised canine larynges were obtained at Key Laboratory of Underwater Acoustic Communication and Marine Information Technology of Xiamen University from animals sacrificed at Xiamen University Zhongshan Hospital for surgical skills practice. The research Institutional Review Board was approved by Xiamen University Zhongshan Hospital Laboratory Animal Management Ethics Committee.

The size of excised canine larynges was controlled by three parameters to decrease the influence of the laryngeal size: L1, length of the epiglottis (27.07 ± 3.05 mm); L2, sagittal length of the larynges (28.82 ± 2.58 mm); and L3, length of the vocal folds (19.88 ± 3.66 mm) (Table 1).

Excised larynges on the experimental platform

Larynges were collected and stored in saline solution before being used in the experiment, and then all of the extrinsic laryngeal muscles and connective tissue was removed. Additionally, the epiglottis and FVFs were reversed to maintain intact subglottal structures. The excised larynges were mounted on a pseudolung experimental platform.

Method and data acquisition

In an anechoic room, the arytenoid muscles of the excised larynges were stabilized by two steel needles to maintain the larynx in a natural position as well as to adduct the vocal folds. The audio signal was obtained with a microphone (Sony ECM-678, Sony Electrics Ins., Park Ridge, NJ), positioned at a distance of 15 cm from the vocal folds and at a 45-degree angle to the vocal folds, while simultaneous analysis of the audio signal was performed by *Cool Edit Pro 2.1 software* (Syntrillium Software Corporation, Phoenix, AZ). The subglottal pressure signal was recorded using a pressure transducer (Shanxi Chuangwei Ltd.-CWY100, Xi'an, China). An air compressor (Xiamen Taixing Electrical Co. Ltd.-2530, Fujian Province, China) produced airflow (MFR recorded by digital airflow machine, MF-5706-N-10, Kongxin Instrument CO. Ltd., Nanning, Guangxi Province, China) through an artificial lung (a homemade cylinder which is 8 cm in radius and 16 cm in height), which was placed upstream of the humidifier (German Bairui Ltd.-022G877S, Starnberg, Barvarian State, Germany) to cause the vocal folds to vibrate. The audio signal was recorded at the subglottal pressures of 1.5 kPa and 2.5 kPa. We chose these two subglottal pressures because our preliminary experiment demonstrated that the audio signal was unstable at and above 3.0 kPa. The SPL was measured with a sound level meter (Shenzhen Wansheng Ltd.-WS1361, Shenzhen, Guangdong Province, China) placed 10 cm from the glottis. The initial air pump pressure was adjusted to ensure an adequate pressure in each data collection to avoid error as a result of the decrease of the air pump pressure. Trials were conducted as a sequence of over 5 seconds of phonation followed by 30 seconds of rest.

TABLE 1.
The Gender and Dimensions of the Excised Larynges

Larynx	Gender	L1 (mm)	L2 (mm)	L3 (mm)
1	Male	29.6	30.1	26.0
2	Male	30.0	27.9	26.1
3	Male	25.6	25.7	15.4
4	Male	25.6	27.9	20.8
5	Male	33.0	30.6	19.7
6	Male	25.2	26.0	16.3
7	Male	27.2	25.9	19.2
8	Male	23.7	33.6	20.0
9	Male	23.3	29.8	17.2
10	Male	27.5	30.7	18.1

L1, length of the epiglottis; L2, sagittal length of the larynges; L3, length of the vocal folds.

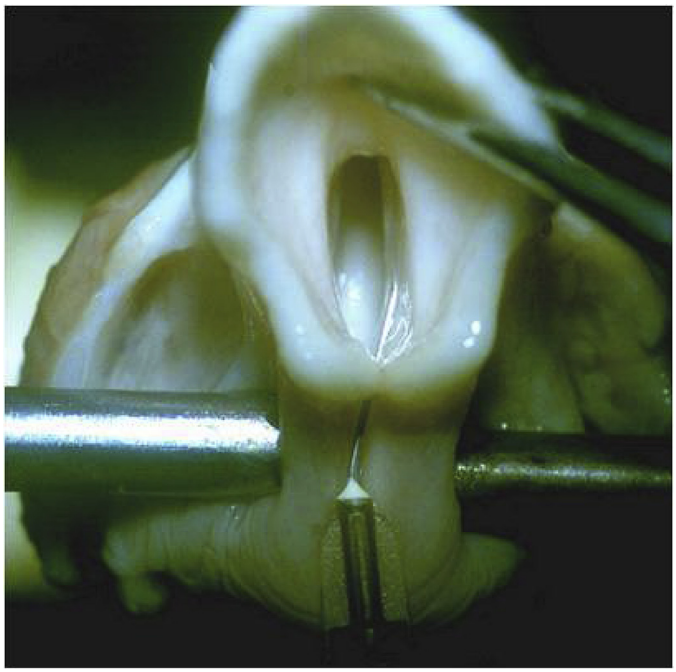

FIGURE 1. Injecting the fructose solution into the medial border of the FVFs.

Phase 1

The MFR, F0, formants, and SPL were measured in the normal FVF anatomic structure of 10 canine models.

Phase 2

Models of degree I FVFs hypertrophy were produced by injecting 0.1 mL of fructose solution (12.5 g fructose solution for intravenous injection dissolved in 50 mL of normal saline) into the superficial layer of the FVFs using a 1-mL syringe (Figure 1),