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Phonation instability flow in excised canine larynges

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Abstract

Objective—Disordered voices are often associated with abnormal changes in aerodynamic parameters of subglottal pressure and airflow. Phonation instability pressure (PIP) has been previously proposed to evaluate subglottal pressure at the onset of chaotic phonation. We propose the concept of and measure phonation instability flow (PIF), the airflow at which phonation becomes chaotic. Phonation flow range (PFR), PIF minus phonation threshold flow (PTF), is proposed to assess the range over which normal vocal fold vibration occurs.

Study Design—Repeated measures with each ex vivo larynx serving as its own control.

Methods—Pressure and airflow were measured at phonation onset and chaos onset in seven excised canine larynges under three experimental conditions: 0% elongation with no glottal gap; 20% elongation with no glottal gap; 20% elongation with a 3 mm posterior glottal gap. Paired t-tests were performed to determine if experimental measurements differed between elongations (0% and 20%) or degrees of abduction (20% elongation with and without a 3 mm glottal gap).

Results—Both PIF and PFR were dependent on abduction but not elongation. PIP was not significantly dependent on either condition. PIF and PFR showed greater differences for abduction than either phonation threshold pressure (PTP) or PTF.

Conclusions—PIF and PFR may be useful parameters in the experimental or clinical settings, particularly when evaluating disorders characterized by a glottal gap such as vocal fold paralysis and presbylaryngis.

Keywords

phonation instability flow; phonation flow range; phonation instability pressure; phonation threshold flow; subglottal pressure; excised larynx

INTRODUCTION

Aerodynamic parameters such as subglottal pressure (P_s), mean flow rate (MFR), and vocal efficiency (V_E)¹ provide valuable information on the inputs to voice production and can be used in the assessment of laryngeal pathology. V_E is equal to acoustic energy divided by aerodynamic energy and reflects how efficiently the larynx transduces energy. Patients with Parkinson's disease and bilateral vocal fold lesions exhibit elevated P_s ^{2,3} while patients with

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vocal fold paresis exhibit increased MFR⁴. Decreases in V_E due to polyps and nodules have also been observed⁵. Aerodynamic assessment may even be more sensitive than acoustic measurements when diagnosing vocal fold nodules⁶.

Current assessment typically involves measuring P_s or airflow during sustained phonation or at the phonation threshold. Measurement of phonation threshold pressure (PTP) and phonation threshold flow (PTF) can provide valuable insights into the effort required to initiate vocal fold oscillation^{7,8}; however, they do not provide information on the aerodynamic characteristics of chaotic voice production.

Jiang and Titze proposed the concept of phonation instability pressure (PIP), the P_s at which vocal fold vibration becomes irregular⁹. Zhang et al. revealed the nonlinear mechanisms underlying PIP and proposed the concept of phonation pressure range (PPR), the difference between PIP and PTP, to describe the range of subglottal pressures over which normal phonation occurs¹⁰. While these parameters may be valuable in assessing chaotic voice, they do not provide a comprehensive picture of aerodynamic activity. Complete characterization of aerodynamic behavior at the instability threshold requires consideration of airflow as well.

Clinically, airflow is easier to measure than P_s as only an external flow transducer and circumferentially vented airflow mask are required. Noninvasive measurement of P_s requires either labial or mechanical interruption^{7,11,12} and can be variable due to difficulties performing the task^{13,14} or the elicitation of laryngeal reflexes¹⁵. Airflow measurement is also more sensitive than pressure in disorders characterized by a glottal gap, such as vocal nodules¹⁶, presbylaryngis, and vocal fold paralysis¹⁷. Hottinger et al. found an increase in PTF while PTP remained unchanged at varying levels of glottal abduction¹⁸. During sustained phonation, airflow is correlated with the efficiency with which the larynx transduces aerodynamic energy into acoustic energy^{1,2}. Investigating the airflow at which vocal fold vibration becomes irregular may provide insight into disordered voice production and produce another airflow parameter which has clinical value.

We measured phonation instability flow (PIF), the minimum airflow required for chaotic voice production. Phonation flow range (PFR), the magnitude of the difference between PIF and PTF, is also proposed to describe the airflow values at which normal vocal fold vibration occurs.

MATERIALS AND METHODS

Larynges

Seven larynges were excised postmortem from canines sacrificed for non-research purposes according to the protocol described by Jiang and Titze⁹. As the size and histological properties of the canine and human larynx are similar^{19,20}, it is an appropriate model for studying human laryngeal physiology. Larynges were examined for evidence of trauma or disorders; any larynges exhibiting trauma or disorders were excluded. Following visual inspection, larynges were frozen in 0.9% saline solution using regular freezing at -20°C .

Apparatus

Prior to the experiment, the epiglottis, corniculate cartilages, cuneiform cartilages, and ventricular folds were dissected away to expose the true vocal folds. The superior cornu and posterosuperior part of the thyroid cartilage ipsilateral to the normal vocal fold were also dissected away to facilitate insertion of a lateral 3-pronged micrometer into the arytenoid cartilage. The larynx was mounted on the excised larynx bench apparatus as specified by Jiang and Titze⁹ (figure 1). A metal hose clamp was used to stabilize the trachea to a tube

connected to a pseudolung which served as a constant pressure source. Stabilization of the larynx was accomplished with two lateral three-pronged devices, one inserted into each arytenoid cartilage. The anterior edge of the thyroid cartilage was then sutured to an additional positioning device, which could be adjusted to facilitate vocal fold elongation. After the larynx was positioned, the length from the glottal anterior to posterior sides was measured with a digital caliper and recorded as the resting vocal fold length at 0% elongation.

The pseudolung used to initiate and sustain phonation in these trials was designed to simulate the human respiratory system. It has a subglottal vocal tract length of approximately 20 cm. Pressurized airflow was passed through two humidifiers (MR-410 Fisher & Paykel Healthcare Inc., Laguna Hills, California) in series to humidify and warm the air. The potential for dehydration was further decreased by frequent application of 0.9% saline solution between trials. Airflow was controlled manually and was measured using an Omega airflow meter (model FMA-1601A, Omega Engineering Inc., Stamford, Connecticut). Pressure measurements were taken immediately before the air passed into the larynx using a Heise Model HPO pressure transducer (Ashcroft Inc., Stratford, Connecticut) mounted perpendicular to the airflow stream.

Acoustic data were collected using a dbx microphone (model RTA-M, dbx Professional Products, Sandy, Utah) positioned at a 45° angle to the vocal folds. The microphone was placed 10 cm from the glottis to minimize acoustic noise produced by airflow. Acoustic signals were subsequently amplified by a Symetrix preamplifier (model 302, Symetrix Inc., Mountlake Terrace, Washington). A National Instruments data acquisition board (model USB6229; National Instruments Corp, Austin, Texas) and customized LabVIEW 8.5 software were used to record airflow, pressure, and acoustic signals on a personal computer. Aerodynamic data were recorded at a sampling rate of 100 Hz and acoustic data at 40,000 Hz. Experiments were conducted in a triple-walled, sound attenuated room to reduce background noise and stabilize humidity levels and temperature.

Experimental Methods

Airflow was gradually increased until the phonation threshold, at which time pressure and flow were recorded as PTP and PTF, respectively. Airflow was then increased to the instability threshold, determined visually using a real-time spectrogram and defined as the point at which stable harmonic frequency structure is lost and only aperiodic phonation with noise-like broadband spectra are observed occurs. This represents the onset of a type 3 voice signal, which is characterized by a lack of apparent periodic structure²¹. Phonation instability can also be described as deterministic chaos featuring a broadband spectrum with energy at many different frequencies²². Pressure and airflow at this point were determined to be the PIP and PIF, respectively (figure 2). After reaching the instability threshold, airflow was gradually decreased until phonation ceased. Trials were followed by five second periods of rest. Phonation flow range (PFR) was calculated by subtracting PTF from PIF. Five trials were performed for each of three conditions: 0% elongation with a 0 mm posterior glottal gap; 20% elongation with a 0 mm posterior glottal gap; and 20% elongation with a 3 mm posterior glottal gap. The glottal gap was achieved via symmetric lateralization of the 3-pronged micrometers. Condition order was varied across larynges to address any potentially confounding effect of fatigue.

To ensure the chaotic vocal fold vibration did not cause damage to the vocal folds, PTP and PTF were analyzed across trials within each experimental condition. Though PTP and PTF are expected to vary across the three experimental conditions due to changes in glottal configuration, each should be consistent across trials within a condition if the tissue is not damaged. One-way analysis of variance (ANOVA) was performed to compare the mean

PTP and PTF of each larynx for the five experimental trials. The tests confirmed that PTP and PTF were not different across trials within a condition (table 1).

Statistical analysis

Paired t-tests were performed to determine if there were significant differences across elongation or abduction. If data did not meet assumptions for parametric testing, a Wilcoxon-Mann-Whitney signed rank test was used. Tests were two-tailed and a significance level of $\alpha=0.05$ was used.

RESULTS

Summary data are presented in table 2. Increasing elongation from 0% to 20% did not affect PTF, PIF (figure 3), or PIP (figure 4). The increase in PTP following increased elongation approached significance ($p = 0.085$). Increasing glottal abduction had significant effects on PTP ($p = 0.016$), PIF ($p < 0.001$) (figure 3), and PFR ($p < 0.001$), as well as near significant effects on PTF ($p = 0.055$). No effect on PIP or PPR was observed.

DISCUSSION

A new phonatory parameter, phonation instability flow, is presented. PIF was sensitive to glottal abduction, but did not vary significantly due to elongation. As airflow parameters are more sensitive to glottal width than vocal fold length, this was expected^{18,23}. For example, PTF has a cubic relationship with neutral glottal half-width, and only a linear relationship with vocal fold length²³. PIP was not responsive to changes in either elongation or abduction. While we did not expect PIP to be dependent on abduction, we expected to observe a greater change following elongation. An increase in PIP did occur when elongating the vocal folds, but this increase was not statistically significant. A slight decrease in PTP occurred due to glottal abduction. This observation was in accordance with the hydraulic analogy of Ohm's law, where subglottal pressure is equal to airflow multiplied by laryngeal resistance. As you abduct the vocal folds and open the glottis, the resistance across the glottis decreases significantly and the pressure decreases accordingly. This change can be overpowered if there is a greater increase in airflow, but this did not occur.

Contrary to our predictions and previous results,^{10,24} there was no significant relationship between vocal fold elongation and PTP or PTF. There are several possible explanations for this disparity. Previous studies examining PTF, PTP, and PIP with increasing elongation did not vary task order; trial sets were performed consistently with increasing degrees of elongation. This may potentially introduce a degree of vocal fatigue, resulting in higher threshold values at greater elongations. By varying task order as we did in this study, we may have introduced subtle changes in glottal configuration that affected the aerodynamic properties of the larynx. Also, previous experiments looked at elongations of 0%, 5%, 10%, and 15% and found that the variance in the data increased somewhat dramatically at greater elongations. It is possible that 20% elongation may be too extreme for experiments analyzing threshold aerodynamics and lesser degrees of elongation should be used preferentially in the future, or multiple degrees of elongation should be used to determine if an observable trend is present. Lastly, inducing chaos in the larynxes may have affected the phonation threshold measurements. While we attempted to analyze this potential phenomenon by evaluating how PTF and PTP changed or did not change within each condition using ANOVA, subtle changes may have occurred which were amplified across conditions. The results from this study coupled with those from previous studies^{10,24} warrant a rigorous investigation evaluating how phonation threshold parameters and instability threshold parameters are affected by vocal fold elongation.

PTF has been the topic of numerous studies recently^{8,18,25,26}. It has been demonstrated previously¹⁸ and in this study to increase with glottal abduction. However, the change in PTF did not quite reach statistical significance, while the change in PIF was highly significant. Continued investigation of the value of PIF in assessing disorders characterized by glottal abduction is warranted. Also, comparing the threshold aerodynamic parameters of interest in a wide variety of experimental conditions may further elucidate which parameters are most sensitive to specific laryngeal pathologies.

Titze described three types of voice signals²¹. Type 1 voice is nearly periodic; type 2 has strong modulations or subharmonics; type 3 is irregular or aperiodic. Increasing airflow resulted in changes in the acoustic spectra, from a periodic voice signal (type 1) to prominent subharmonic frequencies (type 2) to a signal with no apparent periodic structure (type 3). As chaotic acoustic behavior represents the onset of type 3 voice, the boundaries of PFR provide the flow range of type 1 and 2 voice signals. While paired t-tests analyzing the effects of elongation and glottal abduction on PFR yielded no significant differences, a significant effect of abduction was observed for PFR. PFR was significantly increased by abducting the vocal folds. As this glottal configuration is associated with higher flow values, this was expected. In future experiments, studying the relative contribution of each voice signal type to the flow range may provide additional information which may aid in evaluating voice production. One could expect type 1 voice to occur over a relatively greater proportion of the flow range for a larynx with a normal glottal configuration. Though a larynx with an abducted glottal configuration will have a greater PFR, type 2 voicing may occur over much of that flow range. Additional experiments with more precise control over airflow will be conducted to investigate this relationship.

It has been suggested that PTP and PIP may represent important parameters for the initial assessment of vocal pathology¹⁰. While measurement of P_s is valuable, it is more difficult to measure than airflow. Noninvasive measurement of P_s requires interruption via repetitive labial plosives¹¹ or mechanical interruption^{7,12}, while airflow can be measured using only a mask with an external flow transducer²⁷. Evaluating the instability threshold with airflow measurement instead of pressure may offer a simpler means of quantifying aerodynamic activity at the onset of chaotic vocal fold vibration which could provide insight into the mechanism of chaotic voice production.

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Figure 1. Schematic of the excised larynx setup. A: Flow Regulator B: Flowmeter C: Humidifiers (2) D: Pseudolung E: Larynx and positioning mechanism F: Pressure Transducer G: Microphone H: High Speed Camera. Note: Data from B, F, G, and H are recorded and digitized (electrical connections not shown).

Figure 2. Sample data trace displaying selection of phonation onset (left line) and corresponding measurement of phonation threshold flow and phonation threshold pressure as well as chaos onset (right line) and corresponding measurement of phonation instability flow and phonation instability pressure.

Figure 3.

Box plots displaying phonation instability flow (PIF) values across experimental conditions. PIF was significantly different between 20% elongation with 0 mm glottal gap (middle) and 20% elongation with 3 mm glottal gap (right).

Figure 4. Box plots displaying phonation instability pressure (PIP) values across experimental conditions. PIP was not significantly different between elongation conditions or abduction conditions.

Table 1

Phonation threshold pressure (PTP) and phonation threshold flow (PTF) across trials within each experimental condition.

Parameter	Condition	p-value
PTP	0% elongation	0.846
	20% elongation	0.750
	3 mm abduction	0.966
PTF	0% elongation	0.985
	20% elongation	0.750
	3 mm abduction	0.966

P-values represent the results of one-way analysis of variance (ANOVA) tests comparing PTP and PTF across trials within each condition.

Table 2

Summary data for the three experimental conditions.

Parameter	Condition			
	0% elongation	20% elongation	3 mm abduction	p-value
PTP	6.18 ± 3.47	10.23 ± 7.54	4.88 ± 5.58	0.016
PTF	16 ± 12	16 ± 13	35 ± 28	0.055
PIP	33.41 ± 17.54	40.62 ± 20.81	35.23 ± 13.54	0.488
PIF	76 ± 31	67 ± 41	136 ± 43	<0.001
PPR	27.23 ± 15.87	30.39 ± 23.54	30.35 ± 14.12	0.997
PFR	59 ± 29	51 ± 46	101 ± 47	<0.001

P-values represent the results of paired t-tests comparing 0% and 20% elongation, and 20% elongation and 3 mm abduction. PTP = phonation threshold pressure (cmH₂O); PTF = phonation threshold flow (L/min); PIP = phonation instability pressure (cmH₂O); PIF = phonation instability flow (L/min); PPR = phonation pressure range (cmH₂O); PFR = phonation flow range (L/min).