Published in final edited form as:

Ann Otol Rhinol Laryngol. 2008 July ; 117(7): 548–553.

Phonation Threshold Flow in Elongated Excised Larynges

Jack Jiang, MD, PhD, Michael F. Regner, Chao Tao, PhD, and Steven Pauls, MS

University of Wisconsin-Madison School of Medicine and Public Health Department of Surgery -
Division of Otolaryngology, Head and Neck Surgery Madison, Wisconsin 53792-7375

Abstract

Objectives—This study proposes the use of a new parameter of vocal aerodynamics, phonation threshold flow (PTF). The sensitivity of PTF and phonation threshold pressure (PTP) were quantitatively compared to the percent of vocal fold elongation from physiologic length.

Methods—Ten excised canine larynges were mounted on a bench apparatus capable of controlling vocal fold elongation. Subglottal airflow was gradually increased until the onset of phonation. Elongation of the vocal folds was varied from +0% (physiologic length) to +15%, and PTF and PTP were measured.

Results—Mean PTFs at physiologic vocal fold length ranged from 101 mL/s and 217 mL/s. No statistically significant relationship was found to exist between the size of the larynx and the measured PTF values ($p=.404$). The average percentage change of PTF compared to the magnitude of elongation was found to be statistically significant ($p<0.001$). Data indicated that PTF was proportional to the percent of vocal fold elongation.

Conclusion—PTF was positively correlated with vocal fold elongation and PTP for small magnitudes of elongation. The results suggest that PTF may be indicative of the biomechanical properties of the vocal folds, thus providing a possibly valuable tool in the clinical evaluation of laryngeal function.

Keywords

phonation threshold flow; phonation threshold pressure; excised larynx; vocal fold elongation; phonation

INTRODUCTION

During phonation, the vocal folds act as an energy transducer that converts the aerodynamic power produced by the respiratory system into the acoustic power heard as the human voice. Parameters describing this acoustic power can be measured, including fundamental frequency, intensity, jitter, shimmer, and glottal waveform (1). Recently, however, more attention has been placed on investigating the aerodynamic inputs of vocal function, such as subglottal pressure or phonation threshold pressure (PTP) (2,3). PTP is defined as the minimum subglottal pressure required to initiate vocal fold oscillation and produce voice and is reflective of the ease of phonation (4,5,6). The relationships between PTP and the physiological parameters of the vocal folds have been studied using excised larynx experiments (3,7). Specifically, vocal fold pathologies such as dehydration increase the PTP value (7). Accordingly it was suggested that the measurement of PTP in conjunction with other diagnostic tools could improve the precision of initial diagnosis for vocal pathologies.

This shift toward aerodynamic in addition to acoustic measurements of the voice was reinforced by Holmberg, *et al.*, who demonstrated that aerodynamic parameters are more indicative of vocal fold pathologies (8).

Non-invasive methods to measure aerodynamic parameters of the voice have not been easily integrated into the clinician's set of diagnostic tools. PTP could be clinically advantageous but it can be difficult to obtain accurate estimates (2). Previous studies have estimated subglottal pressure via labial interruption (9) but because this technique depends on patient control of airway occlusion, a high variability in timing and duration of closure results. Current PTP measurement techniques require that subjects maintain a consistent glottal configuration and subglottal pressure, even during airflow interruption. This can be difficult for untrained subjects, resulting in unreliable estimates of PTP. Additionally, if nasal airflow exists during oral airflow occlusion this would be a source of error in PTP estimation (2). There have been several attempts to improve upon these less reliable techniques, using balloon valves to mechanically control both complete and incomplete interruptions, with promising results (10,11). However, further development is necessary before these techniques become clinically viable.

Glottal airflow is another aerodynamic parameter dependent upon the physiological characteristics of the larynx. Airflow can be examined at a specific subglottal pressure during phonation to determine the impedance characteristics of the vocal path, which consists of the larynx and vocal tract. Empirical results from excised larynx experiments and clinical observations indicate that a discrete minimum airflow volume velocity threshold exists to achieve phonation. Recently, Jiang and Tao proposed a new aerodynamic parameter, the minimum glottal airflow for phonation (12). They theoretically demonstrated that this phonation threshold flow (PTF) is highly dependent upon the physiological properties and glottal configuration of the vocal folds; their theory predicts that PTF is minimized by decreasing tissue viscosity, vocal fold thickness, mucosal wave velocity, and pre-phonatory glottal area. The reduction of vocal tract resistance and a divergent glottal geometry is also predicted to decrease PTF. Because vocal fold pathologies have been clinically shown to alter vocal fold biomechanics and change pre-phonatory glottal width, PTF may be used to help characterize laryngeal health. Hottinger et al. observed experimentally that PTF was more sensitive than PTP to posterior glottal width changes, demonstrating that PTF may be more sensitive than PTP to pathological conditions (13) PTF has several advantages in the evaluation of vocal function. Unlike subglottal pressure, airflow can be measured non-invasively and directly using an extra-oral flow meter; the ease and directness of measurement of PTF provides a major clinical advantage. Titze reported that peak flow rates vary almost linearly with changing subglottal pressure (6). If this relationship persists in low PTP and PTF ranges, it may be possible to infer PTP values from measured PTF values.

Sufficient experimental studies on PTF are still needed before it can be used clinically. This study proposes to investigate this new parameter, PTF, defined as the minimum glottal airflow to produce phonation. This is accomplished by assessing the reliability and range of PTF measurements using excised canine larynges under controlled elongation and comparing these measurements to their corresponding PTP measurements. By evaluating the effects of varying physical parameters of the vocal folds, we hope to develop a model describing how PTF measurements might vary under a pathological condition of the vocal folds and how PTF and PTP are related. We hypothesize that PTF is directly related to PTP in the ranges of pressure and flow where phonation is initiated. It has been hypothesized that physiological excised canine larynx PTF measurements will fall within a range of values less than those for excised canine larynges with simulated pathologies. As a method of

testing this hypothesis, the vocal folds will be elongated to adjust their biomechanical properties, and PTF and PTP measurements will be recorded.

MATERIALS AND METHODS

Laryngeal Model

Ten larynges were obtained immediately postmortem from adult canines not sacrificed for this study. The larynges were evaluated to rule out the presence of any diseases or lesions. Larynges were stored in 0.9% saline solution until use. The larynges were mounted on a pipe attached to a pseudolung via a metal clamp fixed tightly around the trachea. Two three-pronged devices were inserted into each arytenoid cartilage to stabilize the larynx. The three-pronged devices could be translated and rotated in all Cartesian planes using attached micrometers. Each larynx was mounted in a midsagittally symmetrical position using the micrometer controls. The anterior edge of the thyroid cartilage was sutured to a micrometer used to control elongation of the vocal folds. Figure 1 illustrates the setup. After the larynx was mounted, digital calipers were used to record the physiologic vocal fold length.

Experimental setup

All measurements were carried out in a triple-walled sound-isolated room, designed to eliminate background noise and stabilize room temperature and humidity levels. The experimental bench apparatus shown in Figure 1 was built to create and monitor laryngeal phonation. A pressurized air source and pseudolung with pressure damping characteristics were used to generate air pressure at constant and measurable levels. Subglottal airflow was conditioned to 95% to 98% humidity at 36° to 38° C using two Concha Therm III humidifiers in series.

An Omega airflow meter (model FMA-1610A) was connected to the pseudo-respiratory tract beneath the vocal folds to record airflow. The acoustic signal was recorded using a Sony microphone (model ECM-88) and a Symmetrix pre-amplifier (model 302), mounted 10 cm from the larynx at 45° from the tracheal axis of the larynx, facing the glottis. A Heise digital pressure meter (901 series) was connected to the pseudo-respiratory tract below the vocal folds to monitor subglottal pressure. Data from the voltage output of the airflow meter, microphone, and pressure meter were relayed via baby-N connector cable to a National Instruments data acquisition board (model AT-MIO-16) and then digitized using Labview 7.1 custom-programmed software. The custom Labview software was designed to simultaneously measure airflow, acoustic, and pressure signals. The PTF was calculated by the software as the flow reading when the root mean square of the acoustic signal rises above that of the residual noise present in the system (Figure 2). For comparison purposes, the phonation threshold pressure (PTP) was the subglottal pressure observed at the time the PTF was measured.

Determination of PTF and PTP from excised larynx experiments

For each larynx, the physiologic length of the vocal folds was measured as described by Jiang, *et al* (1). To determine PTF, airflow through each larynx was gradually increased from 0 L/min until phonation could be both heard and observed on the custom Lab View 7.1 program used to record the acoustic signal (Figure 2). The observed airflow and pressure at this time were recorded as the PTF and PTP, respectively. Five trials were performed for each larynx. Figure 2 displays typical airflow, pressure, and acoustic trace graphs collected using the custom Lab View 7.1 software.

Data were also obtained at the threshold levels under conditions of vocal fold elongation. Five trials were performed at different elongation levels with each larynx. To manipulate

vocal fold elongation, a micrometer (Starrett No. 262 Series) was sutured to the laryngeal prominence of the thyroid cartilage (Figure 1). PTF was observed at vocal fold elongation levels in increments of +5% of the physiologic vocal fold length from +0% to +15%. Using this method, the PTF and PTP often canine larynges under each condition of vocal fold elongation (+0%, +5%, +10%, and +15%) were obtained.

The statistical analysis software SigmaStat 3.0 (Jandel Scientific, San Rafael, CA) was used to analyze the data and determine the PTP and PTF. Spearman rank order correlation analysis was performed to determine if a relationship exists between vocal fold length and PTF. Spearman rank order correlation analysis was also performed to determine if a relationship exists between PTF and PTP. Data was then analyzed to determine the range of PTF in the ten sample larynges. A Kruskal-Wallis ANOVA (analysis of variance) on ranks was used to determine if significant differences existed in PTF at different degrees of elongation in comparison to PTF at the physiologic length.

Changes in PTF due to elongation were transformed into a percentage change based on the average PTF at physiologic vocal fold length. Because the observed data were not observed to be normally distributed populations with equal variances, the Mann-Whitney rank sum test was performed, using PTF as the dependent variable and the elongation level (+0%, +5%, +10%, and +15%) as the independent variable, to determine if significant differences existed across all the larynges tested.

RESULTS

Figure 3 shows the mean onset PTF values at physiologic length and the mean PTF values along with standard deviations for each of the 10 larynges. Mean PTF values were between 101 mL/s and 217 mL/s. The overall mean PTF value at physiologic length was 163 mL/s ($\sigma = 34$ mL/s). The correlation coefficient between larynx size and mean PTF was found to be -0.285 , and the p -value for this correlation was 0.404 with a significance level of 0.05, indicating that no significant correlation between larynx size and PTF was observed.

Figure 4 shows the changes in mean PTF values for one larynx (larynx 4). ANOVA confirmed that statistically significant differences in PTF were observed between each elongation level. The mean PTF value increased from 160.56 mL/s at +0% elongation (physiologic length) to 181.14 mL/s at +5% elongation ($p=0.003$). Mean PTF continued to increase, with values of 233.65 mL/s and 313.33 mL/s at elongation levels of +10% and +15%, respectively. P -values for each increase in elongation were less than 0.001.

The results from larynx 4 are typical of the expected relationship between PTF and elongation level of the vocal folds, as demonstrated by the aggregate data (Table I). Comparisons between PTF values at an equal elongation level for a given larynx are expressed as percent deviation from mean PTF of that larynx at physiologic vocal fold length (Figure 5). The mean PTF percent for all larynges increases 14.6% from physiologic length at +5% elongation and is statistically significant ($p=0.0256$). The percentage increases 33.4% from physiologic length at +10% elongation; however, this difference was not found to be statistically significant ($p=0.123$). At +15% elongation, the mean PTF percent increases 131.2% from physiologic length, greater than the mean PTF percent increase at both +5% and +10% elongation levels, and these differences were statistically significant ($p<0.001$ and $p=0.001$, respectively).

Spearman rank order correlation analysis was performed to determine the possible relationship between phonation threshold flow (PTF) and phonation threshold pressure (PTP) values at each elongation level (confidence level $\alpha=0.05$), as seen in Table II. Positive correlation coefficients show that PTP and PTF exhibit some degree of positive

proportionality, and this correlation was found to be statistically significant for the elongation levels of +0% and +5% ($p < 0.001$ for both cases). At +10% and +15% elongation levels, a positive correlation coefficient was observed, but was not statistically significant ($p = 0.054$ and $p = 0.059$, respectively).

DISCUSSION

Research in laryngology has shifted to investigate the use of aerodynamic parameters in addition to acoustic parameters of the human voice in the assessment of laryngeal function. This is illustrated by the measurement of subglottal pressure (SGP) to indicate pathologies may exist. If physiologic and pathologic parameter value ranges are compiled, simple comparisons between a patient's measured value and known values could aid in the diagnostic process. However, SGP measurement is hindered by its impracticality in direct measurement methods and unreliability in indirect measurement methods. Glottal airflow provides the same indication of laryngeal function and when coupled with SGP, can describe the impedance characteristics of an individual's vocal path - the larynx and vocal tract. Accordingly, this study proposed using the minimum glottal airflow for phonation, the phonation threshold flow (PTF), as an indicator of the possible presence of laryngeal pathologies.

Results from this study support theory suggesting that PTF is dependent upon the biomechanical and physiological properties of the vocal folds. Ten excised canine larynges were mounted on a bench apparatus with physiologic thyroarytenoid (TA) muscle tension and no visible pre-phonatory glottal gap. Normal PTF ranged from 101 mL/s to 217 mL/s. Correlation test results indicated each larynx had a consistent, unique PTF, and Spearmann rank sum correlation analysis indicated that no relationship was observed between the physiologic length of the vocal folds and the PTF ($R^2 = -0.285$, $p = 0.404$). This suggests that a normative range of PTF values can be determined for a species, and further suggests that PTF could be used as a diagnostic tool to characterize laryngeal function.

Our preliminary research into the effect of vocal fold elongation on PTF confirmed our hypothesis that PTF increases with increasing elongation. The increased elongation of the vocal folds increases tension – this biomechanical property is positively and directly related to the PTF. PTF values based on elongation percent for individual larynges (Figure 4) and for the mean of the population of ten larynges (Figure 5) substantiate this relationship. In some isolated trials this trend was not observed, illustrating the effect of the numerous variables involved in an excised larynx phonation experiment: the thickness of the vocal folds, the degree of abduction or adduction, and the midsagittal and transverse symmetry of the contralateral folds. It is possible that during the elongation process, these confounding variables were inadvertently affected. The insertion of three micrometers in each arytenoid provided consistent positioning; however, adduction may have been minutely altered during elongation despite accommodative efforts.

The strength of the predicted relationship between PTP and PTF was shown to be statistically significant for physiologic larynges. For larynges mounted with +0% (physiologic length) and +5% elongated vocal folds, positively proportional, statistically significant regressions were found relating PTP and PTF using a Spearmann rank order correlation analysis ($R^2 = 0.481$, 0.591 ; $p < 0.001$). For larynges mounted with +10% and +15% elongated vocal folds, this correlation was weaker and not statistically significant ($R^2 = 0.275$, $p = 0.0536$ and $R^2 = 0.283$, $p = 0.0594$, respectively). Because many vocal pathologies increase the tension of the vocal folds, such as vocal scarring or Parkinson's disease, the degradation of the relationship between PTP and PTF could be indicative of pathology.

Future studies on PTF are required to investigate further its use as a diagnostic tool to characterize laryngeal function. The effects of other variables, such as vocal fold thickness, level of hydration, and glottal configuration, should be investigated to determine their effects on PTF. Computer models may help elucidate the effect of these biomechanical variables on PTF. Future studies should explore the clinical implications of PTF in human models. Normative ranges of values for the PTF in human subjects with physiologic larynges could be compiled, as well as the normative range of PTF for human subjects with vocal pathologies. Naturally, it may be seen that many pathologies have overlapping ranges of PTF values and that this measure may provide an indicator that laryngeal function is likely abnormal. Future studies on the airflow and pressure at phonation onset would also be beneficial to characterize the correlation between PTF and PTP in the case of pathologies.

CONCLUSION

PTF and PTP measurements were obtained from ten canine larynges at four different levels of vocal fold elongation to simulate added tension of the tissue. Statistical analyses of the results indicated that PTF is dependent upon the tension of the vocal folds. Given that PTF is easily measured directly and non-invasively, these data suggest that PTF may be a clinically advantageous diagnostic tool in the general assessment of laryngeal function.

Acknowledgments

This work was supported by NIH Grant No. 1-R01 DC006019.

REFERENCES

1. Jiang J, Chang CB, Raviv JR, Gupta S, Bansali FM, Hanson D. Quantitative study of mucosal wave via videokymography in canine larynges. *Laryngoscope* 2000;110:1567–1573. [PubMed: 10983964]
2. Fischer KV, Swank PR. Estimating phonation threshold pressure. *J Speech Hear Lang Res* 1997;40:1122–1129.
3. Jiang J, Verdolini K, Aquino B, Ng J, Hanson D. Effects of dehydration on phonation in excised canine larynges. *Ann Otol Rhinol Laryngol* 2000;109:568–575. [PubMed: 10855568]
4. Titze IR. On the relationship between subglottal pressure and fundamental frequency in phonation. *J Acoust Soc Am* 1989;85:901–906. [PubMed: 2926005]
5. Titze IR. Phonation threshold pressure: A missing link in glottal aerodynamics. *J Acoust Soc Am* 1992;91:2926–2935. [PubMed: 1629485]
6. Titze IR. Vocal efficiency. *J Voice* 1992;6:135–138.
7. Verdolini K, Min Y, Titze IR, Lemke J, Brown K, Van Mersbergen M, Jiang J, Fisher K. Biological mechanisms underlying voice changes due to dehydration. *J Speech Hear Res* 2002;45:268–281.
8. Holmberg EB, Doyle P, Perkell JS, Hammarberg B, Hillman RE. Aerodynamic and acoustic voice measurements of patients with vocal nodules: variation in baseline and changes across voice therapy. *J Voice* 2003;17:269–282. [PubMed: 14513951]
9. Bard MC, Slavit DH, McCaffrey TV, Lipton RJ. Noninvasive technique for estimating subglottic pressure and laryngeal efficiency. *Ann Otol Rhinol Laryngol* 1992;101:578–582. [PubMed: 1626903]
10. Jiang J, O'Mara T, Conley D, Hanson D. Phonation threshold pressure measurements during phonation by airflow interruption. *Laryngoscope* 1999;109:425–432. [PubMed: 10089970]
11. Jiang J, Leder C, Bichler A. Estimating subglottal pressure using incomplete airflow interruption. *Laryngoscope* 2006;116:89–92. [PubMed: 16481816]
12. Jiang J, Tao C. The minimum glottal airflow to initiate vocal fold oscillation. *J Acoust Soc Am* 2007;121:2873–81. [PubMed: 17550186]

13. Hottinger DG, Tao C, Jiang JJ. Comparing Phonation Threshold Flow and Pressure by Abducting Excised Larynges. *Laryngoscope*. in press.

Posterior

Anterior

Figure 1.

(a) The excised larynx experimental setup; (b) an excised canine larynx mounted on the bench apparatus.

Figure 2. Typical graph traces of acoustic, airflow, and pressure measurements as a function of time. The vertical bar indicates the onset of phonation, and thus PTF and PTP in their respective traces.

Figure 3.

The mean phonation threshold flow values for each larynx. Error bars indicate the standard deviations from the mean.

Figure 4.

The effects of vocal fold elongation from physiologic length on PTF in a typical larynx. PTF significantly increased with increased vocal fold elongation in the majority of the larynges tested. The upper and lower edges of the box represent the 1st and 3rd quartile and the middle lines represent the median PTF for the given elongation level.

Figure 5.

The cumulative effect of vocal fold elongation on PTF from all collected samples. The upper and lower edges of the box represent the 75th and 25th percentile, respectively, and a line within each box marks the median PTF for the given elongation level. Whiskers above and below each box represent the 90th and 10th percentiles, respectively. Statistical outliers are graphed as points.

Table I

Aggregate PTF data for all sample larynges.

Elongation Level	Mean PTF (mL/s)	Standard Deviation
+0%	160.56	6.65
+5%	181.14	4.47
+10%	233.65	48.59
+15%	313.33	32.72

Table II

Strength of correlation between PTP and PTF.

Elongation Level	+0%	+5%	+10%	+15%
Correlation coefficient	0.481	0.591	0.275	0.283
<i>p</i> Value	0.000	0.000	0.0536	0.0594
Number of samples	50	50	50	45