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Variability of the Aerodynamic Measures of Leporine Larynges Exposed to Inhaled Corticosteroids

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Master of Science

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ABSTRACT

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This thesis examined the effects of combination inhaled corticosteroids (ICs) on the stability of six aerodynamic measures of phonation utilizing a traditional benchtop model with leporine larynges. The motivation for this study was based on the increase of voice disorders associated with IC use in recent years. The aerodynamic measures examined were phonation threshold pressure (PTP), phonation threshold flow (PTF), onset resistance, sustained pressure, sustained flow, and sustained resistance. Leporine larynges were selected as the model for this study due to histological similarities between leporine and human vocal folds that make them ideal for translational research. Rabbits were either exposed to saline solution or ICs for 8 weeks before being sacrificed. After being sacrificed, larynges were excised and dissected. After dissection, the larynges were mounted on a benchtop, the aerodynamic data were gathered, and stability over multiple phonation trials was calculated. The results indicate that the variation between individual rabbits across the measures did not differ significantly. However, after controlling for trial, the average variation of the groups across all trials did differ significantly. PTP and sustained pressure were more variable for the inhaler group, while PTF, sustained flow, onset resistance, and sustained resistance were more variable for the control group. These results suggest that some level of variability in aerodynamic measures both within and between subjects is to be expected when using the leporine benchtop model. Furthermore, while IC exposure does not seem to impact within-subject variability, it does influence between-subjects variability.

Keywords: phonation pressure, phonation flow, laryngeal resistance, excised larynx, benchtop model

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DESCRIPTION OF THESIS STRUCTURE AND CONTENT

This thesis, *Variability of the Aerodynamic Measures of Leporine Larynges Exposed to Inhaled Corticosteroids,* is written in a journal-style format. The research for this thesis was funded by research grants awarded to Dr. Kristine Tanner by the David O. McKay School of Education at Brigham Young University and National Institute on Deafness and Other Communication Disorders, National Institutes of Health (1R01DC016269-01A1). The research conducted for this thesis is part of a five-year project conducted under the direction Dr. Kristine Tanner. Data collected across the 5 years will be synthesized for an article that will be published in a peer-reviewed journal. Appendix A contains an annotated bibliography. Appendix B lists the materials used in the dissection, data acquisition, and for the benchtop and phonation trials. Appendix C contains the protocol for setting up LabChart™. Appendix D details the protocol for pressure calibration prior to collecting data and Appendix E contains the protocol for flow calibration. Appendix F lists the protocol for both preparing and dissecting the rabbit tissue. Appendix G details the data acquisition protocol. Appendix H contains the instructions for both data segmentation and analysis. Appendix I contains the raw data used to calculate the coefficient of variation for each parameter. Appendix J lists the geometric and anatomical data for each larynx. Finally, Appendix K contains the thesis timeline.

Introduction

Voice disorders are communication impairments associated primarily with abnormal laryngeal function. Respiratory, phonatory, and resonance changes may contribute to voice disorders. These changes may be structural, functional, or neurological in nature, including potential combinations of these changes. It is estimated that approximately 6% of the general population has a current voice disorder (Roy et al., 2005; Roy et al., 2004). Additionally, incidence and prevalence studies indicate that the frequency of voice disorders is likely underestimated, particularly in children, the elderly, and those populations that have limited access to healthcare (Roy et al., 2007). Individuals who have high occupational voice demands, such as teachers, are particularly at risk for voice disorders (Cohen et al., 2012; Roy et al., 2004). For these individuals voice disorders can have a significant functional impact on their lives.

Adverse Impact of Voice Disorders

Voice disorders are known to have adverse occupational, psychosocial, and health function effects (Merrill et al., 2013; Roy et al., 2005; Roy et al., 2004). Estimates suggest that for around 25% of the work force, voice use is essential (Verdolini & Ramig, 2001). These data suggest that for a high percentage of the population, having a voice disorder could have a significant functional impact on life, including on one's ability to maintain gainful employment. This is supported by survey data collected from individuals with voice-related difficulties, where 7.2% of employed survey participants indicated that they had to miss at least one day of work in the previous year due to voice-related difficulties (Roy et al., 2005). This study further found that other individuals had to limit certain work-related activities due to voice difficulties.

Voice disorders can also lead individuals to limit their participation in a variety of non-work-related activities. One study found that of 174 individuals seeking voice treatments, 75% of

participants identified experiencing social isolation due to the negative impact of voice disorders on social interactions, with 65% identifying an increase in depression after developing a voice disorder (Verdonlini & Ramig, 2001). These findings suggest that voice disorders are not simply an inconvenience but rather have a detrimental impact on social and emotional health.

Asthma and Associated Voice Disorders

One health condition that has received recent attention in the voice disorders literature is asthma (Erickson & Sivasankar, 2010; Gallivan et al., 2007; Sahrawat et al., 2014). Data collected by the Centers for Disease Control and Prevention (CDC) in 2018 suggest that nearly 25 million individuals in the United States suffer from chronic asthma. When an individual has asthma, they have a hyperresponsive airway that responds adversely to certain allergens. These allergens cause inflammation of an individual's airway mucosa, excess mucous production, and the lung's bronchi smooth muscles to contract. This causes a narrowing of the airway, resulting in dyspnea (Doeing & Solway, 2013; Ihre et al., 2004). These reactions cause symptoms such as breathlessness, chest pain, and wheezing. In addition to the more obvious limitations in respiration, phonation, and resonance associated with airway impairment, chronic inflammatory changes may also be related to voice disorders in this population. It has been estimated that as many as 50% of individuals who have asthma have voice disorders (Hassen & Hasseba, 2016). Due to the prevalence of asthma, and trends indicating a rise in prevalence over the past few decades, the population of individuals with asthma is an important group in the study of voice disorders.

Many people with asthma manage their breathing using daily maintenance inhalers. These inhalers most commonly include the combination of a long-acting beta agonist (LABA) and a corticosteroid; together, these are known as combination inhaled corticosteroids (ICs). IC

treatments act by either increasing or decreasing gene transcription leading to alterations in protein synthesis. These changes ultimately decrease the number of inflammatory cells that are recruited in an individual's airway and thus leads to a decrease in airway hyperresponsiveness and an overall decrease in mucosal inflammation (Barnes, 2010; Gallivan et al., 2007). Although IC use helps prevent airway hyperresponsiveness, ICs have a number of both systemic and local side effects (Gallivan et al., 2007). The use of ICs specifically has been associated with voice disorders in the literature (e.g., Erickson & Sivasankar, 2010; Hassan & Hasaba, 2016; Ihre et al., 2004; Lavy, 2000; Sahrawat et al., 2014). Hassen and Hassaba (2016) found that of the 30 individuals who had bronchial asthma included in their study, 53% had some level of dysphonia. Another study examining individuals with dysphonia who used ICs as treatment for respiratory illness found that 79% of individuals had abnormalities in mucosal wave symmetry, 74% had incomplete phase closure, 63% had abnormal glottic closure, 50% had abnormalities in the magnitude of the mucosal wave, and 38% had abnormalities with the free edge of the vocal folds (Gallivan et al., 2007). Although the exact pathogenesis of this dysphonia is not completely understood, some study findings suggest that the LABA portion of the combination inhaler might be the causative factor in vocal fold epithelial changes associated with these disorders (Levendoski et al., 2014; Sivasankar & Blazer-Yost, 2009). This theory is also compatible with the general assumption that steroids produce anti-inflammatory effects in the body. That said, it is unclear how the combination of LABA plus inhaled corticosteroid and its variations might affect the vocal folds.

One complication of studying the influence of ICs on the voice is that there are some data suggesting that asthma in and of itself can cause voice disorders, including use of fewer syllables per breath, increased pause time during speech, and overall vocal fatigue (Lavy, 2000). However,

the prevalence of voice disorders appears to be greater when asthma is treated with ICs. One study with 19,330 participants compared the risk of voice discomfort for individuals with asthma who were medicated, individuals who had asthma but were not medicated, and a control group without asthma. These researchers found that individuals with asthma were at greater risk for voice discomfort than the control group, but that the risk increased even more dramatically when individuals had taken medication for asthma within the past year (Park & Choi, 2016). This large group study suggests that the presence of voice disorders in individuals with asthma may be exacerbated by medication use. These preliminary findings suggest that further investigation of the impact of ICs use on the voice is warranted. Unfortunately, challenges exist in the study of ICs in human subjects because medication necessity limits the types of experimental designs that would offer more conclusive cause and effect data.

Animal Models

Ex vivo animal models provide an alternative to human subjects research and have been used for many years as a method of studying laryngeal pathologies. These models are beneficial because they avoid many problems encountered during in vivo experiments by being noninvasive, affording better visualization of vocal folds, and allowing investigators to more readily control variations in muscle activity that are present in vivo subjects research, and more accurately take measurements of pressure and flow (Döllinger et al., 2011). Benchtop studies have been identified as a useful means for studying excised larynges because they are able to simulate phonation in a controlled manner, allowing for the isolation of variables of interest. Animal models commonly used in benchtop studies include pigs, dogs, sheep, and cows. Each model has its own unique benefits as well as limitations in its translational validity to human models.

Of the models previously mentioned, canine larynges are the most commonly utilized due to the physical similarities between human and canine larynges, especially similarities in size (Jiang et al., 2001). However, the histology of canine larynges is dissimilar from human larynges in that the layers of the vocal folds are composed differently (Kim et al., 2004; Maytag et al., 2013). Both canine larynges and human larynges have a lamina propria composed of three layers. However, the composition of those layers is different. In humans the superficial layer is composed of a gelatinous-like ground substance, covering an intermediate layer composed of elastin, and a deep layer composed of collagen fibers. This is different from the histological composition of canine lamina propria where the superficial layer is composed of sheets of both collagen and elastin covering looser ground substance, with the intermediate and deep layers being less pronounced than in humans (Maytag et al., 2013). The discrepancies in the histological composition of human and canine vocal folds cause differences in vibratory patterns that make extrapolation of data and results collected through canine studies difficult. These difficulties have led researchers to search for alternative animal models.

Among other suggested alternatives, leporine larynges seem particularly well-suited to address the challenges described above due to the histological differences between canine and human larynges, making leporine larynges excellent candidates for phonation research. Unlike canine vocal folds, leporine vocal folds are very similar in composition to human vocal folds. Both human and leporine vocal folds have three layers composed of highly similar extracellular matrix components (Thibeault et al., 2002). Like humans, rabbits have a superficial layer composed of loose ground substance, covering two distinct vocal ligament layers. These histological similarities have been found especially beneficial in research dealing with vocal fold inflammation (Mills et al., 2016).

Maytag and colleagues (2013) established a way to use leporine larynges in benchtop models by modifying the traditional benchtop models used for studying canine larynges in order to accommodate the smaller size of the leporine larynges. With these modifications they were able to establish a reliable method to collect acoustic, aerodynamic, videokymographic, and electroglottographic data using leporine larynges.

Just like any other model, leporine larynges do have potential drawbacks including their smaller size, which may result in additional time required to dissect, mount, and manipulate larynges (Maytag et al., 2013). However, the benefit of being able to more easily translate results to human voice production due to histological similarities far outweighs these potential drawbacks. Thus, it can be concluded that although other models such as canine models are important in research where manipulation of gross anatomy is required, leporine larynges serve as a more viable alternative in research evaluating vocal fold histological changes.

Leporine larynges have been deemed to be particularly useful in asthma research (Keir & Page, 2008). Due to a variety of ethical considerations, including the types of experiments required to accurately understand the mechanisms of asthma and asthma treatment, and the necessity of asthma treatment for individuals suffering from the effects of asthma, animal models have been deemed absolutely essential to furthering our understanding of the implications of IC use (Zosky & Sly, 2007). As noted previously there are a wide variety of animal models available. However, based on preliminary research suggesting that changes in the vocal fold epithelium are linked to the use of ICs, rabbits may be particularly useful in researching the effects of IC use on the voice due to the similarities between leporine and human vocal fold composition. In addition to these vocal fold histology similarities, rabbits who are neonatally immunized respond similarly to humans who have asthma, when given a variety of asthma

treatments (Keir & Page, 2008). Together these factors make leporine larynges the ideal candidate for research examining the effects of ICs on the voice.

Quantification of the Impact of Inhaled Corticosteroid Use

To further understand the detrimental impact of IC use, the current study examined the effects of inhaler use on a variety of aerodynamic measures. Aerodynamic measures are concerned with pressure and flow changes within the larynx and are helpful in phonatory research because they have been shown to provide accurate information on laryngeal function during phonation (Matheron et al., 2017; Sheela, 2013). The larynx essentially acts as a transducer, which converts aerodynamic power into acoustic power (Sheela, 2013). When compared to acoustic measures, aerodynamic measures have been shown to be more sensitive to changes in vocal fold health and are thus more indicative of vocal fold pathology (Hottinger et al., 2007). For instance, one study examining both acoustic and aerodynamic measures for individuals with vocal nodules found that the aerodynamic metrics were more effective than acoustic measures in indicating the presence of vocal nodules (Holmberg et al., 2003).

The aerodynamic measures selected for this study were phonation threshold pressure (PTP), sustained pressure, phonation threshold flow (PTF), sustained flow, and onset and sustained laryngeal resistance. Jiang and Tao (2007) defined PTP as the "minimum subglottal pressure required to initiate phonation" (p. 2873). A higher-than-normal PTP is thought to be indicative of poor vocal fold adduction and thus may indicate changes in vocal fold health (Yiu et al., 2004). PTP is influenced primarily by vocal fold viscosity, vocal fold thickness, prephonatory glottal width, and mucosal wave velocity (Plexico et al., 2011). Thus, any pathology that influences these properties should influence PTP. PTP has been used extensively in research and clinical practice. However, direct measurement of PTP is highly invasive as it

requires subglottal tracheal puncture. Typically, researchers and clinicians opt to use an estimate of PTP rather than direct measurement. This estimate is collected by measuring intraoral air pressure during stop closures in a string of stop consonants (Yiu et al., 2004). Although this method has been shown to provide a fairly accurate estimate of subglottal pressure, some researchers have expressed concern that in order for this measurement to be as accurate as possible, study participants must be trained to maintain a stable glottal configuration and pressure throughout the process, which can be very difficult (Jiang & Tao, 2007).

Phonation threshold flow is defined as "the minimum glottal airflow required to initiate phonation" (Jiang & Tao, 2007, p. 2874). PTF can be derived by measuring airflow from the mouth during phonation using an anesthesia-type mask connected to a flow transducer (Jiang & Tao, 2007). This is a non-invasive procedure and requires little to no patient training, thus making it more viable for use in research and clinical application. PTF has been shown to vary with changes in vocal fold elongation, hydration, and posterior glottal gap size (Zhuang et al., 2013). One study indicated that PTF may be more sensitive than PTP to changes in posterior glottal gap (Hottinger et al., 2007). Zhuang and colleagues (2013) concluded that PTP is a more applicable measure when pathologies involve vocal fold stiffness, whereas PTF is a better indicator when glottal closure is compromised. This highlights the importance of using both PTP and PTF in the current research in order to ensure that any change in vocal fold physiology is captured.

The final outcome measure for this study was laryngeal resistance. Laryngeal resistance is calculated as the quotient of laryngeal pressure divided by laryngeal airflow. Laryngeal resistance is influenced by both the airway's physical structure and the larynx tissue's mechanical properties (Rieves et al., 2009). This means that laryngeal resistance can be

indicative of laryngeal valving efficiency, which aids in determining whether the larynx is either too tight or too loose (Sheela, 2013). As with PTP and PTF this can be helpful in determining the efficiency with which the airway is working. Together, all three measures provide an important understanding of overall laryngeal health.

Statement of Purpose

The current study is part of a larger project being conducted under the direction of Dr. Kristine Tanner that examines the effects of IC use on voice production. As previously noted, the asthma population is increasing, and many diagnosed individuals rely on ICs to manage symptoms. Due to the well documented association between ICs use and voice disorders, further research on this population is warranted. The ultimate goal of the larger project is to prevent future voice disorders among individuals who use ICs as treatment for respiratory diseases. The current study is part of a phase of the project which is attempting to quantify voice function changes secondary to IC use by examining changes in aerodynamic measures from leporine larynges, harvested post-mortem from rabbits that were exposed to ICs prior to being sacrificed.

Specifically, the current thesis sought to determine the stability of PTP, sustained pressure PTF, sustained flow, and laryngeal resistance across repeated trials through the calculation of coefficients of variation. Previous research has shown that some variability in aerodynamic measures is expected when performing experiments on animal models (Mills et al., 2016; Yiu et al., 2004). However, the extent to which this variability is applicable to benchtop studies using leporine larynges has not yet been examined. This research will help identify how much variability is expected and help to quantify the number of trials required to accurately capture aerodynamic measures that are reliable for both control and experimental larynges. Data collected through this thesis will serve as baseline comparisons for future studies.

Research Questions

This study was designed to answer the following questions:

1. How stable are pressure, airflow, and laryngeal resistance following eight-week exposure to inhaled combination corticosteroids?
2. How does stability differ from control leporine vocal folds exposed to inhaled saline?

Method

For this thesis, all operational procedures were conducted at Brigham Young University (BYU) in accordance with risk management guidelines and the Institutional Animal Care and Use Committee. Larynges included in this study were obtained from The University of Utah and were part of a parent project funded by the National Institutes of Health (Kristine Tanner, Ph.D., principal investigator). Larynx preparation and data collection were accomplished in rooms 105 and 106 of the BYU John Taylor Building Annex.

Research Design

This thesis employed a between- and within-groups experimental design. Samples included 22 leporine larynges obtained after sacrifice from male, white New Zealand rabbits. All rabbits were adult, retired breeders and were between 7 and 8 months of age and between 3.1 and 4.8 kg. Upon intake at The University of Utah, all rabbits were quarantined and subsequently randomized to experimental and control groups. For eight weeks, the experimental group received twice-daily administrations of a combination metered dose inhaler (IC; fluticasone propionate 45 mcg and salmeterol 21 mcg) via a spacer and facemask; the control group received twice-daily administrations of nebulized isotonic saline (0.9% $Na^{+}Cl^{-}$) via a facemask. The only exception to the twice-daily administration occurred on two holidays, on which rabbits received a single administration. One rabbit from the control group was found to have structural damage

to the larynx and was excluded from the research; therefore, the experimental and control groups had 11 and 10 larynges, respectively. The independent variables were group and phonation trial and the dependent variables were the coefficients of variation for pressure, airflow, and laryngeal resistance at onset and during sustained phonation.

Larynx Procurement

Following the eight-week treatment or control administration, all rabbits were euthanized as part of the parent project at The University of Utah. Larynges were immediately excised for storage and subsequent transfer to BYU. Excision was accomplished by creating an incision in the anterior portion of the neck to reveal both the trachea and larynx. The incision was held open using hemostats. Fine scalpels were used to dissect away neck musculature. Once the thyroid cartilage and trachea were sufficiently exposed, the larynx and trachea were extracted. These tissues were placed in phosphate-buffered solution-filled containers that were coded and labeled. These containers were placed in a tray of isopropyl alcohol, flash frozen in liquid nitrogen, and stored in a -80° Celsius freezer. Subsequently, larynges were transported to BYU in a foam cooler with dry ice. Upon arrival at BYU, larynges were placed in a -80° Celsius ThermoScientific freezer in room 105 of the John Taylor Building Annex.

Data Collection Preparation

The following procedure was performed at the beginning of each data collection session. Approximately 20 minutes prior to dissection, the containers holding the larynges were placed in a lukewarm water bath. Once tissues were sufficiently thawed, larynges were placed on the dissection table. Each larynx container was refilled with fresh phosphate buffered solution and larynges were frequently submerged to maintain adequate tissue hydration during dissection. Using fine scalpels and X-acto™ knives, any remains of the esophagus and excess tissue were

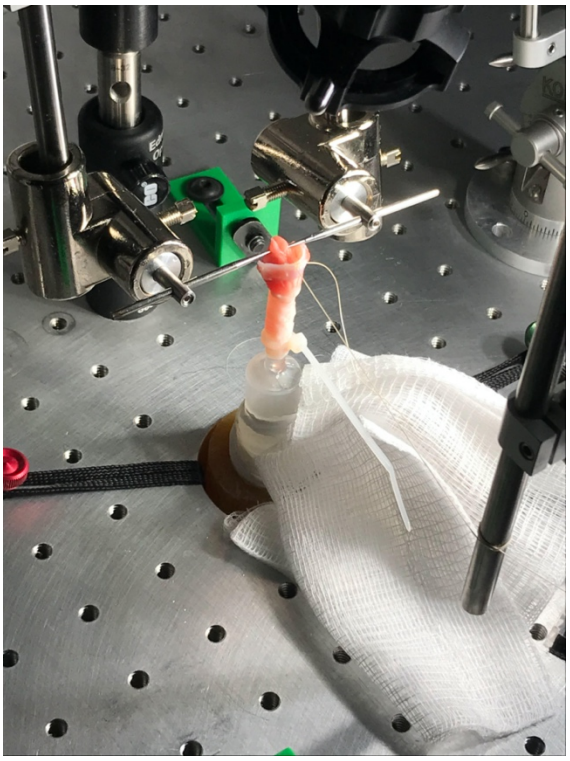
removed from the trachea. The thyroid cartilage superior to the thyroid notch, the epiglottis, and the false vocal folds were also dissected away to expose the true vocal folds. A small overhead light and drawing table sublighting were used to aid researchers in visualizing and distinguishing between false and true vocal folds. Once structures were dissected away, a medical suture was placed in the thyroid cartilage superior to the anterior commissure of the vocal folds to assist in laryngeal mounting. Once fine dissection was completed, larynges were submerged in their respective containers and placed in a refrigerator until they were mounted for data collection.

Benchtop Model

This thesis utilized a benchtop model similar to the one described by Jiang and Titze (1993). Larynges were mounted on a small plastic syringe tip and tubing that protruded through the table. Tubing was suspended above the table using benchtop strings and screws. Each larynx was secured to the plastic tubing to prevent airflow leakage using a single plastic cable tie. The larynx was positioned for phonation using micropositioners which were secured to the benchtop with ¼-20 headless screws. Two micropositioners were positioned on the sides of the larynx. These micropositioners contained a single prong which was gently placed in the lateral edge of the left and right arytenoid cartilages. The positioners' height and lateral displacement could be manually adjusted to best fit each larynx. The positioners were manually positioned to provide adequate pressure to the arytenoid cartilage in order to adduct the vocal folds to prepare for phonation as shown in Figure 1. A third micropositioner was mounted on the benchtop anteriorly to the larynges. The suture string that was placed in the thyroid cartilage during the dissection process was attached to the third micropositioner to provide additional stabilization during phonatory trials. Care was taken to ensure that the larynx remained stable but vocal folds were not manually elongated.

Figure 1

Positioning of the Lateral Micropositioners



Phonation occurred as a result of subglottal airflow provided by a medical grade compressed air tank (50 psi, <1% relative humidity). This tank was anchored to the laboratory wall. Airflow from the tank was directed through an interchangeable, calibrated respiratory flow head transducer (Model MLT300L, AD Instruments, Sydney Australia). After passing through the flow head, air was heated and humidified by a TheraHeat Humidifier (Model RC700000, Smiths Medical, Dublin, OH). The heated and humidified air then passed through plastic tubing, which was surrounded by a custom 20 cm insulated aluminum pseudolung suspended vertically below the surface of the benchtop. This pseudolung was designed to reduce acoustic reverberation. Once tubing had passed through the pseudolung, the plastic tubing protruded through the benchtop and was fitted with a silicone piece that connected plastic tubing to the syringe head where larynges were mounted. A small puncture hole was drilled into the silicone

fixture and tubing, where a calibrated physiological pressure transducer (Model MLT844, AD Instruments, Sydney, Australia) was attached. The benchtop setup is depicted in Figure 2.

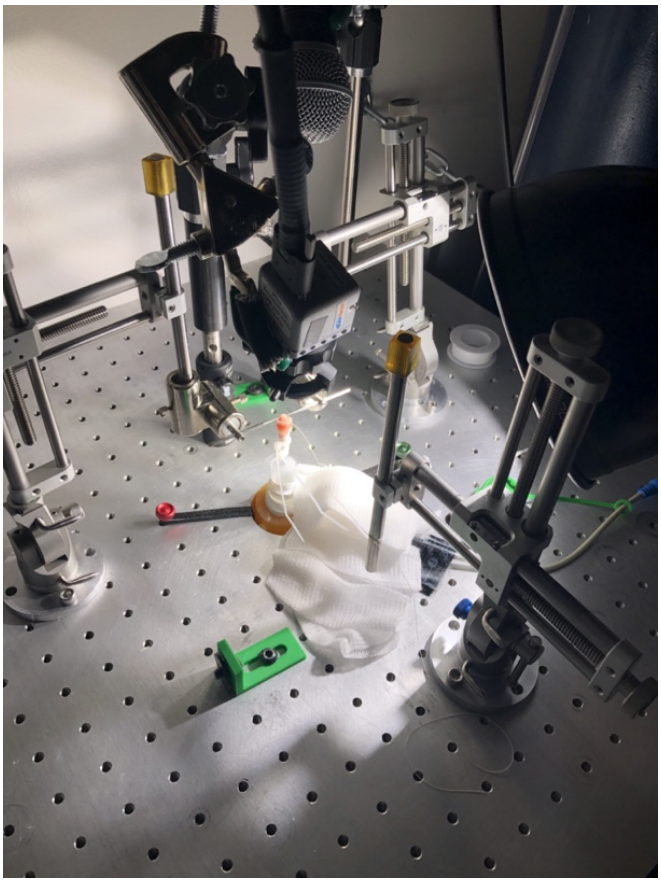
Figure 2

Benchtop Setup

The breadboard benchtop was fitted with a mounting apparatus for both a microphone (Model SM-48, Shure, Niles, IL) and high-speed camera (KayPentax, Montvale, NJ). As depicted in Figure 3, the microphone was positioned approximately six inches above the vocal folds and was used to collect acoustic signals during phonation. The high-speed camera was positioned to collect video footage on the first, fifth, tenth, and fifteenth phonatory trials for each larynx as part of the larger parent project.

Figure 3

Superior View of the Benchtop Setup



Phonation Trials

Once the larynx was mounted to the benchtop, air was supplied subglottally and gradually increased to verify adequate vocal fold adduction and to ensure that phonation would occur. Small adjustments to the lateral micropositioner adduction were made until larynges consistently phonated. Once fine adjustments were completed, phonatory trials were initiated. Each larynx underwent 15 phonatory trials. During each trial, airflow was gradually increased until phonation began. Once phonation began, airflow was held stable for approximately three seconds. After this period of stable phonation, the airflow source was turned off. During each trial, calibrated airflow, acoustic and pressure signals were acquired using version 8 of the

LabChart PowerLab™ hardware and software interface (ADInstruments, 2015). During each trial, rough annotations were made in the software to indicate the approximate timing of phonation onset, steady state, and offset in the acoustic signal. During mounting and phonation trials, larynges were frequently sprayed with saline solution to help maintain tissue surface hydration.

Data Analysis

Saved LabChart™ files were examined carefully and the tentative markings for phonatory onset, stable phonation, and offset that had been placed during phonatory trials were adjusted to more accurately represent the times these events occurred. These adjustments were made by analyzing the oscillations in the acoustic signal waveforms to identify where the quasiperiodic phonation signals began and ended. The audio output from the acoustic signal was also utilized to help verify that marker placement was accurate.

After marker placement had been verified, segmented acoustic and aerodynamic data were imported to a custom Matlab program (The MathWorks, Inc., 2010) written by Christopher Dromey, Ph.D., for waveform analysis. Average pressure and flow values were obtained for phonation onset and sustained phonation using an interval of 10 ms before and after cursor placement. These data were then exported to a spreadsheet for statistical analysis.

Statistical Analysis

For purposes of the parent project, summary data for onset and sustained pressure, airflow, laryngeal resistance, and fundamental frequency (F_0) were examined. Data distributions were examined visually using analysis of covariance. Interjudge reliability was calculated using intraclass correlation coefficients. Intrajudge reliability for each variable resulted in Pearson

correlations greater than or equal to .98, indicating acceptable reliability for the segmentation process.

For each aerodynamic measure, mean scores were obtained across the trials for each rabbit and summary statistics were derived for these scores according to treatment status. For each aerodynamic measure, variances in the means were compared between treatment and control groups using the *F* test. For each aerodynamic measure, the coefficient of variation was obtained over the 15 trials for each rabbit and compared between the treatment and control groups using the Wilcoxon rank-sum test. For each aerodynamic measure, mean, standard deviation, and coefficient of variation scores were derived for each rabbit in each trial. The effect of treatment and trial on each of the aerodynamic mean scores was evaluated using regression analysis. The coefficient of variation was also regressed on treatment and trial variables. The t test evaluated differences in means and the F test evaluated differences in variances. Analyses were conducted in Microsoft Excel (version 16.33, 2019, Microsoft Corp., Redmond, WA) and the Statistical Analysis System (version 9.4, Cary, NC).

Results

As described previously, each larynx was inspected visually and underwent a series of geometric measurements prior to signal acquisition. Of the 22 laryngeal specimens, one larynx was unsuitable for inclusion due to significant structural damage that precluded adequate mounting, vocal fold adduction, and the elicitation of acceptable phonation for purposes of the study. Therefore, this larynx was excluded, resulting in 11 larynges in the inhaler group and 10 larynges in the control group.

Overview of Groups

During data collection, the pressure and flow measurement at phonation onset (i.e., PTP and PTF, respectively), and during sustained phonation were measured for each trial. Upon data analysis, these measures were then used to determine the resistance, which is defined as subglottal pressure divided by laryngeal airflow. Descriptive statistics (mean, median, standard deviation, maximum value, and minimum value) were calculated for each larynx and the distribution of the data was analyzed. The data collected for each parameter were fairly normally distributed for all rabbits. Average aerodynamic values for each larynx, across the 15 trials, are displayed in Table 1 below.

Table 1

Average Aerodynamic Data for Each Rabbit

Group	PTP (cmH_2O)	Sustained Pressure (cmH_2O)	PTF (L/min)	Sustained Airflow (L/min)	Onset LR (cmH_2O/L/min)	Sustained LR (cmH_2O/L/min)
Inhaler Group						
1	7.48	11.24	0.07	0.08	111.23	139.55
2	5.35	8.51	0.08	0.09	65.22	90.26
3	6.53	8.75	0.09	0.10	74.94	92.11
4	5.43	6.81	0.08	0.09	70.97	77.21
5	5.18	10.32	0.05	0.08	100.43	115.79
6	5.49	8.23	0.08	0.09	72.82	92.49
7	11.57	16.62	0.18	0.21	66.06	78.99
8	8.37	14.86	0.12	0.17	70.92	86.63
9	10.98	13.31	0.16	0.17	66.81	76.29
10	7.75	12.58	0.09	0.13	86.04	98.17
11	11.83	15.01	0.14	0.15	83.57	99.99
Control Group						
1	8.35	9.42	0.08	0.07	108.31	127.64
2	7.65	9.53	0.02	0.05	351.23	193.91
3	5.18	8.31	0.04	0.07	133.49	125.72
4	8.23	9.71	0.09	0.09	94.97	106.50
5	8.44	9.47	0.09	0.10	91.31	97.75
6	6.33	8.14	0.05	0.06	125.30	142.73
7	5.77	7.57	0.02	0.04	327.58	183.76
8	6.36	7.73	0.02	0.04	93.75	104.83
9	7.16	8.42	0.107	0.11	66.65	75.32
10	10.94	12.48	0.12	0.12	91.92	102.12

Note. PTP = phonation threshold pressure. PTF = phonation threshold flow. LR = laryngeal resistance. Data for PTP and PTF are from a larger project involving several studies, including separate experimental questions examined in Robison (2021) and Pang (2021).

For each rabbit, mean values were calculated across the trials for PTP, sustained pressure, PTF, sustained airflow, onset laryngeal resistance, sustained laryngeal resistance and F_0 values. Table 2 and Table 3 summarizes these means across the inhaler and saline groups, respectively. A comparison of the mean and the median scores indicate that the data tend to be slightly right-skewed. The variances were compared between the inhaler and control groups using the *F* test. The calculated *F* test (p-value) was 2.39 (0.1031) for PTP, 1.78 (0.2001) for PTF, 46.52 (<.0001) for onset resistance, 5.13 (0.0108) for sustained pressure, 1.78 (0.2001) for sustained airflow, 4.15 (0.0217) for sustained resistance, and 3.85 (0.0274) for F_0. Hence, variability was significantly higher in the control group for onset resistance, sustained resistance, and fundamental frequency, and significantly lower for sustained pressure.

The coefficient of variation (CV) measures relative dispersion of data points around the mean (calculated as the standard deviation divided by the mean and multiplied by 100). That is, it can provide a relative index of the spread of the data, with a larger coefficient of variation indicating greater variability in the data. It allows us to make comparisons of variability among disparate groups and across different metrics. Differences in this measure between the inhaler and saline groups are consistent with the *F* test results.

Table 2

Inhaler Group Descriptive Statistics, (n = 11 larynges)

Statistic	PTP (cmH_2O)	PTF (L/min)	Onset LR (cmH_2O/L/min)	Sustained Pressure (cmH_2O)	Sustained Airflow (L/min)	Sustained LR (cmH_2O/L/min)	F_0 (Hz)
Mean	7.82	0.10	79.0	11.48	0.12	95.23	519.85
SD	2.58	0.04	15.03	3.24	0.04	18.66	66.61
Median	7.48	0.09	72.82	11.24	0.09	92.11	549.50
Minimum	5.18	0.05	65.22	6.81	0.08	76.29	403.15
Maximum	11.84	0.18	111.23	16.62	0.21	139.55	604.61
Range	6.66	0.13	46.01	9.81	0.13	63.29	201.46
CV (%)	32.99	40.00	19.03	28.22	11.67	19.59	12.81

Note. PTP = phonation threshold pressure. PTF = phonation threshold flow. LR = laryngeal resistance. CV = Coefficient of Variation.

Table 3

Control Group Descriptive Statistics (n = 10 larynges)

Statistic	PTP (cmH_2O)	PTF (L/min)	Onset LR (cmH_2O/L/min)	Sustained Pressure (cmH_2O)	Sustained Airflow (L/min)	Sustained LR (cmH_2O/L/min)	F_0 (Hz)
Mean	7.44	0.07	148.45	9.08	0.08	126.03	446.91
SD	1.67	0.03	102.51	1.43	0.03	38.00	130.63
Median	7.40	0.07	101.64	8.92	0.07	116.11	442.12
Minimum	5.18	0.02	66.65	7.57	0.04	193.91	284.51
Maximum	10.94	0.12	351.23	12.48	0.12	75.32	673.73
Range	5.76	0.10	284.58	4.91	0.08	118.59	389.22
CV(%)	22.45	42.86	69.05	15.75	37.5	30.15	29.23

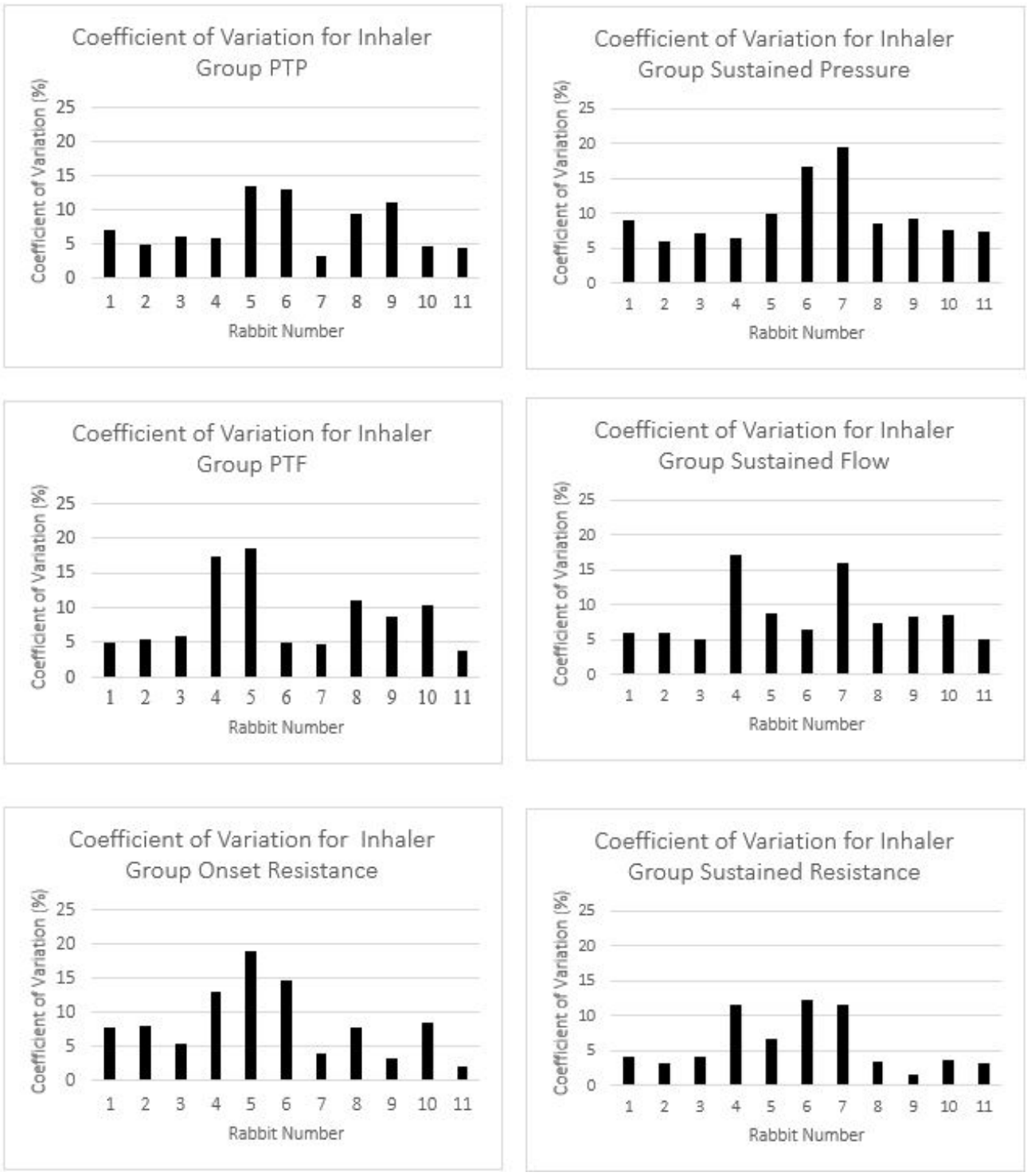
Note. PTP = phonation threshold pressure. PTF = phonation threshold flow. LR = laryngeal resistance. CV = coefficient of variation .

Within Subjects Coefficients of Variation

The variability of the measures, across the 15 trials, for each individual rabbit was examined using the CV. In this study, the CV was calculated for each outcome variable for each rabbit, based on raw data for the 15 trials for each rabbit in the inhaler and control groups. The CV for each outcome variable for rabbits in the inhaler group is displayed in Figure 1, and the CV for each outcome variable for the rabbits in the control group in Figure 2. The raw data used to calculate the CV can be found in Appendix D. For each outcome measure, the Wilcoxon rank-sum test was used to determine whether the variance of the CV significantly differed between the inhaler and control groups, in which no significant difference was found.

Figure 4

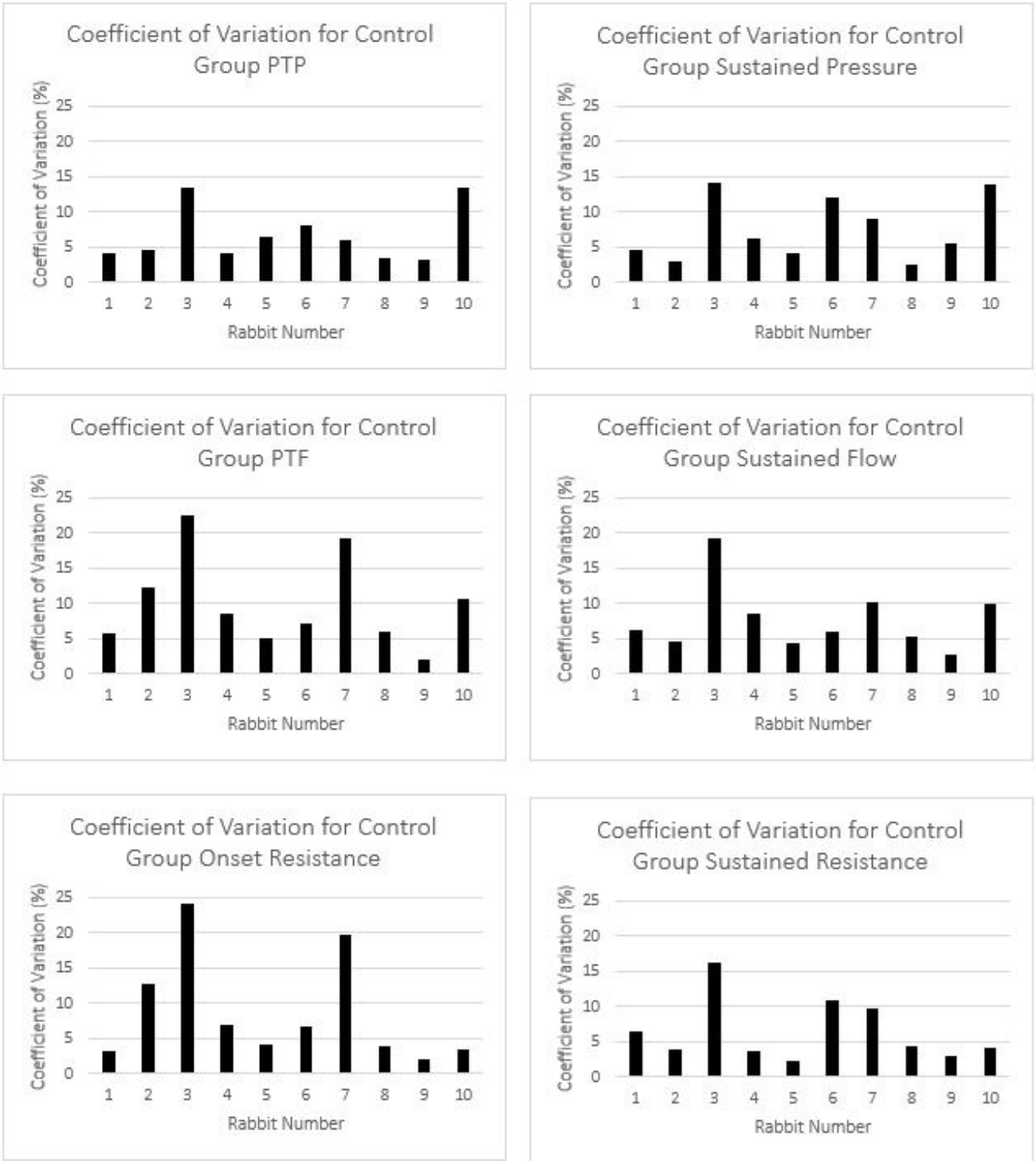
Aerodynamic Measure Coefficients of Variation for Inhaler Group Rabbits



Note. PTP = phonation threshold pressure. PTF = phonation threshold flow.

Figure 5

Aerodynamic Measure Coefficients of Variation for Control Group Rabbits



Note. PTP = phonation threshold pressure. PTF = phonation threshold flow.

Variability by Group and Trial

In addition to examining the variability of each individual rabbit, the average variability of the rabbits in the treatment and control groups, across the 15 trials was also examined. Two approaches were used to examine each outcome variable. The first approach examined differences between the two groups while accounting for variability across trials using the raw outcome data. The second approach was similar except that it employed the CV across the 15 trials. The results of these analyses for each outcome variable are provided below.

Phonation Threshold Pressure

Average PTP values for each trial are presented in Table 4 for the inhaler and control groups. The distributions were approximately normal for both groups. Using the Folded *F* statistic, PTP variances were not significantly different between the groups ($F[14, 14] = 1.19$, $p = 0.755$). An independent sample *t*-test, equal variances assumed, indicated a significant difference in means between the inhaler and control groups (7.82 vs. 7.44, $t[28] = -4.14$, $p = 0.0003$). A regression model showed a significantly higher mean for larynges in the inhaler versus control group (0.374 [$SE = 0.0840$], $t[28] = -4.44$, $p = 0.0001$), after controlling for trial. Mean PTP significantly decreased over the 15 trials (-0.022 [$SE = 0.010$], $t[28] = -2.28$, $p = 0.0310$).

Variances for the CV scores were not significantly different between the groups ($F[14, 14] = 1.37$, $p = 0.5606$). An independent sample *t*-test, equal variances assumed, indicated a significant difference in mean CV scores between the inhaler and control groups (33.79 vs. 23.26, $t[28] = -8.13$, $p < .0001$). A regression model showed a significantly higher mean for larynges in the inhaler versus control group (10.52 [$SE = 1.29$], $t[28] = -8.13$, $p < 0.0001$), after controlling for trial. Mean CV scores did not significantly decreased over the 15 trials (0.148 [$SE = 0.150$], $t[28] = 0.99$, $p = 0.3316$).

Table 4

Phonation Threshold Pressure Descriptive Statistics by Trial

Group	Mean (cmH_2O)	SD (cmH_2O)	CV (%)
Inhaler Group			
1	8.04	2.56	31.80
2	7.79	2.39	30.74
3	7.83	2.52	32.22
4	7.73	2.55	32.98
5	7.94	2.43	30.59
6	8.05	2.49	30.91
7	7.87	2.59	32.84
8	7.62	2.58	33.90
9	7.88	2.72	34.50
10	7.89	2.54	32.19
11	8.00	2.65	33.14
12	7.74	2.78	35.98
13	7.46	2.74	36.80
14	7.25	3.15	43.49
15	8.16	2.83	34.73
Control Group			
1	7.83	1.92	24.54
2	7.68	2.06	26.80
3	7.78	2.65	34.10
4	7.46	1.54	20.59
5	7.66	1.64	21.44
6	7.49	1.46	19.48
7	7.22	1.66	22.94
8	7.19	1.45	20.21
9	7.04	1.37	19.42
10	7.61	1.73	22.72
11	7.12	1.57	22.01
12	7.30	1.62	22.22
13	7.14	1.77	24.77
14	7.64	2.04	26.77
15	7.48	1.57	20.94

Phonation Threshold Flow

The average PTF values for each trial, for both the inhaler and control groups are displayed in Table 5. Distributions for both groups were approximately normal. PTF variances between the groups were not found to be significantly different ($F[14, 14] = 2.43$, $p = 0.1074$). An independent *t*-test, equal variances assumed, indicated that there were significant differences between the mean PTF of the inhaler group and control group (0.10 vs. 0.07, $t[28] = -38.06$, $p =$

0.0001). A regression model showed that mean PTF was significantly higher for larynges in the inhaler group after controlling for trial, (0.347 [*SE* = 0.0009], *t*[28] = -39.82, *p* = 0.0001). Mean PTF values did not differ significantly with trial (0.110 [*SE* = 0.107], *t*[28] = 1.03, *p* = 0.3141).

Variances for the CV scores were not significantly different between groups (*F*[14, 14] = 1.12, *p* = 0.7340). An independent sample *t*-test, equal variances assumed, indicated a significant difference in mean CV scores between the inhaler and control groups (40.44 vs. 51.54, *t*[28] = 12.00, *p* < .0001). A regression model showed a significantly lower mean CV for larynges in the inhaler group versus the control group (11.10 [*SE* = 0.924], *t*[28] = 12.01 *p* < 0.0001), after controlling for trial. Mean CV scores did not significantly change over the 15 trials (0.110 [*SE* = 0.108], *t*[28] = 1.03, *p* = 0.3141).

Table 5

Phonation Threshold Flow Descriptive Statistics by Trial

Group	Mean (L/m)	Standard Deviation (L/m)	CV (%)
Inhaler Group			
1	0.098	0.040	40.403
2	0.099	0.035	35.699
3	0.105	0.039	37.081
4	0.104	0.038	36.689
5	0.101	0.038	37.888
6	0.105	0.041	39.470
7	0.106	0.041	38.694
8	0.101	0.043	42.636
9	0.106	0.044	41.515
10	0.100	0.042	42.418
11	0.105	0.045	42.570
12	0.103	0.044	42.979
13	0.100	0.042	42.376
14	0.104	0.046	43.937
15	0.108	0.046	42.167
Control Group			
1	0.069	0.037	54.708
2	0.067	0.036	52.861
3	0.070	0.038	54.609
4	0.066	0.035	52.720
5	0.069	0.034	49.526
6	0.068	0.034	50.208
7	0.066	0.036	55.127
8	0.066	0.035	53.679
9	0.067	0.032	47.231
10	0.071	0.035	49.098
11	0.066	0.034	52.013
12	0.069	0.034	48.888
13	0.069	0.034	49.639
14	0.072	0.037	51.012
15	0.069	0.036	51.737

Onset Laryngeal Resistance

The average onset laryngeal resistance values for each trial, for both the inhaler and control groups are displayed in Table 6. Distributions for both groups were approximately normal. Onset laryngeal resistance variances between the groups were significantly different ($F[14, 14] = 8.09$, $p = 0.0004$). An independent *t*-test, unequal variances assumed, indicated that

there were significant differences between the mean onset laryngeal resistance of the inhaler group and control group (79.001 vs. 148.500, t[17.41] = 27.08, $p < 0.0001$). A regression model showed that mean onset laryngeal resistance was significantly lower for larynges in the inhaler group versus the control group after controlling for trial, (69.452 [SE = 2.4331], t[17.41] = 28.54, $p < 0.0001$). Mean onset laryngeal resistance scores did not significantly change over the 15 trials (-0.571 [SE = 0.282], t[17.41] = -2.03, $p = 0.0527$).

Variances for the CV scores were significantly different between groups (F[14, 14] = 8.70, $p = 0.0002$). An independent sample t-test, unequal variances assumed, indicated a significant difference in mean CV scores between the inhaler and control groups (21.21 vs. 69.78, t[17.175] = 20.38, $p < .0001$). A regression model showed a significantly lower mean CV for larynges in the inhaler group. (48.58 [SE = 2.388], t[17.175] = 20.34, $p < 0.0001$), after controlling for trial. Mean CV scores did not significantly change over the 15 trials (0.261 [SE = 0.276], t[17.175] = 0.94, $p = 0.3538$).

Table 6

Onset Laryngeal Resistance Descriptive Statistics by Trial

Group	Mean ($cmH_2O/L/m$)	SD ($cmH_2O/L/m$)	CV (%)
Inhaler Group			
1	85.202	17.964	21.084
2	80.344	13.813	17.192
3	76.354	15.357	20.113
4	75.864	15.585	20.543
5	81.757	15.251	18.654
6	79.577	14.340	18.020
7	77.044	15.862	20.589
8	78.829	17.199	21.818
9	77.254	14.108	18.262
10	83.614	19.156	22.910
11	80.293	18.689	23.276
12	80.202	23.617	29.447
13	77.539	17.028	21.960
14	71.699	15.184	21.177
15	79.436	18.308	23.048
Control Group			
1	149.327	90.801	60.807
2	147.332	91.049	61.798
3	142.334	87.806	61.690
4	173.279	152.310	87.899
5	145.664	93.076	63.898
6	155.267	116.895	75.287
7	159.330	121.501	76.257
8	156.916	115.626	73.687
9	142.571	105.752	74.175
10	141.692	96.028	67.773
11	142.618	89.462	62.728
12	143.168	104.694	73.126
13	147.790	123.601	83.633
14	135.560	80.638	59.485
15	143.942	92.886	64.530

Sustained Pressure

Table 7 contains the average sustained pressure values for both the inhaler and control groups, across the 15 trials. The distributions were approximately normal for both groups. Using the Folded F statistic, sustained pressure variances were significantly different between the groups ($F[14, 14] = 5.43$, $p = 0.0032$). An independent sample *t*-test, unequal variances assumed, indicated a significant difference in mean sustained pressure between the inhaler and control

groups (11.28 vs. 9.08, t[18.99] = -12.12, $p < 0.0001$). A regression model showed a significantly higher mean sustained pressure for larynges in the inhaler versus control group (-2.40 [SE = 0.1916], t[18.99] = -1.69, p = 0.1023), after controlling for trial. Mean sustained pressure did not significantly decrease over the 15 trials (-0.037 [SE = 0.022], t[18.99] = -1.69, p = 0.1023).

Variances for the CV scores were not significantly different between the groups (F[14, 14] = 2.29, p = 0.1341). An independent sample t-test, equal variances assumed, indicated a significant difference in mean CV scores between the inhaler and control groups (29.69 vs. 17.46, t[28] = -9.60, $p < .0001$). A regression model showed a significantly higher mean CV for larynges in the inhaler versus control group (-12.24 [SE = 1.17], t[28] = -10.44, $p < 0.0001$), after controlling for trial. Mean CV scores significantly decreased over the 15 trials (-0.34 [SE = 0.1357], t[28] = -2.47, p = 0.0201).

Table 7

Sustained Pressure Descriptive Statistics by Trial

Group	Mean (cmH_2O)	SD (cmH_2O)	CV (%)
Inhaler Group			
1	12.412	4.225	34.039
2	11.633	3.158	27.146
3	11.349	3.490	30.750
4	10.845	3.010	27.755
5	12.789	4.689	36.665
6	11.732	3.332	28.400
7	11.490	3.405	29.639
8	11.244	3.372	29.989
9	11.209	3.134	27.960
10	11.755	3.231	27.483
11	11.482	3.228	28.113
12	11.089	3.355	30.251
13	10.233	2.745	26.829
14	10.456	3.302	31.585
15	12.427	3.577	28.785
Control Group			
1	9.629	2.145	22.277
2	9.239	2.075	22.455
3	9.100	2.575	28.301
4	8.890	1.464	16.467
5	9.380	1.415	15.084
6	9.020	1.455	16.126
7	8.883	1.343	15.122
8	8.774	1.209	13.780
9	8.665	1.425	16.445
10	9.401	1.557	16.563
11	9.338	1.264	13.536
12	8.777	1.327	15.115
13	8.640	1.405	16.261
14	9.094	1.848	20.319
15	9.331	1.306	13.993

Sustained Airflow

The average sustained airflow values for each trial, for both the inhaler and control groups are displayed in Table 8. The distributions were approximately normal for both groups. Sustained airflow variances were significantly different between the groups ($F[14, 14] = 8.68$, $p = 0.0002$). An independent sample *t*-test, unequal variances assumed, indicated a significant difference in mean sustained airflow between the inhaler and control groups (0.12 vs. 0.09,

t[17.182] = 24.32, *p* < 0.0001). A regression model showed a significantly higher mean sustained airflow for larynges in the inhaler versus control group (0.03 [*SE* = 0.0014], *t*[17.182] = 24.34, *p* < 0.0001), after controlling for trial. Mean sustained airflow did not significantly vary over the 15 trials (0.0002 [*SE* = 0.0002], *t*[17.182] = 1.02, *p* = 0.3143).

Variances for the sustained airflow CV scores were not significantly different between the groups (*F*[14, 14] = 2.48, *p* = 0.1008). An independent sample *t*-test, equal variances assumed, indicated a significant difference in mean CV scores between the inhaler and control groups (37.00 vs. 50.89, *t*[28] = -11.24, *p* < .0001). A regression model showed a significantly higher mean CV for larynges in the control versus inhaler group (-13.88 [*SE* = 1.19], *t*[28] = -1.66, *p* < 0.0001), after controlling for trial. Mean CV scores did not significantly decrease over the 15 trials (-0.24 [*SE* = 0.1378], *t*[28] = -1.77, *p* = 0.0885).

Table 8

Sustained Airflow Descriptive Statistics by Trial

Group	Mean (L/m)	SD (L/m)	CV (%)
Inhaler Group			
1	0.129	0.051	39.798
2	0.122	0.040	32.790
3	0.122	0.043	34.902
4	0.117	0.038	32.110
5	0.137	0.068	49.771
6	0.123	0.046	37.094
7	0.124	0.045	36.561
8	0.124	0.045	36.517
9	0.122	0.044	36.163
10	0.124	0.044	35.297
11	0.123	0.047	38.334
12	0.121	0.044	36.303
13	0.120	0.044	36.956
14	0.127	0.048	37.579
15	0.134	0.047	34.884
Control Group			
1	0.089	0.047	53.557
2	0.088	0.047	53.224
3	0.089	0.049	55.188
4	0.085	0.047	55.419
5	0.091	0.045	50.135
6	0.089	0.045	50.872
7	0.089	0.044	49.918
8	0.090	0.046	50.544
9	0.091	0.046	51.074
10	0.092	0.045	49.033
11	0.092	0.044	47.425
12	0.090	0.044	48.622
13	0.091	0.044	48.620
14	0.091	0.047	52.163
15	0.090	0.043	47.507

Sustained Laryngeal Resistance

The average sustained laryngeal resistance values for each trial are presented in Table 9 for both the control and treatment groups. The distributions for both groups were approximately normal. The variances between the groups were not significantly different (F[14, 14] = 2.41, p = 0.1116). An independent sample t-test, equal variances assumed, indicated a significant difference in mean onset pressure between the inhaler and control groups (95.23 vs. 126.0, t[28]

= 18.16, $p < 0.0001$). A regression model showed a significantly lower mean sustained resistance for larynges in the inhaler versus control group (30.80 [*SE* = 1.3584], *t*[28] = 22.67, $p < 0.0001$), after controlling for trial. Mean sustained resistance significantly decreases over the 15 trials (-0.6411 [*SE* = 0.157], *t*[28] = -4.08, $p = 0.0004$).

Variances for the CV scores were not significantly different between the groups (*F*[14, 14] = 1.72, $p = 0.3236$). An independent sample *t*-test, equal variances assumed, indicated a significant difference in mean CV scores between the inhaler and control groups (20.37 vs. 30.94, *t*[28] = 15.64, $p < 0.0001$). A regression model showed a significantly lower mean CV for larynges in the inhaler versus control group (10.57 [*SE* = 0.67], *t*[28] = 15.69, $p < 0.0001$), after controlling for trial. Mean CV scores did not significantly decrease over the 15 trials (0.08 [*SE* = 0.0780], *t*[28] = 1.08, $p = 0.2895$).

Table 9

Sustained Laryngeal Resistance Descriptive Statistics by Trial

Group	Mean ($cmH_2O/L/m$)	SD ($cmH_2O/L/m$)	CV (%)
Inhaler Group			
1	99.278	18.717	18.853
2	98.188	18.104	18.438
3	95.172	18.729	19.679
4	94.800	19.465	20.532
5	97.996	20.201	20.614
6	98.531	19.519	19.810
7	95.690	19.307	20.176
8	93.730	18.437	19.671
9	94.489	17.440	18.457
10	97.744	18.724	19.156
11	96.976	20.406	21.042
12	94.121	19.029	20.217
13	89.511	20.041	22.389
14	85.958	20.755	24.146
15	96.221	21.504	22.348
Control Group			
1	138.076	48.936	35.442
2	130.507	38.170	29.247
3	126.818	36.592	28.854
4	134.413	44.956	33.446
5	128.157	36.411	28.411
6	127.004	39.247	30.902
7	124.117	37.458	30.179
8	121.617	36.918	30.356
9	118.868	39.070	32.869
10	125.389	37.008	29.515
11	124.916	39.805	31.865
12	120.747	39.528	32.736
13	117.342	37.850	32.256
14	124.527	35.669	28.643
15	127.893	37.487	29.311

Discussion

This study was designed to examine the stability of aerodynamic measurements in excised leporine larynges previously exposed to ICs and to compare that stability to control larynges. Specifically, this study aimed to determine how variable leporine phonation was for rabbits that received regular IC treatments versus rabbits that received comparable aerosolized

saline treatments (i.e., 18 breaths, twice-daily for 8 weeks). Although some data exist regarding the variability of aerodynamic measurements in other animal models, this information has not been established for the leporine benchtop model (Hoggan, 2020). Because rabbits are particularly suited for longitudinal voice studies, being small animals with relatively similar vocal folds to humans, understanding variability patterns is important to the design of future leporine benchtop studies (Mills et al., 2016; Thibeault et al., 2002). For purposes of the current study, determining the measurement stability of the IC and control groups was essential to understanding if and how vocal folds might be adversely affected by ICs. As all aerodynamic measurements have some element of variability, it was necessary to learn how much variability might be normally expected in leporine benchtop studies (Mills et al., 2016; Yui et. al., 2004).

Measurement Stability Within Larynges

As described in detail in the Results section, analyses were undertaken to determine how stable aerodynamic outcome measures were within each individual larynx. The CV was calculated across all 15 phonation trials for each larynx, using the standard deviation divided by the mean of those trials, and then multiplied by 100 to obtain the percentage value. For each of the aerodynamic measures included in this study—PTP, PTF, sustained pressure, sustained airflow, onset laryngeal resistance, and sustained laryngeal resistance—the CVs were examined individually and the mean CV for both groups were calculated and compared. The results indicated no differences in the variance of the CVs of the larynges in the control group versus the larynges in the treatment group for the six aerodynamic measures. The similarities of the CVs of rabbits in both groups across all measures indicate that the aerodynamic measures obtained through benchtop studies utilizing the leporine model, remain fairly constant across trials. Additionally, the findings suggest that IC exposure did not have a pronounced impact on within-

larynx aerodynamic measure stability across the 15 phonation trials. Nearly all CVs for individual larynges were below 20%, but many were above 10%. The one exception to this pattern was larynx 3 in the control group, which had CVs greater than 20% but less than 25% for PTF and onset resistance. There is no standard regarding what percent variability is acceptable for benchtop experiments, but these results illustrate the importance of sampling multiple times in order to obtain reliable aerodynamic data. In this study, a protocol including 15 phonation trials was adopted based on previous research involving acoustic voice analysis that found that 15 repeated samples were adequate to acquire stable measures of phonation (Scherer et al., 1995). In general, research has concluded that voice disorders may cause more vocal instability due to fluctuating laryngeal function and so more measures may be needed to capture an adequate sample (Pierce et al., 2021; Scherer et al., 1995). The results from this study suggest that aerodynamic measurement, like acoustic measurement, is subject to variability and that amount of variability differs from specimen to specimen. Due to the measurement variability inherent to phonation, the current study results support recommendations for the acquisition of repeated phonation trials to obtain the most representative sample.

Pressure Stability by Group

PTP is the most heavily studied aerodynamic measure of those included in the current research (Döllinger et al., 2018; Hottinger et al., 2007; Plexico et al., 2011). PTP has been shown to be directly related to vocal effort. As was hypothesized, mean PTP was significantly greater for larynges in the inhaler group versus the control group. This is consistent with previous research indicating that PTP increases with IC use (Erickson & Sivasankar, 2010). Although the precise mechanism for the increase in PTP was not directly examined in the current study, one potential explanation is a decrease in superficial vocal fold hydration. Sivasankar and Blazer-

Yost (2009) found that the LABA portion of ICs was related to decreased chloride secretion in porcine vocal folds. When chloride secretion decreases hydration also decreases and subsequently causes an increase in vocal fold viscosity. Vocal fold viscosity is directly linked to PTP, and so these changes lead to an increase in PTP.

Another factor to consider when interpreting PTP results from this and other benchtop studies is that the CV analysis showed that there is some variability both within and between larynges that must be considered. Some variability in aerodynamic measures is to be expected as there are anatomical differences in any species that contribute to differences in phonation both between and within subjects. The similarity in the PTP CVs for individual rabbits in both groups across the 15 trials suggests that IC treatment did not have a significant impact on the variability of measures within individual specimens. However, when aerodynamic measure stability was examined within each group, the variability of PTP measures indicated that IC treatment was associated with an increase in variability between subjects after controlling for trial. The mean CV for the control group was 23%, suggesting some variability between subjects should be expected; but the CV for the IC group was nearly 34%, indicating significantly greater variability between larynges in that group. Due to the greater variability within the inhaler group, it can be suggested that IC treatment may differentially impact each individuals' phonation. This view is supported by the wide array of vocal fold pathologies that have been associated with IC use, including erythema, edema, leukoplakia, granulation, and laryngeal candidiasis (DelGaudio, 2002). In addition, research conducted by Hassen and Hasseba (2016) suggested that 53% of the individuals they studied using ICs had some level of dysphonia, indicating that some individuals experience more dramatic vocal changes with IC use, while some individuals experience minimal changes. These findings seem to align well with the results from the current study. For

instance, the minimum mean pressure value for rabbits in both groups was 5.18 cmH_2O. Although anatomical differences in specimen make it difficult to draw conclusive data, the equal pressure values between rabbits in the two groups seem to support the conclusion that similarly to human tissue, rabbit vocal folds are differentially impacted by IC exposure.

Compared to PTP, sustained pressure as an indicator of vocal fold health has not been as extensively researched. However, both the CV and mean sustained pressure findings mirrored PTP findings, with both mean sustained pressure and mean CV being higher for larynges in the inhaler group versus the control group after controlling for trial. Mean sustained pressure values were comparatively higher than PTP values. This is to be expected as PTP is defined as the minimum subglottal pressure required to initiate and sustain phonation (Titze, 1992). Thus, it would be expected that sustained phonation might require greater pressure than at onset, when the voice is also soft (Jiang & Tao, 2007). However, comparison of PTP and sustained pressure CVs suggest that for both the inhaler and control groups sustained pressure was less variable than PTP. The magnitude of the differences between groups also differs slightly. Although both differences were statistically significant, on average the mean PTP of the inhaler group was about 0.374 cmH_2O greater than the control group, whereas the mean sustained pressure for the inhaler group was about 2.40 cmH_2O greater than the sustained mean pressure of the control group. The reduced variability between specimens and a slightly larger magnitude in differences between groups suggests that sustained pressure may be a valuable measure in distinguishing between disordered and typical phonation for ex-vivo rabbit larynx benchtop models.

Airflow Stability by Group

The mean group PTF values after accounting for trials were also significantly larger for rabbits in the inhaler group versus the control group. This difference was expected due to Ohm's

law which dictates that as pressure increases, flow will also increase for a given level of resistance. The findings from this study suggest that this relationship holds true for the excised rabbit laryngeal model.

Compared to the mean CV for PTP, the mean CV for PTF was higher for both the inhaler and control group after accounting for trial, with the PTF CV for the inhaler group being 40% and for the control group 51%, compared to the PTP CV of the inhaler group which was 33% and 23% for the control group. These results suggest that PTF measurement may produce more variable values between specimen than PTP measurement. This is consistent with those reported by Hoggan (2020), who also found that flow variability was greater than pressure variability in the leporine model.

Unlike the group PTP CV findings, the mean group PTF CV was significantly lower for larynges in the inhaler group compared to the control group after accounting for trial. That is to say, the PTF values across the 15 trials for the larynges in the inhaler group were not as widely distributed about the mean as values in the control group. Examination of individual larynx means and CVs showed that the variance in the means and CVs between the inhaler and control group were not significantly different. However, the raw data indicated that in general the rabbits in the inhaler group had greater PTF values. The decreased group CV of the inhaler group, combined with the individual elevated flow values suggest that most of the larynges in the inhaler group required increased flow to phonate across trials. Both mean sustained airflow values and mean CV values across the 15 trials were very similar to PTF values for both the inhaler and control groups. Like PTF, the mean sustained flow CV for the inhaler group was less than the mean CV for the control group across the 15 phonation trials.

Resistance Stability by Group

As previously described, laryngeal resistance is defined as the quotient of laryngeal pressure divided by airflow. The laryngeal resistance calculation is derived from Ohm's law. In theory, pressure and flow act as covariates meaning that as pressure increases, flow increases proportionally and results in a stable resistance. The linear pressure and flow relationship has been examined in multiple in vivo and ex vivo studies using canine larynges. Multiple studies found that increases in pressure, resulted in proportional increases in flow (Bielamowicz et al., 1993; Muta & Fukuda, 1988) When a linear pressure/flow relationship is maintained, resistance does not adequately capture differences between disordered and typical phonation. However, in some disorders this relationship is disturbed (Alipour et al., 1997). The statistically significant differences between groups, after controlling for trial with the inhaler group having lower resistance, suggest that this is the case for IC exposure. Ohm's law states that a decrease in laryngeal resistance will result in an increase in flow. Based on group differences IC exposure leads to decreases in laryngeal resistance, resulting in increased flow values. Therefore, the laryngeal resistance data from this study lend additional support for the adverse effects of IC use on phonation as indicated by disruption of the proportional pressure-flow relationship. These results also suggest that laryngeal resistance served as an adequate indicator of vocal fold health in this study.

Group onset resistance CVs after accounting for trial followed a similar pattern to the PTF CV, with the CVs being significantly lower for rabbits in the inhaler group. This supports the idea that increased flow values were tied to a decrease in resistance values in the inhaler group. Compared to the other measures examined in this study, onset resistance for the control group was the most variable. This was expected based on existing research which has found that

resistance is highly variable. Awan and colleagues (2013) found that for 60 healthy adults resistance CV was 28% and that intersubject standard deviations were relatively large for their test-retest reliability measures. Due to these factors, they concluded that resistance may not be as effective as other measures in distinguishing between healthy and disordered phonation. This is corroborated by Hillman et al. (1989) who examined the laryngeal resistance in individuals who had laryngeal hyperfunction caused by either organic lesions, or muscle tension dysphonia. They found that resistance measures of these individuals were not significantly different from resistance norms and thus were ineffective in distinguishing between disordered and typical phonation. In the current research, although group means were significantly different, the ranges of the mean resistance values for individual larynges suggest that the variability of resistance measures makes it difficult to rely on resistance measures to distinguish between disordered and typical phonation. The larynges in the inhaler group had mean resistance values ranging from 64.71-110.15 $cmH_2O/L/min$ and larynges in the control group had resistance values that range from 66.71-338.98 $cmH_2O/L/min$. With the exception of larynges 2 and 7 in the inhaler group, all resistance values for rabbits in the inhaler group were encompassed by the range of the control group, making it difficult to identify disordered phonation on the basis of resistance values alone.

Limitations

This study may be subject to limitations. Although care was taken during the dissection process and all individuals who participated in fine dissection of rabbit larynges were carefully trained prior to dissecting larynges, due to the size of larynges and human error, small errors may have been made during dissection. Vocal folds are very fragile and even small lesions can alter phonation, so some variance in measures may be attributed to error during dissection. In

addition, three individuals participated in the dissection of the larynges. Each individual followed a dissection protocol but small variations in the dissection of tissues may have occurred.

Another potential limitation of this study is related to the translational validity of the data to human subjects. Rabbits are comparatively much quieter than humans. This could be beneficial to the research by minimizing the risk of damage to the vocal folds caused by vocal abuse, thus minimizing a potential confounding variable. However, any difference between rabbits and humans may make it more difficult to generalize the results of the study to humans. In addition, due to the presence of fat pads in female rabbits which make dissection of larynges difficult, only male rabbits were used in this study. Thus, it is impossible to predict whether IC use impacts both genders of rabbits equally.

In addition, data were collected in a room where fluctuating temperature, humidity, and ambient noise may have interfered with some of the measures. Although care was taken to reduce ambient noise, some noise such as the freezer, humidifier, or air fan may have contaminated the acoustic signal. When ambient noise obviously interfered with the acoustic signal, trials were redone to ensure the best signal possible. Waveform analysis suggested that contamination of the acoustic signal was minimal, if not inconsequential. Temperature and humidity were also monitored before the first trial and after the last trial of each rabbit and differences were relatively small. Comparison of the data gathered across trials where small fluctuations in temperature and humidity changes occurred did not yield a consistent pattern, indicating that small fluctuations in temperature and humidity likely had little influence on the pressure, flow, and acoustic signals.

Implications for Future Research

In order to minimize the limitations highlighted above, further research could focus on the optimization of the benchtop model. The model employed has been widely used, but systematic changes may increase the validity of measures taken. Specifically, alternative means of humidifying the air may be examined. In the current study the humidifier occasionally alarmed which interfered with acoustic signals. Care to eliminate other ambient noise should also be taken. For an optimal acoustic signal, the benchtop could be placed in a soundproof booth. In addition, in future studies a more specific and standardized training for fine dissection could be employed to minimize human error and differences in dissection between researchers.

This study focused on aerodynamic changes which serve as indices of vocal fold health. The exact mechanism behind the changes seen was not readily apparent. In future research, it would be helpful to include histological and high-speed video analyses to further determine the mechanisms behind changes observed. Future phases of the study plan to incorporate these measures.

Conclusion

The results of this study suggest that IC exposure impacts aerodynamic measures. The group mean PTP, sustained pressure, PTF, and sustained flow were all elevated for the inhaler group compared to the control group. Mean sustained and onset resistance values were significantly lower for the inhaler group versus the control group. As was hypothesized, this study found that some level of variability in aerodynamic measures is to be expected both within and between subjects. IC exposure did not impact the aerodynamic measure stability within individual larynges, with CVs for individual rabbits in both groups generally being less than 20%. This level of variability was less than the variability seen between subjects. This suggests

that when utilizing the leporine benchtop model, within-subject study designs are preferable to between-subject designs. It also emphasizes the importance of taking multiple measures to truly capture the aerodynamic performance of any given larynx.

Although IC exposure was not related to within-subject variability, it was associated with between-subject variability. Specifically, IC exposure increased the variability of both PTP and sustained pressure measures and decreased the variability of PTF, sustained flow, onset resistance, and sustained resistance. The increase in pressure variability suggests that as with human tissue, individual leporine larynges may respond differently to IC treatment. The ability of the leporine model to capture these nuanced histological changes underscores that it is an ideal model for asthma research. The fact that IC treatment differentially impacted the variability of each of the aerodynamic measures also underscores the importance of including multiple aerodynamic measures in future research. As was mentioned previously, each aerodynamic measure is sensitive to different histological changes, so compiling data from each aerodynamic measure can help detect subtle changes that impact phonation.

This study provides important information about the level of variability that can be expected when performing research using the leporine benchtop model. Due to variability changes that occurred between subjects caused by IC exposure, it additionally highlights the importance of being aware of aerodynamic variability when examining disordered phonation in order to capture adequate aerodynamic data.

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APPENDIX A

Annotated Bibliography

Cohen, S. M., Kim, J., Roy, N., Asche, C., & Courey, M. (2012). Prevalence and causes of dysphonia in a large treatment-seeking population. *The Laryngoscope, 122*(2), 343–348. https://doi.org/10.1002/lary.22426

Purpose of the study. The purpose of this study was to determine the prevalence of dysphonia among the general population; examine the causes of dysphonia and any comorbid diagnoses; and determine the association between age, sex, and presence of dysphonia.

Method. Data in a national medical claims dataset gathered between 2004 and 2008 was examined and the prevalence of dysphonia was determined by collecting information from various ICD-10 codes connected with vocal dysfunction. For individuals with these diagnoses other demographic data was gathered.

Results. A dysphonia prevalence rate of 0.98% for all individuals in the database was determined. Those who had dysphonia were more likely to be female (63.4%) and had a mean age of 46.3. The primary comorbid diagnosis included gastroesophageal reflux, pneumonia, pharyngitis, acute bronchitis and upper respiratory illness.

Conclusion. There is a 1% prevalence rate of dysphonia in the general population. Females are at greater risk for developing dysphonia than males.

Relevance to the current work. This study provides the prevalence of voice disorders in the general population which allows for comparison among those individuals who both have asthma and use ICs to treat that asthma. This justifies the need for the current work.

DelGaudio, J. M. (2002). Steroid inhaler laryngitis: Dysphonia caused by inhaled fluticasone therapy. *Archives of Otolaryngology Head and Neck Surgery, 128*(6), 677–681. https://doi.org/10.1001/archotol.128.6.677

Purpose of the study. The purpose of this study was to examine and describe cases of Steroid Inhaler Laryngitis.

Method. The larynges of 20 individuals diagnosed with Steroid Inhaler Laryngitis between 1998 and 2001 were examined using videofluoroscopy. Symptoms were then treated, and the progress of each patient was noted. The authors recorded the specifics of five of these cases in detail and provided a comprehensive summary of all the other cases.

Results. All individuals participating in this study had reactive airway disorder with complaints of dysphonia. The results of videofluoroscopic examination revealed laryngeal pathologies that ranged from mild to severe. Pathologies included erythema, edema, leukoplakia, granulation, and laryngeal candidiasis. The timing of onset of dysphonia symptoms varied with each individual. When severity of respiratory airway disease allowed, cessation of inhaled fluticasone treatment resolved dysphonia symptoms.

Conclusion. Steroid Inhaler Laryngitis is often associated with the use of inhaled fluticasone treatments. Steroid Inhaler Laryngitis can result in mild to severe dysphonia and can cause a variety of laryngeal pathologies. Where possible cessation of fluticasone treatment is the best option to treat laryngeal pathologies and dysphonia.

Relevance to the current work. The current study examines the effects of ICs on the larynx. This study details some of the laryngeal pathologies that are associated with inhaled steroid treatment which substantiates the relevance of the current study.

Döllinger, M., Kniesburges, S., Berry, D. A., Birk, V., Wendler, O., Dürr, S., Alexiou, C., & Schützenberger, A. (2018). Investigation of phonatory characteristics using ex vivo rabbit larynges. *The Journal of the Acoustical Society of America, 144*(1), 142–152. https://doi.org/10.1121/1.5043384

Purpose of the study. This study quantifies the acoustic and pressure signals of excised rabbit larynges to aid in future phonatory research. It further investigates the effects of glottal closures on phonatory measurements.

Method. Eleven white rabbit larynges were excised and mounted on a benchtop model. Each larynx was vibrated 15 times at three different elongation positions. During vibration high-speed video, acoustic and pressure data was collected. The high-speed video footage was then analyzed to determine glottal closure. Measurements and histological analysis were also performed on each larynx.

Results. It was determined that as elongation and pressure increased, glottal closure also increased as well. Glottal closure impacted the quality of both acoustic and aerodynamic signals, with greater closure resulting in an improved signal. The researchers also determined that the dynamic range of leporine larynges is greater than had previously been reported.

Conclusion. Glottal closure impacts both acoustic and aerodynamic measurements. Greater glottal closure results in better acoustic signals. Better glottal closure is influenced by a greater degree of elongation and an increase in pressure. These measurements paired with histological analysis confirm that rabbit larynges are good models for phonatory research.

Relevance to the current work. The current study utilizes ex-vivo leporine larynges and utilizes a similar benchtop model to the one employed in the current study. The aerodynamic values collected in this study provide a comparison for the current study's data.

Döllinger, M., Kobler, J., Berry, D., Mehta, D., Luegmair, G., & Bohr, C. (2011). Experiments on analyzing voice production: Excised (human, animal) and in vivo (animal) approaches. *Current Bioinformatics, 6*(3), 286–304. https://doi.org/10.2174/157489311796904673

Purpose of the study. The purpose of this article was to review findings from previous in vivo and excised laryngeal studies. By doing so, the authors provide a rationale for using these research designs in future research.

Method. The authors examined and summarized the findings from a large body of articles that used either in vivo or ex vivo excised larynx models.

Results. In vivo studies have been used to examine several topics through simulation including, specific muscle contraction, mucosal wave patterns, and clinical issues like vocal fold paralysis. Excised laryngeal models have been beneficial in analyzing vocal fold vibration, airflow, pressure, and their relationships.

Conclusion. There are benefits to using both in vivo and excised larynx models. While in vivo studies are particularly beneficial in examining neuromuscular function, excised larynx experiments are more helpful in examining airflow and pressure dynamics.

Relevance to the current study. The current study uses excised larynges to examine pressure and flow relationships. This study provides a rationale for the selection

of this experimental design and demonstrates the clinical value of excised larynx benchtop studies.

Erickson, E., & Sivasankar, M. (2010). Evidence for adverse phonatory change following an inhaled combination treatment. *Journal of Speech, Language, and Hearing Research*, *53*(1), 75–83. https://doi.org/10.1044/1092-4388(2009/09-0024)

Purpose of the study. The purpose of this study was to examine the effects that IC treatment had on the phonation threshold pressure PTP and perceived phonatory effort (PPE) of individuals with asthma.

Method. Fourteen adults with asthma, who had been prescribed ICs treatment, were recruited for this study. Each individual was given either a sham or an actual IC treatment for 2 consecutive days. Prior to receiving the treatment, their vocal range was measured. Phonation threshold pressure was then measured at their 10^{th}, 20^{th} and 80^{th} percent pitches. Each individual also rated their PPE, before the treatment, immediately after the treatment, and 1 and 2 hours post-treatment.

Results. The data indicated an increase in PTP for individuals receiving IC treatment at all pitches as the time post-treatment increased. However, these increases were only significant at the 80^{th} percent pitch. These increases were not seen in sham treatment measures. No significant increases in PPE were found.

Conclusion. Inhaled corticosteroid treatment for asthma does have an acute negative effect on the vocal effort required to produce phonation as measured by PTP.

Relevance to the current work. This study examined the negative effects of ICs treatment on voice parameters and calls for further research to examine the mechanism

behind these changes. These mechanisms are what the current study is attempting to quantify.

Gallivan, G. J., Gallivan, K. H., & Gallivan, H. K. (2007). Inhaled corticosteroids: Hazardous effects on voice—An update. *Journal of Voice, 21*(1), 101–111. https://doi.org/10.1016/j.jvoice.2005.09.003

Purpose of the study. The purpose of this study was to determine the side effects of IC use on laryngeal function. This study employed strobovideolaryngoscopy (SVL) to analyze the impact of IC use on the mucosal wave and glottic closure of the vocal folds.

Method. Thirty-eight patients diagnosed with of bronchial asthma, that was being treated through IC use, were recruited for this study. Each of these individuals was experiencing hoarseness and dysphonia. Comprehensive histories were gathered from each patient and they received laryngeal examination through at least one SVL. These SVL were then analyzed for presence of abnormalities.

Results. Presence of abnormal SVL findings and symptom incidence increased with increased dosage and frequency of medication use. 79% of individual had abnormalities in mucosal wave symmetry, 74% had incomplete phase closure, 63% had abnormal glottic closure, 50% had abnormalities in the magnitude of the mucosal wave, and 38% had abnormalities with the free edge of the vocal folds.

Conclusion. Use of ICs results in various changes to the structure and function of the vocal folds. Increased dosages can cause greater damage.

Relevance to the current work. Both this study and the current study examine the effects of ICs on the voice. This study examines the effects of IC use of the structure of human subjects' larynges. This data in this study provides a comparison to the data

collected through the leporine model utilized in the current study. Furthermore, this study provides suggestions for the identification of physiological changes that may lead to the voice being altered or disordered that can be compared to the aerodynamic results of the current study.

Hassen, H. E., & Hasseba, A. M. A. (2016). Voice evaluation in asthma patients using inhaled corticosteroids. *The Turkish Journal of Ear Nose and Throat*, *26*(2), 101–108. https://doi.org/10.5606/kbbihtisas.2016.79740

Purpose of the study. The purpose of this research was to examine the presence of dysphonia and laryngeal abnormalities in patients who use ICs to treat their asthma.

Method. Thirty individuals with a diagnosis of bronchial asthma, that was treated with ICs between May and December of 2013, participated in this study. Each participant's larynx was examined using videofluoroscopy and a comprehensive voice history was also collected for each individual. Each individual's vocal quality was analyzed using the grade, roughness, breathiness, asthenia and strain (GRBAS) scale, and acoustic analysis was performed on recordings of sustained vowels and pitch glides. After all data was collected, statistical analysis was performed to determine the significance of the data.

Results. It was determined that 53% of participants had some level of dysphonia. Some participants complained of other symptoms such as phonasthenic symptoms (47%) and regurgitation or cough history (36.7%). Videofluoroscopic data indicated that 56.7% of participants had interarytenoid thickening, and 56.7% had vocal fold erythema. Other prominent findings included the presence of vocal fold edema, supraglottic

hyperfunction, and vocal fold edge irregularity. In addition, acoustic analysis revealed jitter values that were significantly higher than expected.

Conclusion. IC treatment is correlated with some level of dysphonia in many patients. This dysphonia may be partially due to anatomic changes that have an adverse phonatory impact.

Relevance to the current work. This study describes physiological and anatomical changes experienced by IC users. This provides rationale for observed changes in PTP and PTF, and for the necessity of the current study.

Holmberg, E. B., Doyle, P., Perkell, J. S., Hammarberg, B., & Hillman, R. E. (2003). Aerodynamic and acoustic voice measurements of patients with vocal nodules: Variation in baseline and changes across voice therapy. *Journal of Voice, 17*(3), 269–282. https://doi.org/10.1067/s0892-1997(03)00076-6

Purpose of the study. The aim of this study was to determine whether acoustic and aerodynamic measurements accurately represented changes in vocal fold health and appearance.

Method. Ten females with a diagnosis of bilateral vocal nodules were recruited for this study. Each underwent videolaryngoscopy to confirm diagnosis and then participated in voice therapy once a week for 4–6 months. Each subject's baseline performance on aerodynamic and acoustic tasks was determined prior to beginning treatment, and then again after each therapy phase. Statistical analysis was performed to determine relationships between measures.

Results. Differences in pre- and post-therapy videolaryngoscopic data showed decreases in nodule size and edema. Some significant differences in aerodynamic

measurements were observed between baseline and post-therapy measures for individuals, however significant group differences were not observed. This may be, in part, due to large session-to-session variation in all measures. When compared to previously gathered normative data, aerodynamic measurements were more accurate in predicting laryngeal pathology than acoustic measurements.

Conclusion. Aerodynamic measures are a better indication of vocal fold pathology than acoustic measures. Neither aerodynamic nor acoustic measurements changed significantly between pre- and post-therapy measurements, indicating that other analysis is needed to indicate progress during therapy.

Relevance to the current work. This study indicates that aerodynamic measurements have predictive validity in demonstrating the presence of vocal fold pathologies. This supports the use of PTP, PTF, and laryngeal resistance as measures of vocal fold health in the current study.

Hottinger, D. G., Tao, C., & Jiang, J. J. (2007). Comparing phonation threshold flow and pressure by abducting excised larynges. *Laryngoscope*, *117*(9), 1695–1699. https://doi.org/10.1097/MLG.0b013e3180959e38

Purpose of the study. This study examines the sensitivity of PTP and PTF to changes in prephonatory glottal width.

Method. Ten canine larynges were harvested post-mortem. Using a benchtop model, trials were run on each larynx at five different prephonatory glottal widths. Five trials were run at each width, and the mean PTP and PTF data were calculated for each of the five trials. An ANOVA was run to determine whether mean PTP and PTF values, at

each width, were significantly different from each other. A linear model was developed to explain differences in PTF at different widths.

Results. It was determined that PTF differences between prephonatory glottal widths were statistically significant. Differences in the PTP values were not found to be statistically significant. A linear model was also able to be developed to match PTF data but not PTP data.

Conclusion. PTF is more sensitive to changes in prephonatory glottal width than PTP is. This has important implications for the use of PTF to diagnose physiologic changes in the vocal folds.

Relevance to the current work. PTP and PTF are two of the outcomes measured in the current study. This study provides a rationale for the use of PTF when examining vocal fold pathology. It further discusses situations when PTF may be a more appropriate measure than PTP establishing that there is a need for both outcome measurements.

Ihre, E., Zetterström, O., Ihre, E., & Hammarberg, B. (2004). Voice problems as side effects of inhaled corticosteroids in asthma patients - A prevalence study. *Journal of Voice 18*(3), 403–414. https://doi.org/10.1016/j.jvoice.2003.05.003

Purpose of the study. The purpose of this study was to determine the prevalence of voice disturbances in individuals who use ICs as a prescribed treatment for their asthma.

Method. A 25-question questionnaire was distributed to 350 patients who had a confirmed diagnosis of asthma at three different allergology and asthma hospitals in Stockholm, Sweden. The questionnaire was comprised of questions concerning an individual's asthma symptoms, voice disturbances and problems, and use of medication.

Results. Two-hundred-eighty patients responded to the questionnaire. Results indicated that there was a positive correlation between the use of corticosteroids and voice problems. Voice hoarseness and throat clearing were the most frequently indicated voice problems. No differences were found in voice difficulties experienced by males and females, however, patients with higher cortisone prescriptions, as well as those who had more vocally demanding professions indicated more frequent voice related difficulties.

Conclusion. There is a positive correlation between voice problems and the use of corticosteroids to treat asthma.

Relevance to the current work. This study quantifies the prevalence of voice disturbances caused by the inhalation of corticosteroids in one population. The current study is attempting to further quantify these disturbances through the collection of aerodynamic measurements.

Jiang, J., Raviv, J., & Hanson, D. (2001). Comparison of the phonation-related structures among pig, dog, white-tailed deer, and human larynges. *Annals of Otology, Rhinology, & Laryngology, 110*(12)*,* 1120–1125. https://doi.org/10.1177/000348940111001207

Purpose of the study. This study compares the anatomy of the phonation-related structures of pig, dog, white-tailed deer, and human larynges. These comparisons were used to determine which animal models most closely resemble humans.

Methods. The resting vocal fold length and height, cricothyroid angular range of motion, and vocal fold stiffness were measured in the excised larynges of two humans, three dogs, three white-tailed deer, and three pigs. The differences in measurement among these larynges were then compared.

Results. The vocal fold length of dogs was closest to humans. Both dog and pig vocal fold heights, and ranges of motion were similar to that of humans, but deer vocal fold height was much smaller. Vocal fold stiffness was most similar between the deer and human models.

Conclusion. Of the examined models, pig and dog appear most similar to human larynges, suggesting that they may be beneficial as models in phonatory research.

Relevance to current work. The current study also uses ex-vivo animal larynges to study human phonation. This study provides rationale to support using excised larynges and animal models in phonation research.

Keir, S., & Page, C. (2008). The rabbit as a model to study asthma and other lung diseases. *Pulmonary Pharmacology & Therapeutics*, *21*(5), 721–730. https://doi.org/10.1016/j.pupt.2008.01.005

Purpose of the study. The purpose of the article was to establish a rationale for the use of rabbits in research concerning asthma and other lung diseases.

Method. The authors reviewed benefits of using rabbits over other small animals. They also examined the similarities in the reactions of human subjects with asthma and rabbits who had been neonatally immunized, to various stimuli. Furthermore, they compared asthma subjects with asthma and rabbits' reactions to treatment drugs.

Results. There are a variety of similarities between neonatally immunized rabbits and humans with asthma that make rabbits an ideal model for studying asthma and lung diseases. Among these similarities is the similarity in airway inflammation in response to a variety of stimuli and sensitivity to similar drug treatments.

Conclusion. Rabbits are a good model for studying asthma and other lung disease's pathophysiology and treatment.

Relevance to the current work. This paper provides rationale for using rabbit models in asthma research. This information helps to justify the selection of the rabbit model in the current study.

Lavy, J. (2000). Dysphonia associated with inhaled steroids. *Journal of Voice, 14*(4), 581–588. https://doi.org/10.1016/S0892-1997(00)80014-4

Purpose of the study. The purpose of this study was to quantify the effects of inhaled steroid use on the voice through the use of acoustic analysis and videolaryngoscopy.

Method. Twenty-two individuals, who were using inhaled steroids and experienced subsequent hoarseness, were referred to this study. Each individual underwent videolaryngoscopy, voice acoustic analysis, and answered questions in a survey. Investigators analyzed supraglottic hyperfunction, quality of mucosa, vocal fold apposition, and stroboscopic wave quality using the videolaryngoscopy data. In addition, they used acoustic data to analyze the mean fundamental frequency, maximum phonation time, and jitter.

Results. Results from videolaryngoscopic investigation suggest that nine individuals had poor vocal fold apposition, and eight experienced some degree of supraglottic hyperfunction. For various reasons, the mucosal quality could only be assessed in 14 individuals, but of those who could be assessed, five individuals' mucosal quality was described as abnormal. Acoustic data suggested that six individuals

experienced reduced maximum phonation time, and that changes in jitter were quite common.

Conclusion. Although more information is needed to rule out other underlying causes, videolaryngoscopic and acoustic data suggest that there may be structural and physiological changes secondary to use of inhaled steroids.

Relevance to the current work. The current study also seeks to quantify changes secondary to IC use.

Maytag, A. L., Robitaille, M. J., Rieves, A. L., Madsen, J., Smith, B. L., & Jiang, J. J. (2013). Use of the rabbit larynx in an excised larynx setup. *Journal of Voice*, *27*(1), 24–28. https://doi.org/10.1016/j.jvoice.2012.08.004

Purpose of the study. The purpose of this study was to establish a reliable method for the utilization of rabbit larynges in benchtop studies. The histological similarities between rabbit and human vocal folds gives this method practical utility in the current and in future research.

Method. Five New Zealand White Rabbit larynges were harvested, stored, and dissected. The epiglottis and extraneous thyroid cartilage and tissue were cut away to reveal the true vocal folds. The larynges were mounted on a Luer Lock and were secured using glue and a zip tie. Humidified air was blown through the vocal folds while acoustic, video, electroglottograph, and aerodynamic data was gathered. The data collected from the rabbits was compared to five excised canine larynges that had been phonated in a similar manner.

Results. All rabbits successfully phonated. Phonation threshold pressure, flow, mucosal wave amplitude, and F_0 were reported. Coefficients of variance were reported and compared for the rabbit and canine larynges.

Conclusion. This method of dissection and data collection is reproducible and reliable. This model will be helpful in future studies that examine changes in vocal fold tissues.

Relevance to the current work. The benchtop model used in this study is very similar to the model used in the current study. This study also provides information on the similarities between human and rabbit vocal fold histology that justifies the selection of rabbit larynges as the animal model in the current study.

Mills, R., Dodd, K., Ablavsky, A., Devine, E., & Jiang, J. (2016). Parameters from the complete phonatory range of an excised rabbit larynx. *Journal of Voice*, *31*(4), 517.e9–517.e17. https://doi.org/10.1016/j.jvoice.2016.12.018

Purpose of the study. This study quantifies the airflow, subglottal pressure, F_0, sound pressure level and vibratory amplitude across the full phonatory range of excised rabbit larynges. These values serve to further support the idea that rabbit larynges are ideal for examining vocal fold inflammation due to the similar vocal fold histology between rabbit and human larynges.

Method. Seven rabbit larynges were mounted on a benchtop and data was collected on each larynx at 0%, 5%, 10%, 15%, and 20% elongation. At each position airflow level at PTP was recorded and then gradually increased in .25 L/min increments until phonation instability pressure was reached. The average parameters gathered during

each elongation phase were compared using a one-way repeated measures analysis of variance.

Results. Data indicated that as elongation increased PTP also increased significantly. PTF values did not differ significantly as elongation changed. Both phonation instability flow and phonation flow range had an inverse relationship with elongation, decreasing significantly as elongation increased. F_0 also increased with elongation, while vibratory amplitude decreased.

Conclusion. Changes in airflow and elongation of the vocal folds have a direct impact on pressure, F_0, and vibratory amplitude. This seems to indicate that the rabbit is a good model for studies involving vocal fold inflammation.

Relevance to the current work. This study utilizes a similar benchtop model to the current study. It also provides support for the utilization of leporine larynges in benchtop studies when investigating histological changes to vocal fold tissues. The current thesis can be classified as this type of experiment as it attempts to quantify changes in laryngeal health secondary to IC use.

Park, B., & Choi, H.G. (2016) Association between asthma and dysphonia: A population-based study. *Journal of Asthma, 53*(7)*,* 679–683, https://doi.org/10.3109/02770903.2016.1140181

Purpose of the study. The purpose of this study was two-fold. 1) To determine the prevalence of organic laryngeal disease in individuals with asthma, as compared with the general population. 2) To determine the prevalence of voice-related difficulties for individuals who have asthma and are either medicated or not medicated as compared to the general population.

Method. Data on 19,330 participants gathered across four years was analyzed. Each of the subjects underwent laryngoscopic examination, during which all endoscopic findings and diagnoses were recorded. Participants also answered questions concerning whether they had an asthma diagnosis and reported any voice discomfort or dysphonia they were experiencing. Collected data underwent statistical analysis and adjusted odds ratios (AOR) were calculated for several factors.

Results. Results indicated that presence of laryngeal lesions was similar for both the asthma group (7.8%) and the control group (7.0%). However, the presence of dysphonia in individuals without organic laryngeal lesions was significantly higher for the asthma group (11.3%) than for the control group (5.5%). Adjusted odds ratios indicated that those of the female sex and those who reported having greater stress levels had higher risk for dysphonia. Presence of asthma also showed a greater AOR, with the AOR being the greatest for those who had been medicated for asthma within the past year.

Conclusion. Presence of asthma places one at a greater risk for experiencing dysphonia. This risk is increased when asthma symptoms are treated with medication.

Relevance to the current work. This study illuminates some potentially confounding variables in drawing an association between ICs use and voice difficulties. This research will be useful in ensuring sure these variables are controlled for in the current study.

Plexico, L., Sandage, M., & Faver, K. (2011). Assessment of phonation threshold pressure: A critical review and clinical implications. *American Journal of Speech-Language Pathology, 20*(4), 348–366. https://doi.org/10.1044/1058-0360(2011/10-0066)

Purpose of the study. The purpose of this study is to review current literature about PTP to determine its clinical applicability and establish data collection standards to be used in future research.

Method. The authors begin by defining what PTP is and explaining that it is influenced by vocal fold stiffness and viscosity, mucosal wave velocity, and prephonatory glottal width. They then transition to explanation of current methods for gathering and assessing PTP. They expound on both direct and indirect measures and highlight procedural differences that may make comparing results difficult. The researchers support this with a critical review of articles examining PTP. They also performed a survey of current practitioners to determine how they use PTP clinically and in research.

Results. The critical review illuminated that PTP has been used to study a variety of topics including hydration and vocal fatigue. The majority of studies were performed on young adults with a few studies including older adults and no studies including children. Differences were found in consonant vowel sequences used to elicit PTP, quiet phonation, and other procedural explanations. The survey of practitioners found the 55.9% of respondents did not use PTP clinically. Again, procedural differences were seen in PTP data collection among those who did use it. Differences found in both the survey and literature review included difference in consonant vowel trains used, number of syllables used to illicit PTP and which pressure peaks are used to calculate PTP.

Conclusion. In order to compare research employing PTP and increase the clinical utility of PTP, a more standardized method for gathering PTP is needed. The

researchers of this study argue that the syllable train /pi/, with 5 syllables, has the greatest evidence to support it.

Relevance to the current work. This article provides important information on what laryngeal characteristics that influence PTP. PTP is one of the primary outcome measurements employed in the current study and an understanding of the laryngeal characteristics that influence PTP is crucial in interpreting changes secondary to IC use.

Regner, M. F., Robitaille, M. J., & Jiang, J. J. (2010). Interspecies comparison of mucosal wave properties using high-speed digital imaging. *Laryngoscope, 120*(6), 1188–1194. https://doi.org/10.1002/lary.20884

Purpose of the study. This aim of this study was to determine which animal model most closely simulates human laryngeal vibration. This study compared the vocal fold vibratory characteristics of pig, cow, dog and sheep larynges with human laryngeal vibration.

Method. Porcine, ovine, bovine, and canine larynges were mounted on a benchtop model. Each larynx was vibrated at three different subglottal pressures and ten high-speed videos were recorded at each of the pressure levels. Information from the videos was used to make a kymograph. The kymograph data was used to analyze the frequency, amplitude, and superior-inferior phase difference of vibration. The data gathered on each species was then compared using statistical analysis.

Results. Comparisons between species revealed that the oscillation frequency of canine larynges was the most similar to humans. Porcine models have an amplitude range most like humans, however neither porcine nor canine oscillation amplitudes were significantly different then human oscillation amplitude. Comparison between animal

models indicated that the porcine's phase difference was significantly different from all other species.

Conclusion. The authors concluded that where physical vibration is concerned canine and porcine models are the best models to compare to in vivo human larynx vibration.

Relevance to the current work. This study utilizes a benchtop model similar to the current study. It also provides the limitations of other animal models which can serve as a comparison for the leporine model utilized in the current study.

Roy, N., Merrill, R., Gray, S., & Smith, E. (2005). Voice disorders in the general population: Prevalence, risk factors, and occupational impact. *Laryngoscope, 115*(11), 1988–1995. https://doi.org/10.1097/01.mlg.0000179174.32345.41

Purpose of the study. The purpose of this study was to identify the prevalence of voice disorders among the general adult population, and to identify any variables that increase one's risk of developing a voice disorder.

Method. The general population in Iowa and Utah were sampled and interviewed using random digit dialing. Teachers in both states were also sampled and interviewed randomly from a list of currently employed teachers in both states. Each survey participant was asked questions about current voice use patterns and potential risk factors for developing voice disorders. Data gathered went through statistical analysis.

Results. Of the survey respondents, 29.9% indicated that they had experienced a voice disorder during their lives, with 6.6% reporting current voice disorders. Risk factors associated with voice disorders included being female and more educated, experiencing exposure to chemicals, and increased frequency of talking, talking loudly,

and talking quietly. Results also suggested voice disorders have an occupational impact with 7.2% of employed participants noting at least one missed day of work due to their voice difficulties.

Conclusion. Data gathered in this study provides important information about the prevalence of voice disorders and associated risk factors. This data can be used to help prevent voice disorders.

Relevance to the current work. This study examines some of the occupational impacts of voice disorders. This information helps provide insight into the potential functional impact of voice disorders caused by ICs use.

Sahrawat, R., Robb, M., Kirk, R., & Lutz, B. (2014). Effects of inhaled corticosteroids on voice production in healthy adults. *Logopedics Phoniatrics Vocology*, *39*(3), 108–116. https://doi.org/10.3109/14015439.2013.777110

Purpose of the study. The purpose of this study was to examine the effect that ICs had on the acoustic parameters of the voice for 15 healthy females and 15 healthy males, and to examine if the effects were gender specific.

Method. Fifteen healthy male and 15 healthy female college students were recruited to participate in this study. These individuals were administered ICs over a 5-day period in both the morning and the evening. Audio recordings of the individuals producing sustained vowels and "The Rainbow Passage" were recorded an hour after administration on the morning and evening of the first day, on the evening of the fifth day, and on the sixth day of the experiment. The audio recordings were analyzed to look for changes in F_0, first and second formant frequency, and bandwidth for sustained

vowels. A long-term spectral analysis was performed to determine first spectral peak and spectral tilt for reading passages.

Results. No significant changes were found for F_0 or formant bandwidth. There were small changes seen in the first formant for the vowel /i/ between pre-IC recordings and the first recording and second recordings. There were also significant changes in first spectral peak and spectral tilt. With the first spectral peak increasing from pre-IC recordings to the third recording and spectral tilt lowering across recordings. These changes disappeared a day after IC treatment was stopped.

Conclusion. These results suggest that for healthy individuals receiving IC treatment, there are some changes in acoustic parameters. These acoustic parameter changes are more obvious during connected speech than for sustained phonation.

Relevance to the current work. This study quantifies some of the acoustic parameter changes seen when ICs are used in healthy individuals, which supports that the voice changes as a function of ICs use. The current work uses aerodynamic measurements rather than acoustic measures, but the data provided in this study can provide a good comparison.

Witt, R. E., Regner, M. F., Tao, C., Rieves, A. L., Zhuang, P., & Jiang, J. J. (2009). Effect of dehydration on phonation threshold flow in excised canine larynges. *Annals of Otology, Rhinology & Laryngology, 118*(2), 154–159. https://doi.org/10.1177/000348940911800212

Purpose of the study. The purpose of this study was to determine the effect of surface dehydration on PTP values and to help determine whether PTF is a viable measure of vocal fold health.

Method. Eleven canine larynges were harvest post-mortem. Excised larynges were mounted onto a bench top and PTF data was gathered on each larynx. Eight larynges were subjected to a dehydration trial where they were phonated for 10 seconds using dry airflow, followed by a 3 second rest period for 5 minutes. Two of these larynges continued trials until the larynges were no longer able to phonate. The two control larynges received similar treatment to the dehydration trials, but airflow was 100% humidified. One larynx was subjected to both control trials followed by dehydration trials. PTF data was gathered for each trial. Initial and final PTF data were then compared using a t-test to determine if differences were significant.

Results. Statistically significant differences were found between initial and final PTF values for all dehydration trials. Significant differences in PTF were also found between each trial cycle. Significant differences were not found in initial and final PTF values for control trials. The PTF values between the control and dehydration trials for the larynx that received both treatments were also significantly different.

Conclusion. PTF values are correlated with vocal fold dehydration. This indicates that PTF may have clinical application as a determinant of vocal fold health. The differences seen between the dehydration and control values of the larynx that received both treatments suggest that differences were due to dehydration and not caused by group differences.

Relevance to the current work. The data collected in this study suggests that PTF varies with vocal fold health and thus supports the use of PTF as a primary outcome measurement in the current study. It further highlights the importance in maintaining

adequate tissue hydration when collecting data during the current study in order to limit confounding variables.

Yiu, E. M., Yuen, Y. M., Whitehill, T., & Winkworth, A. (2004). Reliability and applicability of aerodynamic measure in dysphonia assessment. *Clinical linguistics & phonetics, 18*(6–8), 463–478. https://doi.org/10.1080/0269920041000170359

Purpose of the study. The purpose of this study was to create normative pressure and flow data for a homogenous group of individuals, and to establish the accuracy of these measurements in distinguishing between normal and pathological voices. A secondary aim of this study was to identify whether increasing the number of trials when gathering aerodynamic measurements increases accuracy of measurements.

Method. Twenty-eight females between the age of 20 and 40 with diagnosed laryngeal pathologies and dysphonia were recruited to the study. These subjects were then matched with an individual of a similar age in a non-dysphonic group. Aerodynamic measurements were taken on each subject as they performed maximum phonation time, comfortable vowel phonation, vowel-consonant syllable string, and sentence production tasks. Information on the mean flow rate, and peak intra-oral pressure as an estimate for subglottal pressure were extracted from these phonation tasks.

Results. Airflow rates and subglottal pressure estimates were significantly higher for the dysphonic group when compared to non-dysphonic group. However, there was individual variability. Aerodynamic measurements were shown to be 91.1% accuracy in discriminating between dysphonic and non-dysphonic groups when five clinical trials were used to calculate measures but dropped to 87.5% accuracy when three trials were used.

Conclusion. Aerodynamic measures are quite accurate in discriminating between disorder and non-disordered voices. Accuracy of discrimination increases with repeated measures or aerodynamic trials.

Relevance to current work. This article examines the clinical utility of PTP as a measure of laryngeal functions, which supports inclusion of the measure as an outcome measurement in the current study. This study also discusses variability in acoustic measurement and why repeated measures is necessary. The current work is also investigating variability within excised larynges.

Zhuang, P., Sprecher, A. J., Hoffman, M. R., Zhang, Y., Fourakis, M., Jiang, J. J., & Wei, C. S. (2009). Phonation threshold flow measurements in normal and pathological phonation. *Laryngoscope*, *119*(4), 811–815. https://doi.org/10.1002/lary.20165

Purpose of the study. This study examines the effects of vocal nodules and polyps on PTF and mean flow rate (MFR). It also examines the potential of using PTF to distinguish between healthy and disordered vocal folds.

Method. PTF and MFR measurements were gathered on 40 individuals with healthy vocal folds, 21 individuals with vocal nodules, and 23 individuals with vocal polyps. T-tests were performed to determine the effects of gender on these measures and an ANOVA was performed to determine the effects of vocal fold pathology on PTF.

Results. Gender was found to significantly influence PTF and MFR. ANOVA tests indicated that PTF does differ significantly with vocal fold pathology. Further analysis found that while there were significant differences in PTF values between the normal and polyp groups, there was little difference between the polyp and nodule group, or the nodule and normal groups.

Conclusion. The identified difference suggests that PTF is reflective of the biomechanical properties of the vocal folds. The differences further suggest that with further research PTF could be clinically useful in distinguishing between pathological and normal vocal folds.

Relevance to the current work. The current study uses PTF as an outcome measure in indicating differences in vocal fold health. This study supports the idea that PTF can be used in differentiating between normal and pathological folds, particularly if pathology effects vocal fold mass or prephonatory glottal width as it did in this study.

Zhuang, P., Swinarska, J. T., Robieux, C. F., Hoffman, M. R., Lin, S., & Jiang, J. J. (2013). Measurement of phonation threshold power in normal and disordered voice production. *Annals of Otology, Rhinology & Laryngology, 122*(9), 555–560. https://doi.org/10.1177/000348941312200904

Purpose of the study. The purpose of this study is to determine whether phonation threshold power (PTW) is effective in distinguishing between individuals with normal voices, vocal fold masses and vocal fold motility disorders.

Method. PTP and PTF data was gathered for 100 individuals with normal vocal folds, 72 individuals with polyps, 22 individuals with cysts, and 19 individuals with mobility disorders. Additional data was also gathered on 41 individuals in the polyp group who underwent polyp excision and then repeated trials. The products of PTP and PTF values for each individual were calculated to yield PTW. The values for each group were compared using statistical analysis.

Results. There were statistically significant differences between the control group and all other groups. In addition, all three values decreased significantly after polyp

excision. PTW was found to be the most sensitive measurement in distinguishing between groups.

Conclusion. PTW could potentially be used to distinguish between individuals who have polyps or motility disorders and those who do not.

Relevance to the current work. This study discusses outcomes that can be determined through the use of PTP and PTF measures. These measures are two primary outcome measures in the current study and will be used to indicate vocal fold health.

Zosky, G. R., & Sly, P. D. (2007). Animal models of asthma. *Clinical & Experimental Allergy*, *37*(7), 973–988. https://doi.org/10.1111/j.1365-2222.2007.02740.x

Purpose of the study. This paper examines the research utility of animal models in the study of asthma. It outlines the benefits and limitations of some of the animal models that are currently in use and calls for further research to examine the benefits of different animal models.

Method. The authors reviewed the benefits of using various animal models, detailing the benefits and drawbacks of the use of mice, rats, guinea pigs, sheep, and dogs in asthma studies. They further outlined several of the difficulties in generalization to human subjects. This included the anatomical variation between animal and human subjects and the fact that most animals do not naturally develop asthma symptoms as humans do, which means asthma symptoms must be induced.

Results. Mice serve as a good model due to detailed knowledge available about their genetics, however their response to asthma triggers is dissimilar from that of humans. Rats possess similar advantages and drawbacks to mice but have the added benefit of being larger than mice. Guinea-pigs have a response to some irritants that is

more similar to humans than other rodentia models, however it is still dissimilar enough to make generalization difficult. Dogs are more likely to develop allergic reactions that are similar to humans, however these are typically not airway responses making the direct study of asthma using dogs difficult. Furthermore, dog models are much more expensive than alternative models. Sheep can demonstrate allergic responses similar to asthmatic humans however response to treatments manifests differently than in humans. All these differences in models make extrapolation of data to human models difficult.

Conclusion. Animal models allow us to examine the effects of asthma in an intact system. Although there are limitations to using animal models, using them is still essential to further our understanding of asthma pathophysiology.

Relevance to the current work. This study highlights the necessity of animal research in furthering our understanding of asthma despite the limitations of many animal models. This information will help justify the design of the current study.

APPENDIX B

Materials

Materials for Dissection

- Dissection table
- Dissection mats
- Lab sink
- Room temperature water
- Overhead light and drawing table
- #11 size X-acto™ knife
- Stainless steel disposable scalpels (size 15)
- Hemostatic forceps (4)
- Manicure scissors
- Medical suture (silk black braided 45 cm suture, 24 mm needle)
- White, nitrile, powder free gloves
- Face masks
- Disposable plastic aprons
- Safety goggles
- Phosphate-Buffered Saline (PBS) solution
- Test tubes
- ThermoScientific ™ freezer
- Food grade refrigerator
- Styrofoam box
- Cryogenic gloves
- Sharpie Permanent Marker
- Red hazardous waste box (for scalpel and suture needle disposal)
- Sani-Cloth™ germicidal disposable wipes
- Digital caliper (UltraTECH™ no. 1433)
- Digital scale (Ozeri Model ZK14-S™)

Materials for data acquisition

- Dell computer
- Dell computer monitor
- PowerLab™ data acquisition hardward (AD Instruments)
- LabChart™ data acquisition softward (AD Instruments, 2015)
- Microphone (Model SM-48, Shure, Niles, IL)
- High-speed camera (KayPentax, Montvale, NJ)
- Medical-grade air tank (2) containing compressed, low-humidity air (50 psi, <1% relative humidity)
- Physiological pressure transducer (Model MLT844, AD Instruments)
- Sphygmomanometer (AD Instruments)
- Syringe (25 cc/ml)
- Pressure calibration block

- Gauze (decrease reverberation under pressure transducer)
- Velcro™ for securing transducers during calibration and data collection
- Pneumotach Calibration Unit (MCU-4, Glottal Enterprises)
- Audio Output Extension
- Bose™ Amplifier
- Pulse transducer (AD Instruments)
- AcuRite™ Hygrometer (Model 01083M)

Materials for benchtop and phonation trials

- Anterior (one) and lateral (two) Micropositioners (Model 1460, Kopf Industries)
- Micropositioner single prong attachments (Kopf Industries)
- Plastic syringe tip (25 cc/ml)
- Tubing
    - Vinyl: 1 ½” ID outer diameter (OD), 1” inner diameter (ID)
    - Clear Vinyl: 1 1/8” OD, 7/8” ID; 1”OD, ¾"ID; ¾" OD, ½" ID; 7/8” OD, 5/8” ID; 5/8” OD, ½" ID; ½" OD, 3/8” ID; 3/8” OD, ¼" ID; 5/16” OD, 3/16” ID; 3/16” OD, 1/8” ID
- Respiratory flow head transducer (Model MLT300L, AD Instruments, Sydney Australia)
- Flow head meters (Model MLT300L, AD Instruments)
- TheraHeat™ Humidifier (Model RC700000, Smiths Medical, Dublin, OH)
- Distilled water
- 20 cm foam-insulated aluminum custom pseudolung
- Teflon tape™
- Cable ties
- Screwdriver

APPENDIX C

LabChart™ Protocol, Computer Set-up

1. Power on the computer (Dell™), desktop (Dell™),, then PowerLab™ unit.
2. Open LabChart™ 8 Application (AD Instruments, 2015)
    a. See pop-up, “Scanning for Devices”
    b. “Powerlab 8/35” and “Playback File” should be selected, if not, verify that power to PowerLab is turned on and then select “device scan” again
    c. Click “OK”
    d. On the “Welcome Center” screen, select “New”
    e. On the upper right corner, select “start”
        i. Allow LabChart to run for 15 minutes—the program requires sufficient time to warm up
3. Input channel settings
    a. On the upper left corner of LabChart window, select “Setup” tab --> channel settings
    b. Verify that the following settings are applied:
        i. Microphone: sampling rate 40 k/s; range 10 mV; units mV
        ii. Pressure: sampling rate 1 k/s; range 20 mV; units mmHg
        iii. Flow: sampling rate 1 k/s; range 200 mV; units mV
        iv. High speed trigger: sampling rate 1 k/s; range 2 V; units V
    c. Units will be set during specific pressure and flow calibration
    d. Press “OK” in the bottom right corner when settings are accurate
4. Add a comment that settings were double- checked
    a. See a word box on the upper right part of the screen
        i. Type in “settings”
        ii. In the drop-down box to the left of the text box, make sure it is set to “All”
        iii. Press the “Add” button to the right of the text box
            1. You can drag the comment to be closer to the actual moment of change by hovering the mouse over the small black box at the bottom of the screen, directly below the comment. When a white left/right arrow pops up, you can drag the comment
5. To return to the live recording of data, press the button in the bottom right corner entitled “Show latest data”

APPENDIX D

Pressure Calibration, LabChart™ Protocol

1. Zero the pressure transducer before collecting data
    a. Attach the pressure transducer to the clear piece with the white cap
        i. Pinch clear prongs together and fit circle around the golden piece's rim
    b. Attach the pressure transducer to a small wooden block for stability.
    c. Fasten the transducer wire between Velcro on the benchtop.
    d. Attach the manometer (sphygmomanometer dial piece) via the blue stop cock
        i. The air-tight screw end should attach to the outlet on the stop cock that is 180 degrees from the tube that attaches the manometer
        ii. Remove the white stop cock on the pressure transducer to open it to atmospheric pressure
        iii. The hand within the manometer dial should be within the small rectangle at the bottom when zeroing
    e. Make sure that the pressure transducer is stable
    f. On LabChart, press the start button to collect data for approximately 3 seconds
        i. Press stop
        ii. Highlight most recent section of blue data
            1. Click on "Pressure" drop down box on right side of screen
            2. Select "Bridge Amp"
            3. Set range to 20 mV
            4. Do not set a low pass value
            5. Do not check "Mains filter" box
            6. Press "zero" button
            7. Click "OK"
        iii. Leave a comment noting that pressure has been zeroed
            1. Alt+ p (pre-set comment)
            2. Add the white cap back to the clear piece
2. Take the syringe (25 cc/ml) and pull the plunger out to the end of the syringe
3. Add the syringe to the open outlet on the stop cock
4. Press "start" on LabChart
5. Insert plunger into syringe until the manometer dial reads 40 mmHg—hold this for 5 seconds
    a. Add a comment: Alt+ 4 (pre-set comment indicating 40 mmHg)
6. Press stop
7. At the bottom of the screen, adjust the horizontal scaling to approximately 50, or until the bump is visible without needing to scroll
8. Highlight the bump by starting at the "zero pressure" plateau and finishing at the 40 mmHg plateau
9. Click the pressure drop down box (on right side)
    a. Click "Units Conversion"
    b. On the bottom left side of the popup window should be a + and – box; press the + button until you can see both bumps on the small graph

c. Click the Units Conversion “on” button on the right upper corner of the popup window
d. Click your cursor on the first plateau
    i. Click the arrow button next to “Point 1”—a value should automatically appear
    ii. Manually insert a “0” in the next text box
    iii. In the “Units” drop down box, select “mmHg”
e. Click on the second plateau
    i. Click the arrow button next to “Point 2”—a higher value should automatically appear
    ii. Manually insert a “40” in the next text box
f. Click “OK”
g. Insert pre-set comment “40 mmHg”: Alt+ c
h. Disconnect pressure transducer from pressure calibration box and attach to the tracheal mount located on the benchtop

APPENDIX E

Flow Calibration, LabChart™ Protocol

1. Zero the spirometer before collecting data
    a. Remove the tubes from both sides of the flow head meter located on the benchtop apparatus.
        i. Keep the position of the flow head steady while you run 3 seconds of data collection
        ii. Click “stop”
        iii. Highlight the most recent flow signal (green line)
        iv. On the “Flow” dropdown box, click “Spirometer”
            1. Set the Range to 200 mV
            2. Set the Low Pass to 100 Hz
            3. Do not check the “Invert” box
            4. Click “Zero” button
            5. Click “Ok”
    b. Using the pre-set comment Alt+F, leave comment that zeroing occurred (after pressing the “start” button)
2. Attach the flow head meter (via the blue piece) to the input on the top of the pneumotach calibration unit.
    a. Switch on the pneumotach calibration unit power using the switch on the back of the unit; it should make a few beeps
    b. Using the switches on the calibration unit, set the Flow rate to “½” and the liter to “1”
    c. Default mode on unit should be on “flow”
    d. Select “start” on LabChart software
    e. Flip up the “start” switch on the calibration unit; you should hear the machine take 3 inhalations and 3 exhalations
    f. Once the calibration unit has completed inhalations and exhalations stop data acquisition on LabChart software
    g. Select the middle exhalation (“up” plateau) whole single signal
    h. Click the “Flow” dropdown box
    i. Select “Spirometry Flow”
    j. Next to “Flow Head”, click MLT 300 L
    k. Click “Calibrate”
    l. Insert 1L in injected volume
    m. Click “ok”
3. Leave a comment noting that calibration occurred (after pressing “start” button)
    a. Alt+ 1 (pre-set comment)
4. Verify that channel 3 (flow channel) is now in L/s
5. Reattach the flow head meter to the tubes under the benchtop setup. The arrow on the flow head meter should point in the direction of flow (left). Do not remove the clear tube attachments between the Lab Chart box and the flow head meter.

APPENDIX F

Rabbit Tissue Dissection and Preparation Protocol

Procure rabbit larynges

1. Obtain all animal tissues from the University of Utah. All in vivo animal procedures were completed at the University of Utah. They administered twice-daily doses of either inhaled combination corticosteroids (salmeterol fluticasone propionate) or nebulized isotonic saline to in vivo experimental and control rabbits, respectively. Then, they sacrificed animals and flash froze rabbit larynges in phosphate buffered solution.
2. Transport larynges to the Taylor Building Annex on Brigham Young University campus using a Styrofoam container with dry ice, supplied by researchers from the University of Utah
3. Store rabbit larynges procured from the University of Utah in a commercial ThermoScientific™ freezer at –80° Celsius

Thaw frozen larynges

1. Remove larynges from freezer approximately 30 minutes before beginning dissections.
2. Fill lab sink with lukewarm water. Leave frozen larynges in water until completely defrosted.

Fine dissection

1. Use manicure scissors and size 11 X-acto™ knife
2. Spare posterior cricoarytenoid, lateral cricoarytenoid, cricothyroid, and thyroarytenoid muscles
3. Resect esophagus from posterior trachea and larynx, inferiorly to superiorly
4. Resect tissue superior to false vocal folds
    a. Resect epiglottis
    b. Resect portion of thyroid cartilage approximately 4mm superior to vocal folds
5. Identify fat pads, lateral to vocal folds and superior to anterior commissure
6. Resect false vocal folds
    c. Abduct false vocal folds using forceps
    d. Resect false vocal folds with anterior to posterior incision, starting at anterior commissure
4. Resect excess tissue lateral, superior, and posterior to true vocal folds that may affect vocal fold vibration
    a. Resect ventricular folds

Suture

1. Insert suture needle through anterior thyroid cartilage, approximately 1 mm superior to anterior commissure
2. String through thyroid commissure, using two loops to secure suture
3. Dispose of needle in hazardous waste box

Storage

1. Temporary storage prior to data collection for no more than four hours

b. Place completed larynges in coded vials of fresh phosphate buffered solution
c. Store vials in food-grade refrigerator to maintain tissue hydration

APPENDIX G

Data Acquisition Protocol

These procedures occur immediately following pressure and flow calibration and specimen fine dissection. To collect data on pressure and flow of phonation, at least two research assistants must work together, one using (1) LabChart on the computer and the other performing (2) Mounting and Air responsibilities at the benchtop:

1. LabChart:
    a. Press “start” before trial begins
    b. Manually type “trial 1” in text box, insert at channel 1 (microphone channel) by pressing enter
    c. At the onset of phonation, press Alt+ O (pre-set comment)
    d. At the steady-state of phonation, press Alt+ S (pre-set comment)
    e. At the cessation of phonation, press Alt+T (pre-set comment)
    f. Press “stop” button if needed
        i. Ex. need to spray the larynx, adjust the micro-positioners, etc.
    g. When moving on to trial 2, adjust text box to say “trial 2”, click enter to leave comment
    h. Repeat until 15 trials are complete
    i. Ensure signals look normal during phonation
    j. Leave additional comments regarding difficulty in phonation, extra steps for mounting, re-recording trials for irregular signals, etc.
    k. Take notes for data sheet
        i. Ex. Perceptually pressed phonation, used Teflon tape, air leakage initially—fixed by lowering micro-positioners, etc.

2. Mounting and Air:
    a. Mount the rabbit larynx on a custom bench-top set-up. Use Zip Tie™ at base of trachea to secure trachea to air flow tube and prevent air leakage. Wrap and secure the trachea with Teflon tape as needed to prevent air leakage. Insert micro-positioners at the same level into the arytenoid cartilages to adduct the vocal folds. Tie suture string to anterior elongation post; pull until string is taut, but not too tight. Ensure larynx is sitting up straight and is secure.
    b. Using a commercial light and iPhone camera, take still images of mounted larynges for purposes of later visual-perceptual analysis
    c. Turn air tank on using hand-dial until steady phonation is perceived. After approximately 4 seconds, turn the air tank off quickly.

APPENDIX H

Data Segmentation and Analysis Protocol

1. Selecting Signals for Segmenting
    a. Open Lab Chart ™ version 8
    b. Open the file from Desktop folder "LabChart Data"
    c. Select the pre-collected animal signals that you want to segment
    d. Select "File" –> "Save Selection"
        i. Rename file and save in designated folder
        ii. Do not save changes to main LabChart Data File
    e. Open new file to segment
2. Placing Onset and Offset
    a. Zoom in to 2:1
    b. Analyze the waveform and place onset on the second peak after the waveform begins to look semi-periodic.
    c. Examine both periodicity and amplitude of waveform to determine where offset is and place marker on the last semi-periodic peak before signal dies out
        i. Note: You can use the audio from the acoustic signal to help identify the approximate location of onset and offset.
3. Marking trial errors
    a. Identify any trials where errors occurred and trials were repeated
    b. Change all of the markers in discarded trials so that they are not tagged "phonation onset" and "phonation offset". Change "phonation onset" to "signal start" and "phonation offset" to "signal end". This is so that these trial errors will not be accounted for when Matlab analysis is performed.
    c. Keep detailed notes on which trials were in error and where they are in the data.
4. Export Segments
    a. Click "File" -> "save" and save segmented file as a new file
    b. Select "File"-> "export" to convert file to txt file
    c. Save the txt files in the correct folder and upload to custom Matlab program for further analysis
5. Open Matlab application
    a. Click "Open File" -> select segmented txt file
    b. Drag the yellow boxes on the screen out of the way
    c. Count trials to verify that all 15 trials have been included in txt file
6. Selecting Results
    a. Move red markers on microphone signal data to surround one trial of phonation
        i. Note the placement of the vertical lines between pressure signal peaks. The red markers should be placed as closely to these lines as possible but must be within the vertical markers.
    b. Select "play" in order for application to register line placement
7. Select "save"
    a. Save as "rabbit#_trial#"
    b. It will save as a CSV file (both sound and excel file)

8. Open excel file to see pressure, flow, and resistance values for phonation onset, steady phonation, and offset phonation 9.

Repeat steps with each trial

APPENDIX I

Coefficient of Variation Raw Data by Treatment and Trial

Group	Mean	Standard Deviation	CV (%)
	Phonation Threshold Pressure (cm H_2O)		
Inhaler Group			
1	8.04	2.56	31.80
2	7.79	2.39	30.74
3	7.83	2.52	32.22
4	7.73	2.55	32.98
5	7.94	2.43	30.59
6	8.05	2.49	30.91
7	7.87	2.59	32.84
8	7.62	2.58	33.90
9	7.88	2.72	34.50
10	7.89	2.54	32.19
11	8.00	2.65	33.14
12	7.74	2.78	35.98
13	7.46	2.74	36.80
14	7.25	3.15	43.49
15	8.16	2.83	34.73
Control Group			
1	8.54	2.97	34.82
2	8.35	2.95	35.35
3	8.50	3.48	40.92
4	8.17	2.77	33.94
5	8.36	2.81	33.62
6	8.20	2.74	33.38
7	7.96	2.92	36.70
8	7.97	2.92	36.66
9	7.83	2.94	37.48
10	8.28	2.77	33.40
11	7.83	2.78	35.54
12	8.02	2.84	35.42
13	7.88	2.97	37.75
14	8.37	3.10	37.07
15	8.16	2.71	33.15
	Phonation Threshold Flow (L/m)		
Inhaler Group			
1	0.098	0.040	40.403
2	0.099	0.035	35.699
3	0.105	0.039	37.081
4	0.104	0.038	36.689
5	0.101	0.038	37.888
6	0.105	0.041	39.470
7	0.106	0.041	38.694
8	0.101	0.043	42.636
9	0.106	0.044	41.515
10	0.100	0.042	42.418

Group	Mean	Standard Deviation	CV (%)
11	0.105	0.045	42.570
12	0.103	0.044	42.979
13	0.100	0.042	42.376
14	0.104	0.046	43.937
15	0.108	0.046	42.167
Control Group			
1	0.081	0.053	66.307
2	0.079	0.052	65.170
3	0.082	0.055	66.357
4	0.078	0.053	67.402
5	0.081	0.051	62.834
6	0.080	0.051	64.280
7	0.078	0.053	68.042
8	0.078	0.054	68.569
9	0.080	0.052	64.986
10	0.082	0.051	61.556
11	0.078	0.052	66.353
12	0.081	0.051	62.509
13	0.081	0.052	63.684
14	0.084	0.053	62.998
15	0.080	0.050	62.091
	Onset Laryngeal Resistance ($cmH_2O/L/m$)		
Inhaler Group			
1	85.202	17.964	21.084
2	80.344	13.813	17.192
3	76.354	15.357	20.113
4	75.864	15.585	20.543
5	81.757	15.251	18.654
6	79.577	14.340	18.020
7	77.044	15.862	20.589
8	78.829	17.199	21.818
9	77.254	14.108	18.262
10	83.614	19.156	22.910
11	80.293	18.689	23.276
12	80.202	23.617	29.447
13	77.539	17.028	21.960
14	71.699	15.184	21.177
15	79.436	18.308	23.048
Control Group			
1	142.829	88.796	62.170
2	140.874	88.993	63.172
3	136.364	85.621	62.789
4	164.410	147.457	89.688
5	139.503	90.633	64.968
6	148.111	113.408	76.570
7	151.874	117.889	77.623
8	149.616	112.332	75.080
9	136.503	102.324	74.961
10	135.694	93.247	68.718

Group	Mean	Standard Deviation	CV (%)
11	136.420	87.325	64.012
12	137.096	101.342	73.920
13	141.215	119.269	84.459
14	130.172	78.559	60.350
15	138.006	90.292	65.426
	Sustained Pressure (cm H_2O)		
Inhaler Group			
1	12.412	4.225	34.039
2	11.633	3.158	27.146
3	11.349	3.490	30.750
4	10.845	3.010	27.755
5	12.789	4.689	36.665
6	11.732	3.332	28.400
7	11.490	3.405	29.639
8	11.244	3.372	29.989
9	11.209	3.134	27.960
10	11.755	3.231	27.483
11	11.482	3.228	28.113
12	11.089	3.355	30.251
13	10.233	2.745	26.829
14	10.456	3.302	31.585
15	12.427	3.577	28.785
Control Group			
1	10.382	3.220	31.020
2	10.040	3.307	32.938
3	9.953	3.739	37.570
4	9.706	3.041	31.334
5	10.150	2.884	28.416
6	9.837	3.041	30.917
7	9.662	2.881	29.822
8	9.639	3.089	32.053
9	9.598	3.378	35.195
10	10.243	3.160	30.852
11	10.103	2.806	27.776
12	9.568	2.910	30.409
13	9.504	3.160	33.253
14	9.917	3.246	32.731
15	9.990	2.513	25.159
	Sustained Airflow (L/m)		
Inhaler Group			
1	0.129	0.051	39.798
2	0.122	0.040	32.790
3	0.122	0.043	34.902
4	0.117	0.038	32.110
5	0.137	0.068	49.771
6	0.123	0.046	37.094
7	0.124	0.045	36.561
8	0.124	0.045	36.517
9	0.122	0.044	36.163

Group	Mean	Standard Deviation	CV (%)
10	0.124	0.044	35.297
11	0.123	0.047	38.334
12	0.121	0.044	36.303
13	0.120	0.044	36.956
14	0.127	0.048	37.579
15	0.134	0.047	34.884
Control Group			
1	0.089	0.047	53.557
2	0.088	0.047	53.224
3	0.089	0.049	55.188
4	0.085	0.047	55.419
5	0.091	0.045	50.135
6	0.089	0.045	50.872
7	0.089	0.044	49.918
8	0.090	0.046	50.544
9	0.091	0.046	51.074
10	0.092	0.045	49.033
11	0.092	0.044	47.425
12	0.090	0.044	48.622
13	0.091	0.044	48.620
14	0.091	0.047	52.163
15	0.090	0.043	47.507
	Sustained Laryngeal Resistance ($cmH_2O/L/m$)		
Inhaler Group			
1	99.278	18.717	18.853
2	98.188	18.104	18.438
3	95.172	18.729	19.679
4	94.800	19.465	20.532
5	97.996	20.201	20.614
6	98.531	19.519	19.810
7	95.690	19.307	20.176
8	93.730	18.437	19.671
9	94.489	17.440	18.457
10	97.744	18.724	19.156
11	96.976	20.406	21.042
12	94.121	19.029	20.217
13	89.511	20.041	22.389
14	85.958	20.755	24.146
15	96.221	21.504	22.348
Control Group			
1	133.588	48.752	36.495
2	126.738	38.308	30.226
3	123.422	36.496	29.570
4	130.162	44.918	34.509
5	124.515	36.594	29.389
6	123.559	38.947	31.521
7	120.801	37.198	30.793
8	118.600	36.425	30.712
9	116.167	38.132	32.825

Group	Mean	Standard Deviation	CV (%)
10	122.253	36.617	29.951
11	121.564	39.365	32.382
12	117.720	38.820	32.977
13	114.699	36.962	32.225
14	121.237	35.554	29.326
15	124.170	37.646	30.318

APPENDIX J

Geometric and Anatomical Data for Each Larynx

Table J1

Anatomical Tracheal Dimensions

Group	Trachea length (mm)	Trachea width (mm)
Inhaler		
1	12.52	7.15
2	14.52	5.81
3	14.10	5.80
4	15.84	6.08
5	12.32	5.64
6	16.54	8.20
7	14.35	6.03
8	19.10	7.18
9	13.18	6.75
10	13.68	7.16
11	14.56	6.08
Control		
1	15.15	6.64
2	19.53	7.04
3	18.89	5.50
4	16.24	7.16
5	18.10	6.94
6	16.00	7.09
7	17.40	5.96
8	15.47	7.28
9	15.32	7.28
10	12.56	7.75

Table J2

Vocal Fold Anatomical Size and Dimensions

Group	Length of vocal folds	Width of vocal folds	Width from vocal fold to thyroid cartilage
Inhaler			
1	5.73	1.10	3.58
2	6.37	1.88	2.78
3	6.65	1.56	3.30
4	6.51	1.70	3.29
5	6.37	1.68	3.65
6	5.87	1.62	2.51
7	6.82	1.61	3.64
8	7.88	1.73	2.78
9	7.33	1.73	2.78
10	6.59	1.34	3.58
11	7.63	2.00	3.76
Control			
1	6.48	1.44	3.82
2	6.62	1.61	3.76
3	6.69	2.10	3.15
4	7.15	1.84	3.53
5	7.31	1.65	2.85
6	6.44	1.59	3.73
7	7.07	1.76	3.43
8	7.03	2.03	3.23
9	5.77	1.72	3.01
10	6.68	1.85	3.42

Table J3

Thyroid Cartilage Anatomical Measurements

Group	Protuberance to bottom	Width
Inhaler		
1	3.35	13.81
2	2.44	13.75
3	2.44	13.75
4	1.77	12.93
5	2.04	12.82
6	4.32	12.85
7	3.82	13.47
8	2.80	14.19
9	4.81	14.32
10	4.75	14.02
11	3.98	12.51
Control		
1	2.71	13.81
2	2.93	13.45
3	3.54	14.46
4	4.58	14.39
5	3.66	13.72
6	3.80	15.76
7	4.38	15.11
8	4.92	15.14
9	3.78	13.52
10	5.54	14.22

APPENDIX K

Thesis Timeline

6/19

- Training in fine dissection of rabbit larynges and benchtop setup. Training in collecting acoustic, aerodynamic, and visual data.

10/19

- Fine dissection and collection of acoustic, aerodynamic, and visual data for experimental larynges

11/19

- Training for data segmentation of raw data on LabChart™ to prepare for upload to MatLab ™ program for analysis

12/19

- Preparation for control rabbit acquisition for further data collection

1/20

- Fine dissection and collection of acoustic, aerodynamic, and visual data for all control larynges

2/20-3/20

- Maintain lab
    - Back-up collected data on hard drive
    - Computer maintenance via crash-plan download
    - Medical grade compressed air USP gas cylinder replacement
    - Reset precautionary ThermoScientific™ battery

4/20

- Complete data analysis of phonation pressure and flow using MatLab and Audacity programs performed by Amber Prigmore and Meg Hoggan

6/20

- Analyze data for significant differences between experimental and control groups in phonation pressure and flow completed by Dr. Ray M. Merrill, Ph.D., using SPSS (version 24) and the Statistical Analysis System (version 9.4)

8/20

- Write prospectus

11/20

- Edit prospectus document

12/20

- Complete Prospectus meeting with thesis committee, discussing specific thesis questions, importance of current study, and protocol for completing statistical analysis

1/21

- Edit Prospectus documents to align with feedback received from thesis committee

2/21-5/12

- Complete drafts and revisions of thesis document