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Phonation Threshold Pressure and Phonation Threshold Flow in Rabbits Treated
With Inhaled Corticosteroids Versus Controls

Heidi Joan Robison

A thesis submitted to the faculty of
Brigham Young University
in partial fulfillment of the requirements for the degree of
Master of Science

Kristine Tanner, Chair
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ABSTRACT

Phonation Threshold Pressure and Phonation Threshold Flow in Rabbits Treated With Inhaled Corticosteroids Versus Controls

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Master of Science

This thesis is part of a larger series of studies being conducted by Kristine Tanner, PhD, Associate Professor in the Department of Communication Disorders at Brigham Young University (BYU). The larger project is funded by the National Institute on Deafness and Other Communication Disorders at the National Institutes of Health. This thesis primarily investigated the effects of combination inhaled corticosteroids (ICs) on aerodynamic measures of the voice. In recent years, an increase in the localized laryngeal side effects from IC treatment, including dysphonia, have been reported. This study employed a between-groups experimental design, with two groups of rabbit larynges having been exposed to either ICs or nebulized isotonic saline two times each day for eight weeks at The University of Utah. For this study, the independent variable is group condition (i.e., IC versus saline) and the dependent variables are two aerodynamic measurements made at the onset of phonation using a benchtop experimental setup, namely phonation threshold pressure (PTP; cmH₂O) and phonation threshold flow (PTF; L/min). The results of this study indicate a significant difference in PTP and PTF between vocal folds treated with IC as compared to vocal folds treated with nebulized isotonic saline solution. Implications of this study suggest negative changes in the voice due to IC treatment.

Keywords: phonation threshold pressure (PTP), phonation threshold flow (PTF), inhaled corticosteroids, rabbit phonation, benchtop model, asthma

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DESCRIPTION OF THESIS STRUCTURE AND CONTENT

This thesis, *Phonation Threshold Pressure and Phonation Threshold Flow in Rabbits Treated With Inhaled Corticosteroids Versus Controls*, is written in a journal style format. This paper was part of a larger, long-term project being conducted by Dr. Kristine Tanner. Funding was received for the project from the David O. McKay School of Education and the National Institute on Deafness and Other Communication Disorders, National Institutes of Health (1R01DC016269-01A1).

The preliminary pages of the thesis reflect requirements for submission to the university. This thesis contains one reference list found before the appendices. The corresponding annotated bibliography is found in Appendix A. Appendix B contains materials necessary for the data collection and analysis. Appendix C contains LabChart™ protocol and computer set-up information. Appendix D includes protocol for pressure calibration in LabChart™ with Appendix E containing protocol for flow calibration in LabChart™. Appendix F details rabbit tissue dissection and preparation protocol. Appendix G includes data acquisition protocol and Appendix H contains data segmentation and analysis protocol.

Data from this thesis were accepted as a poster presentation for the Annual Convention of the American Speech-Language-Hearing Association in San Diego, CA (Convention canceled due to Covid-19). The data will from this thesis will be used in the long-term study conducted by Dr. Kristine Tanner and ultimately submitted to a peer-reviewed journal with the author of this thesis listed as one of multiple coauthors.

Introduction

In the most recent report from the Centers for Disease Control (CDC), approximately 7.7% of the total population, or 1 out of every 13 individuals of all ages in the United States has an asthma diagnosis (Centers for Disease Control, 2018). Asthma is a lung disease that affects people of all ethnicities, genders, living environments, and other demographic variables. It is reported that over 40 million adults and children in the US suffer from chronic pulmonary diseases, including 25 million cases of asthma (Akinbami & Lie, 2011; Mannino et al., 2000). For example, New Zealand has reported one of the highest prevalence of asthma diagnosis in the world, affecting approximately 15%-20% of children and adults (Sahrawat et al., 2014). According to Sahrawat et al. (2014), asthma is the third highest cause of disease-related long-term disability in New Zealand.

One of the hallmark characteristics of asthma is inflammation of the bronchi (Ihre et al., 2004). Inflammation causes mucus to collect in the airway, causing many individuals to cough and clear their throats frequently to achieve their adequate voice for communication. Asthma attacks causing coughing, shortness of breath, chest tightness or pain among other symptoms vary from person to person and can be triggered by exercise, occupational irritants, or allergies (Mayo Foundation for Medical Education and Research, 2020).

Inhaled Corticosteroid Treatment

In an effort to reduce inflammation and the effects of asthma on quality of life, inhaled corticosteroids (ICs) are commonly prescribed. Short-acting inhalers, or rescue inhalers are used during asthma attacks as a quick relief, while longer acting inhalers are used provide control of asthma symptoms (Dong et al., 2015). The goal of IC treatment is to decrease the chronic inflammation of asthma and reduce airway hyperresponsiveness. This goal is targeted by

combining corticosteroids with long-acting beta agonist (LABA). Corticosteroids are bronchodilators used to reduce airway inflammation. Additionally, LABA are bronchodilators, but also inhibit mast (white blood) cell release and may reduce sensory nerve activation. The combination of these two drugs addresses the pathophysiology of asthma and is commonly referred to as combination IC. (Barnes, 2002). The positive effects of IC treatment can be seen as early as 30 minutes to two hours after treatment (Sahrawat et al., 2014), reducing the frequency and severity of asthma attacks. IC treatment have been effective in reducing morbidity and improving the health of asthma patients allowing many to live a normal life with improved lung function and reduced exacerbations (Barnes, 2010; Erickson & Sivasankar, 2010). While there are many advantages to IC treatment, the prevalence of systemic and local side effects must be considered when using ICs to treat asthma.

Systemic side effects occur as a consequence of up to 80% of IC treatment dosage being swallowed. These side effects include glaucoma, cataracts, osteoporosis, bruising, adrenal suppression, growth suppression, psychiatric disturbances, and metabolic abnormalities (Barnes, 2010; Bhalla et al., 2008). Local side effects include pharyngitis, laryngitis, throat irritation, oropharyngeal candidiasis, and dysphonia (Barnes, 2010; Bhalla et al., 2008; Sahrawat et al., 2014). The degree of local side effects varies depending on the dose and frequency of IC treatment. While all of these side effects of IC treatment are important to consider, the most common complaint from up to 50%-55% of individuals using ICs is hoarseness (Ihre et al., 2004; Hassen & Hasseba, 2016).

Pulmonologists, allergists, otolaryngologists, and pharmacologists as well as corresponding literature on asthma indicate that in addition to cough and throat clearing, vocal fatigue and different dysphonic voice qualities are associated with IC use (Ihre et al., 2004).

Dysphonia is characterized by a change in pitch, vocal quality, loudness, or effort that can impair functional living and quality of life (Galván & Guarderas, 2012). Dysphonia impacts individuals' ability to effectively communicate in their home, work, and social environments. IC treatment induced dysphonia is likely caused by deposits of the medication in the oropharynx after administration as it has been reported that up to 80% of the treatment dosage may be deposited in the oropharynx (Erickson & Sivasankar, 2010; Galván & Guarderas, 2012).

To prevent systemic and local side effects, researchers have suggested that when taking ICs in large doses (>800 µg), individuals should rinse with mouthwash immediately after treatment and use a spacer device, a holding chamber attached to the inhaler to slow the delivery of medication (Barnes, 2010). However, rinsing with mouthwash and using a spacer does not address the potential changes leading to dysphonia at or below the level of the vocal folds caused by IC treatment. More research needs to be completed on effective prevention of side effects from the treatment; however human research limitations must be addressed. For example, IC treatment cannot be withheld from those who need them, so it is difficult to determine why certain individuals experience what seems to be IC-associated voice disorders (Erickson & Sivasankar, 2010). For this reason, alternative models have been utilized in studying the effects of IC on the voice which include *in vivo* and *ex vivo* animal models.

Animal Models of Human Phonation

Considerable research has assessed the effectiveness of IC for treating asthma. However, only recently have the local side effects of IC treatment been a topic of research. Because of the invasive nature of studying the human voice, alternative methodologies including animal, cadaveric, and computational models have been used throughout the past decade to further our understanding of the voice (Howard et al., 2015). Both *in vivo* and *ex vivo* animal studies have

been performed, each with their individual advantages. *In vivo* models provide physiologic activation of the intrinsic muscles of the larynx, where *ex vivo* models use a simulated approach for muscle contraction and relaxation (Birk et al., 2017).

In general, animal models involving pig, dog, cow, and rabbit larynges have been extensively studied in attempt to better understand the human voice and laryngeal pathologies (Maytag et al., 2013). Each of these animals have benefits in studying the human voice; but pig, dog, and cow histological laryngeal composition differs notably from the human histological structures, making the translation to human research on vocal fold layers and histology questionable. Maytag et al. (2013) proposed that rabbit larynges are the most accurate model to study the human voice due to similar tissue composition. Humans have three layers of lamina propria, each composed of different fibers. Human vocal folds have a superficial layer of loose, pliable, gelatin like substance, an intermediate layer of dense ground substance and elastin, and a deep layer of collagen. In most animals, the composition of the lamina propria layers differ. In rabbits, however, the composition is the same, making them a more ideal model for understanding the human voice and the potential effects medications may have on the vocal fold tissue (Maytag et al., 2013). Using rabbits to study the effects of ICs on the voice provides an opportunity to reliably quantify vocal fold vibrations and aerodynamic measures (Maytag et al., 2013). In addition to their having similar tissue properties, the rabbit is small and ideal for *in vivo* drug administration.

In 1993, Jiang and Titze established a benchtop setup to study *ex vivo* larynges. The model established by Jiang and Titze (1993) has been repeatedly used by researchers in the field to study a variety of aspects of the human voice. Maytag et al. (2013) reported a modified benchtop setup for studying *ex vivo* rabbit larynges. Benchtop setups allow research to be

conducted on a variety of measurements that may be used to compare animal phonation to voice production in humans. *Ex vivo* models provide a reliable alternative to *in vivo* experiments when using the benchtop setup. In comparison to *in vivo*, *ex vivo* experiments require static and manual control of the larynx via sutures or screws, whereas *in vivo* utilizes a complex connection of muscle contractions and movement of cartilages (Birk et al., 2017). Using a benchtop, researchers have found that they can manually apply force to the thyroid cartilage via sutures to provide a simulation of cricothyroid muscle contraction. Adduction is achieved in *ex vivo* larynges by applying force laterally to the arytenoid cartilage, simulating the lateral cricothyroid muscle movement (Birk et al., 2017). The benchtop setup has been well researched and adapted to make it as reliable as possible for translating to *in vivo* and human voice research. Additionally, the benchtop setup proposed by Jiang and Titze (1993) provides a way to simulate different voice disorders to effectively study the nature and characteristics of the voice without relying on human subjects (Birk et al., 2017; Döllinger et al., 2018; Hottinger et al., 2007).

Benchtop Measures of Voice Function

Aerodynamic and acoustic parameters as well as high speed imaging are frequently used to examine voice production. The acoustic signal is typically used in benchtop work to inform aerodynamic measures as it represents the vocal sound source but not the source-filter interactions. Phonation threshold pressure (PTP) and phonation threshold flow (PTF) are two aerodynamic measures that have recently been used in combination to provide insight into the effort required to initiate vocal fold oscillation, or phonation onset (Hoffman et al., 2012; Jiang et al., 1999).

The aerodynamic measure most routinely used in benchtop work is PTP, measured in cm H₂O (Jiang et al., 1999). Phonation threshold pressure (PTP) is defined as the minimum

subglottic pressure needed to initiate and sustain phonation (Jiang et al., 1999). Phonation threshold pressure has been used for many years to quantify disorders caused by vocal fold swelling and masses (Jiang et al., 1999) and has advantages of longstanding translational use that can be compared to humans. To measure PTP, direct or indirect measures can be taken. Three methods of directly measuring PTP including a translaryngeal catheter inserted through the nasal passageway down into the trachea passing through anesthetized vocal folds, a percutaneous catheter inserted into the trachea, and an intraesophageal catheter swallowed in a small balloon (Plexico et al., 2011; Plant, 2005; Plant et al., 2004). Measuring PTP directly has been done for a number of years, but it is very invasive in nature. Hence, direct measurements are not generally used in clinical settings.

To facilitate the use of PTP as one measurement of voice function, indirect measurements were developed that are noninvasive and which can be used clinically. The common indirect approach is to measure intraoral pressure while having the speaker produce a string of voiceless bilabial stop consonants (Smitheran & Hixon, 1981). When using the indirect measure, subglottic pressure is equal to the intraoral pressure at the initial point of lip separation (Plant, 2005). An advantage of studying an *ex vivo* larynx on the benchtop model is that PTP can be measured directly by applying a pressure sensor directly below the vocal folds, making it an accurate measurement and not just an estimate (Birk et al., 2017).

Phonation threshold pressure has been established as a valuable aerodynamic measure for many years and is used in the assessment of vocal fold pathologies (Hottinger et al., 2007; Jiang et al., 1999; Plant, 2005). Jiang et al. (1999) found statistical differences in PTP when evaluating individuals with vocal fold polyps compared to individuals with normal, healthy voices. Studies have shown that when there is an increase in vocal fold mass, caused by growths or swelling,

PTP will rise (Jiang et al., 1999). In general, there are four contributing factors to PTP values. These factors are 1) vocal fold viscosity, 2) velocity of the mucosal wave, 3) prephonatory glottal width, and 4) vocal fold thickness (Jiang & Titze, 1993; Plexico et al., 2011). An increase in vocal fold viscosity will increase PTP, a primary reason why vocal fold hydration is a key factor in maintaining a healthy voice (Plexico et al., 2011). There are many other physiological or biomechanical changes that can occur to the vocal folds due to vocal trauma or laryngeal pathologies, all of which change PTP in a predictable manner (Plexico et al., 2011).

In 2007, PTF was introduced as an additional aerodynamic measure to evaluate the voice (Jiang & Tao, 2007). PTF, measured as mL/s, is defined as the minimum amount of airflow to initiate stable phonation (Jiang & Tao, 2007). Similar to PTP, PTF is dependent on glottal configuration, tissue properties, and vocal tract loading (Jiang & Tao, 2007). In contrast to PTP, PTF has been shown to be more sensitive to changes in glottal width, leading some researchers to suggesting PTF is more clinically valuable than PTP (Hottinger et al., 2007). PTF is measured noninvasively by averaging the glottal flow rate during vowel production (Jiang & Tao, 2007).

PTP and PTF have different diagnostic advantages but are both valuable aerodynamic measures. Pressure and flow can be measured at onset, during sustained phonation, and at the offset of phonation, with sustained and offset pressure being measured through tracheal puncture. The measures of PTP and PTF are specific to phonation onset. Subglottic air pressure is the key driving force for airflow through the larynx and both play a key role in phonation (Plant, 2005; Plant et al., 2004). Threshold values can be influenced by a number of factors including fundamental frequency (F_0), vocal intensity, and laryngeal resistance (Plant et al., 2004). Abnormal changes in aerodynamic functions often indicate vocal fold pathologies and provide

diagnostic information regarding speech system dysfunctions, which has clinical significance (Hoffman et al., 2012; Hottinger et al., 2007; Jiang & Tao, 2007).

Context for Current Study

This thesis is part of a larger study being completed under Dr. Kristine Tanner with the long-term goal to prevent and cure voice disorders caused by IC treatment. The study examines the adverse effects of inhaled corticosteroids on the vocal folds and it aims to quantify threshold for voice function changes associated with ICs.

Statement of Purpose

The purpose of this study is to quantify aerodynamic measures of PTP and PTF in rabbit vocal folds treated with IC. Asthma prevalence is increasing by 10% each decade, with ICs becoming the treatment of choice. The increase in prevalence and administration of ICs has resulted in an increase of voice disorders caused by IC treatment in up to 50% of individuals with asthma (Galván & Guarderas, 2012; Williamson et al., 1995).

In any study, a control group is necessary to know what changes occur in the experimental group. In many voice studies nebulized isotonic saline is used as the control treatment. In a study completed by Tanner et al. (2015), it was found that nebulized saline (0.9% Na⁺Cl⁻) improved voice production in individuals with Sjögren Syndrome, an autoimmune disease-causing vocal fold dehydration. An additional study completed by Welham et al. (2009) compared vocal fold scar healing in a treatment, sham, and control group. The sham group was treated with saline, while the control group received no treatment. Results of this study indicate that PTP, glottal resistance, and glottal efficiency were of similar values in the sham group and the control group, indicating reliability in using saline as a sham and/or control group for future studies.

Research Questions

Based on the extant literature, it was hypothesized that IC usage would cause adverse effects on voice function in an excised larynx model. Specifically, it was hypothesized that rabbits that received IC treatment over time would have higher (i.e., worse) airflow characteristics as measured using PTP and PTF as compared to controls. This study will address the following research questions:

1. What are the onset pressure and airflow characteristics of rabbit vocal folds following eight-week exposure to inhaled corticosteroids?
2. How do these onset pressure and airflow characteristics differ from control rabbit vocal folds exposed to inhaled saline?

Method

This thesis was part of a larger series of studies being conducted by Kristine Tanner, PhD, Associate Professor in the Department of Communication Disorders at Brigham Young University (BYU). The larger project was funded by the National Institute on Deafness and Other Communication Disorders at the National Institutes of Health. This thesis included a between-groups experimental design, with two groups of rabbit larynges having been exposed to either ICs or nebulized isotonic saline two times each day for eight weeks at The University of Utah. For this study, the independent variable was group (i.e., IC versus saline); the dependent variables were two aerodynamic measures at the onset of phonation using a benchtop experimental setup, namely PTP (cmH₂O) and PTF (L/min).

Statement of Research Ethics

All data collection and analysis procedures complied with research ethics for animal research. The Institutional Animal Care and Use Committee approved the project procedures at

both The University of Utah and BYU. The thesis methods were also compliant with BYU Risk Management policies.

Larynx Samples

Pre-Study Treatment Administration

The larynges for this thesis were obtained after all living animal procedures were completed. Preparation for this study was as follows. At The University of Utah, 22 male, white New Zealand retired breeder rabbits, ages seven to eight months, were obtained. Each rabbit weighed between 3.1 and 4.8 kg prior to quarantine. Randomization of rabbits was performed using a random number generator and 11 rabbits were assigned to each group. For the IC group, rabbits were administered one puff of fluticasone propionate (45 mcg) and salmeterol (21 mcg) from a metered dose inhaler with spacer (i.e., an animal “puffer” with facemask) twice daily. The animals inhaled the ICs transnasally for 18 breaths. The 11 control rabbits received aerosolized saline solution twice daily, 18 transnasal breaths per administration, using an ultrasonic nebulizer and facemask. During the administration, the rabbits were awake and carefully handled using standard procedures. After receiving treatment for 8 weeks, the rabbits were euthanized. Immediately after euthanizing, a vertical incision was made on the anterior portion of the neck to expose the trachea and larynx after which the neck opening was maintained using metal hemostats and a fine scalpel was used to dissect away neck musculature. Tissue was dissected until the superior border of the thyroid cartilage and the inferior border of the trachea were exposed. Excision of the full length of the trachea, false vocal folds, true vocal folds, epiglottis, arytenoid cartilage, and the thyroid cartilage was performed. Once extracted, the larynges were placed in small vertical plastic tubes in phosphate-buffered saline (PBS) solution. The tubes were then placed in a tray of isopropyl alcohol to prevent ice crystals forming while flash freezing in

liquid nitrogen. Larynges were stored at -80°C in a research lab at The University of Utah until ready for transportation to BYU for data collection.

Larynx Procurement

Larynges were transported to BYU by the thesis study team in a foam cooler to room 105 of the John Taylor Building Annex where they were stored. The larynges were placed in a ThermoScientific Freezer at -80°C for approximately 12 to 15 hours before data collection. Upon completion of all thesis data collection, samples were returned to The University of Utah.

Fine Dissection

On the day of data collection, each larynx was removed from the freezer and placed in a lukewarm bath to thaw approximately 30 minutes before trials were to be run. Once fully thawed, the larynges were removed from the PBS solution and finely dissected using X-Acto™ knives, scalpels, and small surgical scissors. Throughout the dissection process, larynges were kept hydrated by dipping them into PBS solution and spraying them with a 0.9% isotonic saline (Na^+Cl^-). Figure 1 is a photo of larynges in their PBS containers during the thawing process.

Figure 1*Thawing Larynges Prior to Fine Dissection*

The trachea was transected, leaving approximately 2 cm to facilitate optimal stabilization on the benchtop set up. The epiglottis and superior portion of the thyroid cartilage above the thyroid notch were dissected. The false vocal folds were dissected along with any excess tissue to reveal the true vocal folds. Hemostats were used to hold tissue in place and provided more accurate dissection. Once finely dissected, a surgical suture was threaded above the anterior commissure to allow for anterior attachment and stability. After dissection, larynges were placed back into a small tube filled with PBS until ready to be mounted for data collection. Figure 2 is a photo of a rabbit larynx during the fine dissection process.

Figure 2*Fine Dissection***Benchtop Setup**

The benchtop setup designed by Jiang and Titze (1993) and modified by Maytag et al. (2013) was used to guide the benchtop setup for this study. A medical-grade air tank provided compressed air with low humidity (50 psi, <1% relative humidity). The tank was secured to the wall via chains in accordance with Joint Commission on Accreditation of Healthcare Organizations and the Occupational Safety and Health Administration standards. Air passed from the air tank through a respiratory flow head transducer (Model MLT300L, AD Instruments, Sydney, Australia) to a ThereaHeat heated humidifier (Model RC7000, Smiths Medical, Dublin, OH). Air was humidified and traveled through tubing connecting to a 20 cm aluminum foam-

insulated custom pseudo lung attached beneath the breadboard benchtop (Thorlabs, Ann Arbor, MI). The pseudolung was suspended under the benchtop via benchtop pins and strings. Tubing protruded up from the pseudolung where two silicone devices designed to taper the airflow and narrow the passage from the tubing rested on the benchtop. A 10 mL syringe approximately the average width of rabbit larynges was situated over the two silicone devices and provided a location for the larynges to be mounted to the benchtop.

On the surface of the breadboard, three adjustable single prong micropositioners were mounted on the benchtop used to stabilize the larynx and adduct the vocal folds. Two micropositioners were placed lateral to the syringe head and one was placed anterior. The anterior micropositioner provided a location for the string attached to the thyroid via suture in the thyroid cartilage to attach. A subtracheal outlet was drilled into the tubing directly above the benchtop where a physiological pressure transducer (Model MLT844, AD Instruments, Sydney, Australia) attached perpendicular via a stop cock to the upward vertical airflow. A microphone was placed approximately 10 cm at a 45° above the mounted larynx to collect acoustic data. A digital hygrometer was used to monitor and report room temperature and humidity during data collection. Figure 3 is a photo of the benchtop setup and its components.

Figure 3*Benchtop Setup***Acquisition System Calibration**

Prior to running trials, pressure and flow were zeroed and calibrated using LabChart™ version 8 (PowerLab™, AD Instruments, Colorado Springs, CO). The respiratory flow head was attached to a Glottal Enterprise™ pneumotach calibration unit to sample the airflow in L/min. The flow was then calibrated to 1 L/min. The pressure transducer was calibrated by the PowerLab™ system to 40 mmHg.

Mounting the Larynx

When ready to run trials, a finely dissected larynx was mounted on the syringe head on the benchtop setup. The trachea was secured on the setup using Zip Ties™ and Teflon™ tape if needed to prevent air leakage. The two lateral micropositioners were inserted into the arytenoid cartilage at equal levels to stabilize the larynx and adduct the vocal folds. The suture thread in

the anterior commissure of the thyroid was tied to the anterior micropositioner to stabilize the larynx. The micropositioner was tightened just enough to stabilize but not to lengthen the vocal folds. Figures 4 illustrates the mounted rabbit larynx and three micropositioners; Figure 5 is a close-up photo of the mounted larynx.

Figure 4

Larynx and Micropositioners

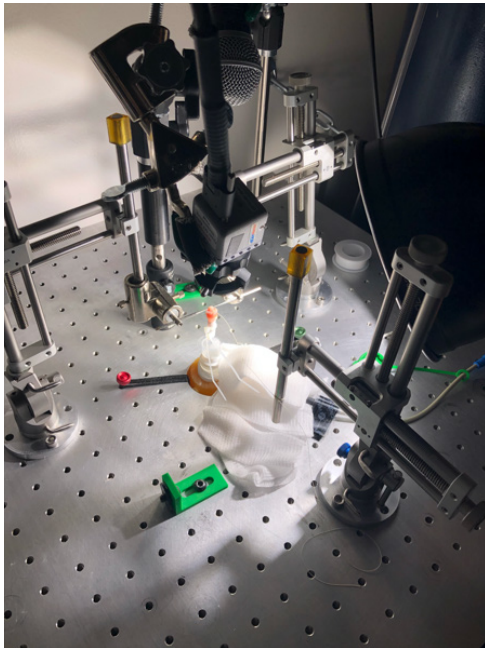
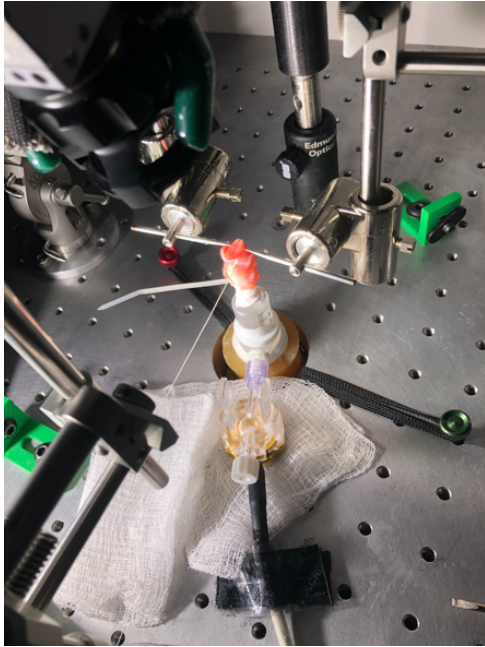


Figure 5*Close-Up of Benchtop Mount***Data Collection**

Once mounted, 15 phonatory trials were run on each rabbit larynx. Air was supplied from the compressed air tank and gradually turned on by twisting a knob until phonation began. Air passed from the air tank, through the tubing and humidifier, up the pseudolung and syringe tip to vibrate the vocal folds. Once phonation was initiated, the air flow was held at a constant state for approximately 3 seconds and then turned off. During each trial, the acoustic signal, pressure, and flow were acquired. During all 15 trials the micropositioners and position of the larynx remained stable. Pressure was measured by the pressure transducer prior to air reaching the vocal folds and flow was measured by the flow meter. Tissue was kept hydrated throughout the 15 phonatory trials by spraying 0.9% saline solution every two to three trials as needed.

Data Analysis

After collecting data, using a previously established methodology (Prigmore, 2020), markers were placed at phonation onset, steady state, and offset for each trial. The signals were then averaged within 10 ms of phonation onset. Phonation onset was determined acoustically and visually by listening to acoustic signals and analyzing recurring oscillations in the acoustic signal. Analysis was performed using a custom Matlab program (MathWorks, Natick, MA) written by Christopher Dromey, PhD, who is a member of this Thesis Committee. Phonation was analyzed for each phonatory trial using Praat, version 6.0.49 (Boersma & Weenink, 2020).

Statistical Analysis

For purposes of the parent project, summary data for onset and sustained pressure, airflow, laryngeal resistance, and F_0 were examined. Data distributions were examined visually using box plots. One-way analysis of variance was conducted for each of these variables. For this thesis, Student Newman-Keuls analyses were conducted for PTP and PTF using an alpha level of .05. Analyses were conducted using the Statistical Analysis System (version 9.4, Cary, NC) by Ray M. Merrill, PhD, in Life Sciences at BYU.

Results

As described in the Method section of this document, several analyses were undertaken to address the proposed experimental questions. First, descriptive characteristics of the laryngeal specimens and environmental conditions in this study are provided. Second, descriptive statistics for PTP and PTF values are included. Lastly, this investigation's experimental hypotheses are examined using parametric statistics as described below. Of note, one control group specimen was excluded from all analyses due to structural damage to the larynx that resulted in the

inability to properly mount and elicit phonation. Therefore, the final data set included 11 larynges in the inhaler group and 10 in the control group.

Specimen and Environment Data

Measurements were taken using a digital caliper for information regarding tracheal, laryngeal, and vocal fold dimensions. Tables 1, 2 and 3 include precise measurements of each rabbit larynx used in the study reported in mm. Each of these measurements supports a thorough representation of the size of each specimen in this study and for comparison and replication purposes in other investigations.

Table 1*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	5.5
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 2*Vocal Fold Anatomical Size and Dimensions*

Group	Length of vocal folds (mm)	Width of vocal folds (mm)	Width from vocal fold to thyroid cartilage (mm)
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 3*Thyroid Cartilage Anatomical Measurements*

Group	Protuberance to bottom (mm)	Width (mm)
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

For each data collection session, both room temperature and environmental humidity levels were recorded. Specifically, baseline and any changes in temperature were tracked.

Additionally, percent relative humidity was documented at baseline and at the conclusion of data collection for each laryngeal specimen. These data are provided in Table 4.

Table 4

Room Temperature and Humidity

Group	Session Date	Initial Humidity	Final Humidity	Initial Temperature (°F)	Final Temperature (°F)
Inhaler					
1	9/27/2020*	35%	35%	75	75
2	9/27/2019	38%	38%	75	75
3	9/27/2019	37%	37%	75	77
4	9/27/2019	37%	39%	75	75
5	9/27/2019*	36%	36%	75	76
6	10/11/2019	12%	14%	80	80
7	10/11/2019	12%	12%	80	80
8	10/11/2019	12%	15%	78	80
9	10/11/2019	11%	14%	79	80
10	10/11/2019	12%	13%	80	80
11	10/11/2019	14%	14%	80	80
Control					
1	1/10/2020	22%	24%	75	76
2	1/10/2020	22%	21%	75	75
3	1/10/2020	19%	21%	75	75
4	1/10/2020	19%	20%	76	76
5	1/24/2020	19%	20%	79	80
6	1/24/2020*	19%	20%	79	79
7	1/24/2020	21%	23%	79	79
8	1/24/2020	23%	22%	80	79
9	1/24/2020	20%	23%	79	80
10	1/24/2020	23%	23%	79	79

Note. *Replaced by approximates based on series of rabbits and time-frame comparisons.

Descriptive Statistics

Descriptive statistics for PTP and PTF were generated for both the experimental (inhaler) and control (saline) groups. The mean PTP for the experimental group was 7.82 cmH₂O (SD = 2.58; Range = 5.18-11.84); mean PTP for the control group was 7.44 cmH₂O (SD = 1.67; Range = 5.18-10.94). Regression line analysis for PTP in the experimental and control group is shown in Figure 6. The mean PTF for the experimental group was 0.10 L/min (SD = 0.04; Range = 0.05-0.18); mean PTF for the control group was 0.07 L/min (SD = 0.03; Range = 0.02-0.12). Regression line analysis of PTF in the experimental and control group is shown in Figure 7. Average F₀ for the inhaler group was 519.85 Hz (SD=66.61); average F₀ for the control group was 446.91 Hz (SD=130.63). Visual inspection of PTP and PTF outcome data using box plots and distribution examination were compatible with the assumptions for parametric analysis of variance.

Figure 6

Regression Line Analysis for PTP Inhaler and Control Group

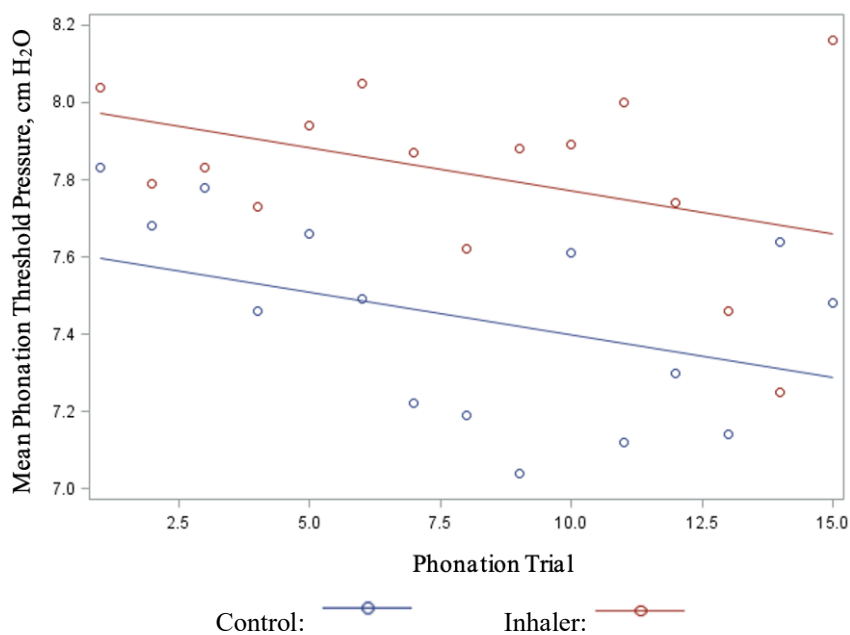
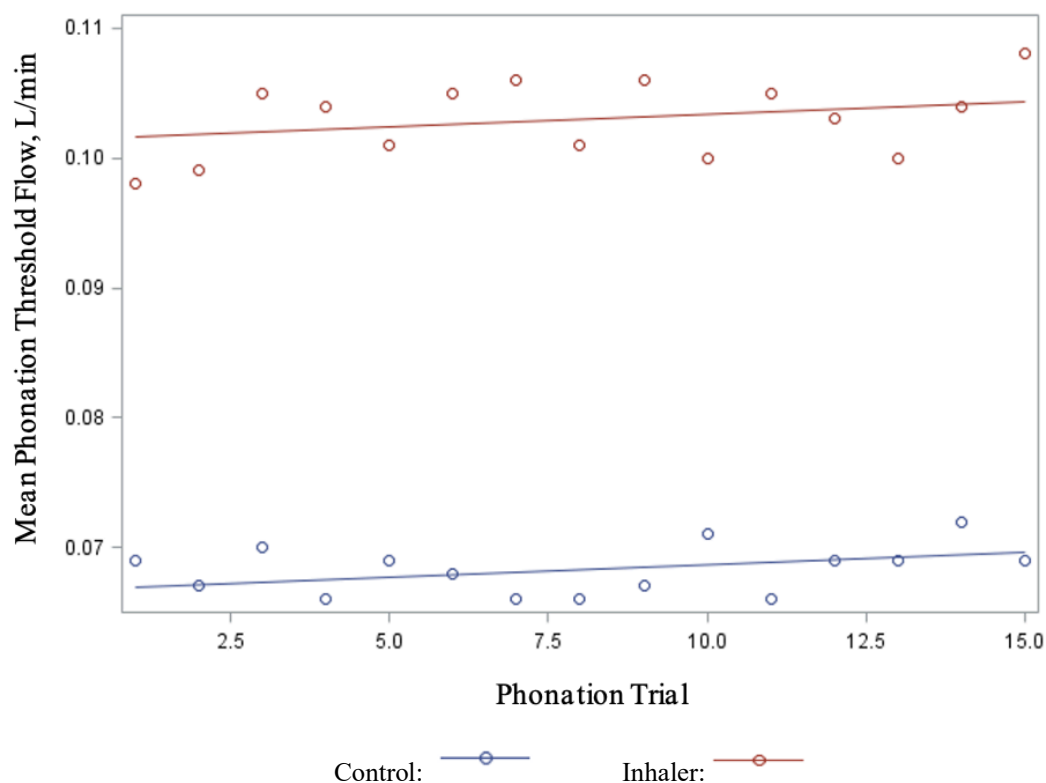


Figure 7

Regression Line Analysis for PTF Inhaler and Control Group



Group Comparisons

A repeated measures one-way analysis of variance (ANOVA) was undertaken to examine the effect of inhaler use on PTP. The results indicated that inhaler use significantly influenced PTP across phonation trials for both groups [$F(35, 279) = 124.09, p = 0.0001$]. A post-hoc Student-Newman-Keuls analysis indicated that mean PTP for the inhaler group was significantly different than the mean PTP for the control group, $p < .05$.

Additionally, a repeated measures one-way ANOVA was undertaken to examine the effects of inhaler use on PTF. The results indicated that inhaler use significantly influenced PTF across phonation trials for both groups [$F(35, 279) = \infty, p = 0.0001$]. (Note: an infinity f value is defined as an f-value that is so large it cannot be estimated but is an indication of high likelihood of differences among the groups.) A post-hoc Student-Newman-Keuls analysis

indicated that mean PTF for the inhaler group was significantly different than the mean PTF for the control group, $p < .05$.

Segmentation Reliability

Although data reduction and aerodynamic analysis procedures were largely automated via a custom Matlab program, segmentation in LabChart was required for original identification of phonation onset. Two investigators with extensive training in segmentation procedures identified phonation onset for each trial. To ensure accuracy of segmentation prior to automated Matlab analysis, 10% of samples were re-segmented by each examiner for purposes of intrajudge reliability. Intrajudge reliability for each variable resulted in Pearson correlations greater than or equal to .98. Additionally, each examiner re-segmented 10% of inhaler and control samples originally completed by the other examiner (i.e., interjudge reliability). Interjudge reliability was calculated using ICC and reported to be greater than or equal to .948. These results indicate acceptable reliability for the segmentation process.

Results of this study indicate significant differences in PTP and PTF measures in rabbit vocal folds treated with ICs over 8 weeks as compared to rabbit vocal folds treated with nebulized isotonic saline solution.

Discussion

The current study sought to measure the effects of ICs on aerodynamic measures of the voice. The purpose of this research was to quantify aerodynamic changes in the voice due to ICs treatment as there has been an increase in voice disorders caused by ICs in recent years. Rabbits treated with ICs for 8 weeks were compared to rabbits treated with nebulized isotonic saline solution. The independent variable was group (IC versus saline) while the dependent variable were PTP (cmH₂O) and PTF (L/min). The primary questions of this study were 1) what are the

onset pressure and flow characteristics of rabbit vocal folds following 8-week exposure to inhaled corticosteroids and 2) how do these onset pressure and airflow characteristics differ from control rabbit vocal folds exposed to inhaled saline? In collecting data, a benchtop setup was used. Operational procedures remained consistent throughout each dissection, trial, and analysis using current research on benchtop setups to optimize the results of the study. As hypothesized, analysis of the data showed significant differences between PTP and PTF in the inhaler and experimental group.

PTP and PTF Characteristics

Aerodynamic measurements of voice (i.e., PTP and PTF) are frequently used to examine vocal fold health due to ability to reflect differences in voice productions that can arise from a variety of factors (Hoffman et al., 2012; Jiang et al., 1999). The minimum amount of subglottal pressure and airflow needed to initiate phonation is defined respectively as PTP and PTF. Extensive research has been completed on how to interpret PTP values regarding vocal fold health. Researchers have found that when PTP increases it is commonly due to growths or swelling of the vocal fold, specifically in changes to vocal fold viscosity, velocity of the mucosal wave, prephonatory glottal width, and/or vocal fold thickness (Jiang & Titze, 1993; Plexico et al., 2011). Results of this study indicate higher PTP values in vocal folds treated with ICs as compared to vocal folds treated with nebulized isotonic saline. Onset pressure characteristics of rabbit vocal folds following 8-week ICs treatment revealed a rise in PTP, standard deviation was higher, and on average the range of values increased, showing greater variability in PTP values when treated with ICs. Likewise, while PTF is a relatively new measurement, it has been found that when PTF increases it is commonly due to changes in glottal width due to glottal configuration, tissue properties, and vocal tract loading (Jiang & Tao, 2007). The results of this

study revealed PTF values in rabbit vocal folds treated with ICs to have higher PTF on average with a larger standard deviation and range.

It might be hypothesized that the increase in PTP and PTF values in rabbit vocal folds treated with ICs over 8 weeks is due to histological changes of the vocal folds. The purpose of IC treatment is to decrease chronic inflammation of the airways caused by asthma. While ICs are effective in decreasing inflammation in the lower airway, local upper airway—and specifically laryngeal—side effects such as dysphonia can occur. These laryngeal changes may be characterized by laryngitis and hoarseness in a majority of individuals who use ICs. It has been reported that ICs may cause the vocal folds to swell, leading to increased mass and histological changes to the vocal folds (Ihre et al., 2004; Hassen & Hasseba, 2016; Sahrawat et al., 2014). The results of this study are compatible with the literature, indicating that ICs increase the mass and stiffness of the vocal folds, therefore increasing PTP and PTF and producing a negative impact on phonation.

Central Tendency and Acoustic Differences Between Groups

It is notable that the inhaler group had a greater standard deviation than the control group. Greater variation among rabbits in the inhaler group for PTP and PTF is similar to the variation that is observed among individuals with certain types of voice disorders. It is logical to presume that individuals with voice disorders associated with ICs would vary in their clinical presentation and voice characteristics. Several different voice characteristics and presentations have been reported in the literature (Barnes, 2002; Bhalla et al., 2008; Dogan et al., 2007; Galván & Guarderas, 2012; Gallivan et al., 2007). Therefore, the increased standard deviation observed in the inhaler group as compared with the control group supports the overall generalizability of the animal model used in this study (Erickson & Sivasankar, 2010; Holmberg et al., 2003).

In the current study, it was also observed that the inhaler group had a slightly higher F_0 on average than the control group. It is anticipated that with increased swelling F_0 would be lower as it has been repeatedly found that as mass increases fundamental frequency decreases; however, results of this study indicate F_0 increased in the inhaler group with an average of 519.85 (SD=66.61) as compared to the control group with an average F_0 of 446.91 (SD=130.63). The increase in F_0 may be due to not having thyroarytenoid (TA) muscle contraction on the benchtop setup as the TA muscle is primarily responsible for lowering pitch. It is anticipated that TA muscle contraction may play a bigger part in what causes disordered vocal folds to have lower F_0 . Additionally, TA muscle contraction has been found to be a key factor in individuals with voice disorders including hoarseness (Chhetri & Neubauer, 2015).

Relationship Between PTP and PTF

Phonation occurs when there is enough pressure (PTP) and flow (PTF) for the vocal folds to vibrate. A number of theories and laws govern this principle and provide further understanding of the physiology of phonation. Specifically, the Bernoulli Effect provides clarity on the relationship between pressure and flow when it comes to phonation. According to Bernoulli's effect, velocity of airflow increases as it travels through narrowing vocal folds, upon which negative pressure causes the vocal folds to be drawn together, closing the glottis. Air pressure builds up beneath the vocal folds until it reaches a threshold level high enough to separate the vocal folds. When the pressure is high enough, airflow occurs through the glottis, separating the vocal folds and releasing air. The separation of the vocal folds occurs until the myoelastic characteristics of the vocal folds and intrinsic muscles of the larynx take over to draw the vocal folds back together. This process of drawing the vocal folds together, building up pressure, increasing velocity, decrease in pressure, and drawing the folds back together is

repeated to create phonation. Each cycle produces a single pulse of air and when combined produces phonation (Seikel et al., 2010).

The amount of pressure needed to initiation phonation is PTP, one of the primary measures of this study. Similarly, the amount of flow needed to initiation phonation is PTF. Pressure and flow are related in such a way that pressure drives flow through the glottis. It is expected that as pressure increases flow will also increase if resistance stays constant. This study found consistent results as both PTP and PTF increased on average compared to the control group.

Translation Potential to Human Subjects

The goal of this study was to quantify aerodynamic changes in the voice due to ICs usage to take further steps to understanding changes in the human voice due to ICs usage. In translating this study to humans, it has been found that rabbit vocal folds a valid model of human vocal folds due to similar tissue composition. Using rabbit vocal folds, this study was able to independently examine how possible changes to vocal fold tissue composition changes phonation. As the layers of the vocal folds change, the nature of vocal fold vibration will also change. In this study, it was hypothesized that vocal folds swell due to ICs usage, therefore pressure and flow measurements would be adversely impacted. With an increase in mass of the vocal folds, higher pressure and flow is required to initiate phonation. This pattern found in the rabbit larynges can be hypothesized to be identical to how human vocal folds would react to ICs usage considering the similar tissue composition.

Limitations

Despite best efforts of the researchers, there are limitations to this study. Using rabbit vocal folds as a model for human voice production has been found to be reliable due to similar

tissue composition; however, the quiet nature of rabbits may be a limiting factor. When translating the results to human models, there are many additional factors that influence the health of the vocal folds including voice usage and intensity of phonation. This study aimed at identifying only aerodynamic measures; therefore, isolating this measure was effective in rabbit vocal folds.

The benchtop setup established by Jiang and Titze (1993) was utilized in this study; however, in order to use the benchtop setup fine dissection of excess tissue was completed to reveal the true vocal folds. Despite best efforts, human error in dissection is a limitation. Using knowledge from classes, training from other lab members, and researching additional information, lab members attempted to consistently dissect equal amount of tissue for each larynx. Due to human error, some larynges may have had more or less tissue, possibly adversely impacting measurements. Additionally, while using the benchtop setup, a small microphone was used to collect acoustic data. During the trials, the microphone was frequently repositioned in hopes of optimizing the acoustic signal. Because of moving the microphone, acoustic signal could be different for larynges worked on different days. Only male rabbits were included in this study. While this was an effective way of controlling experimental factors, it is unknown if results would be similar in female rabbits. Female rabbits were not included due to prominent fat deposits that can obscure laryngeal view.

Despite these and other limitations such as small sample size and general methodological constraints with benchtop models, this study represents an important initial step toward understanding how voice function is affected by IC use.

Implications for Future Research

This study provides a foundation for future work regarding the effects of ICs on the voice. This study showed that there is a significant difference in aerodynamic measures when using rabbit models to study the voice. Future studies may utilize additional animal and human models to further examine the effects of ICs on the voice. Rabbits are the most valid model; however, how this translates to human research is still unclear. Therefore, future research could look at transitioning these results to human models and comparing the differences between aerodynamic measures in human and rabbit larynges that have been treated with ICs.

In addition to using human models, further research could be completed utilizing rabbit vocal folds. This study exclusively used data obtained *ex vivo*. Future studies could further look at *in vivo* acoustic parameters of the voice in comparing ICs and inhaled saline solution and well as videostroboscopy to visualize the health of the vocal folds as they receive treatment. Due to the limiting factor of rabbit fold vocal intensity, an additional implication for future research could be to determine the impact of voice usage in combination with ICs has on vocal quality.

The benchtop setup used for this study has been well researched over the years; however, in the voice lab where trials were run, continued modification of the benchtop setup would be beneficial. Future studies could specifically look at automating a saline solution hydration system to have controlled hydration for each larynx and standardizing the location of the microphone for acoustic signal. Additionally, future studies could look at detailing a protocol with specific videos/pictures of what tissue to dissect and how much

Because this study is part of a larger, five-step process, future steps of this project that have been outlined include using an *in vivo* rabbit model to determine the threshold for and reversibility of ICs effects on the voice using transnasal videoendoscopy. Measurements will be

taken at baseline and every 2 weeks during treatments with the purpose of testing to see if the effects of ICs can be reversed.

Conclusion

Results of this study show there is a significant difference in aerodynamic measures of PTP and PTF due to ICs usage as compared to a control group. It was found that PTP and PTF values on average increased with ICs treatment as well as in the standard deviation and range. The findings of the current study are vital for future research regarding the effects of ICs on the voice. Quantifying aerodynamic changes at threshold in the voice due to ICs usage allows further research to be done that can translate to human subject research.

References

- Akinbami, L. J., & Liu, X. (2011). Chronic obstructive pulmonary disease among adults aged 18 and over in the United States, 1998-2009. *NCHS Data Brief*, (63), 1–8.
- Barnes, P. J. (2002). Scientific rationale for inhaled combination therapy with long-acting b2-agonists and corticosteroids. *European Respiratory Journal*, *19*, 182–191.
<https://doi.org/10.1183/09031936.02.00283202>
- Barnes, P. J. (2010). Inhaled corticosteroids. *Pharmaceuticals*, *3*(3), 514–540.
<https://doi.org/10.3390/ph3030514>
- Bhalla, R. K., Jones, A. S., & Roland, N. J. (2008). Prevalence of pharyngeal and laryngeal complications in adult asthmatics using inhaled corticosteroids. *The Journal of Laryngology & Otology*, *122*(10), 1078–1083.
<https://doi.org/10.1017/s0022215107001272>
- Birk, V., Döllinger, M., Sutor, A., Berry, D. A., Gedeon, D., Traxdorf, M., Wendler, O., Bohr, C., & Kniesburges, S. (2017). Automated setup for ex vivo larynx experiments. *The Journal of the Acoustical Society of America*, *141*(3), 1349–1359.
<https://doi.org/10.1121/1.4976085>
- Boersma, P., & Weenink, D. (2020). Praat: doing Phonetics by Computer.
<https://www.fon.hum.uva.nl/praat/>.
- Centers for Disease Control and Prevention. (2018). *FastStats - Asthma*. Centers for Disease Control and Prevention. <https://www.cdc.gov/nchs/fastats/asthma.htm>.
- Chhetri, D. K., & Neubauer, J. (2015). Differential roles for the thyroarytenoid and lateral cricoarytenoid muscles in phonation. *The Laryngoscope*, *125*(12), 2772–2777.
<https://doi.org/10.1002/lary.25480>

- Dogan, M., Eryuksel, E., Kocak, I., Celikel, T., & Sehitoglu, M. A. (2007). Subjective and objective evaluation of voice quality in patients with asthma. *Journal of Voice*, *21*(2), 224–230. <https://doi.org/10.1016/j.jvoice.2005.11.003>
- 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>
- Dong, Y. H., Hsu, C. L., Li, Y. Y., Chang, C. H., & Lai, M. S. (2015). Bronchodilators use in patients with COPD. *International Journal of Chronic Obstructive Pulmonary Disease*, *10*, 1769–1779. <https://doi:10.2147/COPD.S86198>
- 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\(2009/09-0024\)](https://doi.org/10.1044/1092-4388(2009/09-0024))
- 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>
- Galván, C. A., & Guarderas, J. C. (2012). Practical considerations for dysphonia caused by inhaled corticosteroids. *Mayo Clinic Proceedings*, *87*(9), 901–904. <https://doi.org/10.1016/j.mayocp.2012.06.02>
- 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>

- Hoffman, M. R., Rieves, A. L., Budde, A. J., Surender, K., Zhang, Y., & Jiang, J. J. (2012). Phonation instability flow in excised canine larynges. *Journal of Voice*, *26*(3), 280–284. <https://doi.org/10.1016/j.jvoice.2011.03.007>
- 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](https://doi.org/10.1067/s0892-1997(03)00076-6)
- Hottinger, D. G., Tao, C., & Jiang, J. J. (2007). Comparing phonation threshold flow and pressure by abducting excised larynges. *Laryngoscope*, *117*(9), 1695-1699. doi: 10.1097/MLG.0b013e3180959e38
- Howard, N. S., Mendelsohn, A. H., & Berke, G. S. (2015). Development of the ex vivo laryngeal model of phonation. *The Laryngoscope*, *125*(6), 1414–1419. <https://doi.org/10.1002/lary.25149>
- 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>
- Jiang, J. J., & Tao, C. (2007). The minimum glottal airflow to initiate vocal fold oscillation. *The Journal of the Acoustical Society of America*, *121*(5), 2873–2881. <https://doi.org/10.1121/1.2710961>
- Jiang, J. J., & Titze, I. R. (1993). A methodological study of hemilaryngeal phonation. *The Laryngoscope*, *103*(8). <https://doi.org/10.1288/00005537-199308000-00008>

- Jiang, J., O'mara, T., Conley, D., & Hanson, D. (1999). Phonation threshold pressure measurements during phonation by airflow interruption. *The Laryngoscope*, *109*(3), 425–432. <https://doi.org/10.1097/00005537-199903000-00016>
- Mannino, D. M., Gagnon, R. C., Petty, T. L., & Lydick, E. (2000). Obstructive lung disease and low lung function in adults in the United States: Data from the National Health and Nutrition Examination Survey, 1988-1994. *Archives of Internal Medicine*, *160*(11), 1683–1689.
- Mayo Foundation for Medical Education and Research. (2020, August 11). *Asthma*. Mayo Clinic. <https://www.mayoclinic.org/diseases-conditions/asthma/symptoms-causes/syc-20369653>.
- 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>
- Plant, R. L. (2005). Aerodynamics of the human larynx during vocal fold vibration. *The Laryngoscope*, *115*(12), 2087–2100. <https://doi.org/10.1097/01.mlg.0000184324.45040.17>
- Plant, R. L., Freed, G. L., & Plant, R. E. (2004). Direct measurement of onset and offset phonation threshold pressure in normal subjects. *The Journal of the Acoustical Society of America*, *116*(6), 3640–3646. <https://doi.org/10.1121/1.1812309>
- Plexico, L. W., Sandage, M. J., & Faver, K. Y. (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\(2011/10-0066\)](https://doi.org/10.1044/1058-0360(2011/10-0066))

- Prigmore, A. (2020). A comparison of phonation threshold pressure and phonation threshold flow between pig and rabbit benchtop-mounted larynges. *Theses and Dissertations*. 8404. <https://scholarsarchive.byu.edu/etd/8404>
- Sahrawat, R., Robb, M. P., Kirk, R., & Beckert, L. (2013). 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>
- Seikel, J. A., King, D. W., & Drumright, D. G. (2010). *Anatomy & physiology for speech, language, and hearing* (4th ed.). Delmar Cengage Learning.
- Smitheran, J. R., & Hixon, T. J. (1981). A clinical method for estimating laryngeal airway resistance during vowel production. *The Journal of speech and hearing disorders*, 46(2), 138–146. <https://doi.org/10.1044/jshd.4602.138>
- Swanson, E. R., Abdollahian, D., Ohno, T., Ge, P., Zealear, D. L., & Rousseau, B. (2009). Characterization of raised phonation in an evoked rabbit phonation model. *The Laryngoscope*, 119(7), 1439–1443. <https://doi.org/10.1002/lary.20532>
- Tanner, K., Nissen, S. L., Merrill, R. M., Miner, A., Channell, R. W., Miller, K. L., Elstad, M., Kendall, K.A., & Roy, N. (2015) Nebulized isotonic saline improves voice production in Sjogren’s Syndrom. *The Laryngoscope*, 125(10), 2333-2340. <https://doi.org/10.1002/lary.25239>
- Welham, N. V., Montequin, D. W., Tatiya, I., Tateya, T., Choi, S. H., & Bless, D. M. (2009). A rat excised larynx model of vocal fold scar. *Journal of Speech, Language, and Hearing Research*, 52(4), 1008–1020. [https://doi.org/10.1044/1092-4388\(2009/08-0049\)](https://doi.org/10.1044/1092-4388(2009/08-0049))

Williamson, I. J., Matusiewicz, S. P., Brown, P. H., Greening, A. P., & Crompton, G. K. (1995).
Frequency of voice problems and cough in patients using pressurized aerosol inhaled
steroid preparations. *The European Respiratory Journal*, 8(4), 590–592.

APPENDIX A

Annotated Bibliography

Phonation threshold pressure has been extensively researched in the voice field; however, phonation threshold flow is a relatively new measurement. A comprehensive analysis of the literature regarding PTP, PTF, ICs use, animal models to study the voice, and the benchtop setup provided relevant information necessary for the current study. This literature review includes relevant articles pertaining to different aspects of the study.

Bhalla, R. K., Jones, A. S., & Roland, N. J. (2008). Prevalence of pharyngeal and laryngeal complications in adult asthmatics using inhaled corticosteroids. *The Journal of Laryngology & Otology*, 122(10), 1078-1083. doi:10.1017/s0022215107001272

Purpose of the Study: Inhaled corticosteroids have systemic and local side effects. Systemic side effects have been well reported, however the literature and research on local side effects is lacking. The purpose of this study was to examine and report on the prevalence of local side effects resulting from the use of inhaled corticosteroids. It is essential that researchers understand the prevalence of local side effects so that therapeutic strategies can be developed to manage the effects and risk factors of ICS usage.

Method: This study was a questionnaire based observational design. Participants were recruited from two local general practices using asthma nurse specialists to assist in selecting participants based on who was currently prescribed ICs. A pilot study of 20 individuals who had symptoms of pharyngolaryngitis due to ICs completed a respiratory symptom questionnaire. After the pilot study, 190 patients were administered the questionnaire via mail. Asthma severity was classified as mild, moderate, or severe using

the British Thoracic Society guidelines for each participant. Areas assessed in the survey included smoking history, co-morbidities, preventative measures, and side effects of ICs including hoarseness, voice weakness, voice loss, sore throat, throat irritation, and long-standing cough. Additionally, the number, type, strength, dosing regime, and duration of ICs use were assessed. Participants recorded their throat discomfort using a 10-point visual analogue scale.

Results: The survey had a 75.8% response rate, and the majority of the responses were from patients with mild or moderate asthma who had been using ICS for 1-5 years. It was found that asthma severity (mild, moderate, or severe) was directly correlated with a longer duration of ICS use. The mean discomfort score on the 10-point visual analogue scale was 4.26, with 0 indicating no discomfort and 10 indicating severe discomfort. In general, side effects from ICS were observed immediately after use or after 3 months. Results show that the longer an individual used an inhaler, the more susceptible they were to have dysphonia including weakness of their voice, throat irritation, and hoarseness. Overall, hoarseness, sore throat, cough, and throat irritation were found to be more prevalent than the researchers initially expected.

Conclusion: This observational study found that there are both pharyngeal and laryngeal local side effects caused by using ICs. Some side effects of inhalers use, such as voice weakness, are directly attributable to the use of corticosteroids. Additionally, some side effect, such as throat irritation, are a result of using combined corticosteroids (steroids and non-steroids). It was determined that there is a correlation between ICs use and local side effects in the oropharynx and larynx.

Relevance to the Current Work: This study provides evidence of the negative local side effects associated with the use of ICs in asthma treatment. The current study is specifically looking at the aerodynamic side effects of using ICs that indicate dysphonia. Further examining the aerodynamic side effects will expand our understanding of local side effects and how they manifest.

Birk, V., Döllinger, M., Sutor, A., Berry, D. A., Gedeon, D., Traxdorf, M., Wendler, O., Bohr, C., & Kniesburges, S. (2017). Automated setup for ex vivo larynx experiments. *The Journal of the Acoustical Society of America*, *141*(3), 1349–1355
<https://doi.org/10.1121/1.4976085>

Purpose of the Study: The purpose of this study was to minimize the duration of experimental procedures in ex vivo larynx studies. The researchers aimed to ease the variation of parameters in experiments so as to minimize the influence of degeneration and dehydration of the larynx tissue due too extensive experimental time. The possibility of achieving an automated onset detection was also examined during this study. Researchers attempted to create a standardized experimental procedure to maximize the number of experimental conditions implemented in a single larynx in the shortest possible amount of time. Automating a set up in attempts to minimize dehydration while still acquiring sufficient data.

Method: A customized, computer-controlled setup was developed for this study using a time-dependent variation of vocal fold elongation and adduction. Additionally, an electro-mechanical device was custom developed for positioning the laryngeal cartilage and mounting the larynx. The *ex vivo* porcine larynx was mounted on an artificial stainless-steel trachea with a hold drilled 130mm below the glottis to measure subglottal

pressure via an XCS-93-5PSISG pressure sensor. To stimulate cricothyroid muscle contraction, an electro-mechanical setup would apply a defined amount of force on the thyroid cartilage, tilting it. An additional device was developed to achieve arytenoid cartilage rotation to adduct the vocal folds.

Results: The results of this study indicate the effectiveness and functionality of using a computer controlled, standardized setup for *ex vivo* larynx studies. Acoustic and subglottal pressure signals were selected in data acquisition and captured at a sampling rate of 50 kHz for 5 seconds each. The influence of vocal fold elongation and adduction on phonation were showed by automated onset detection. In addition, the aerodynamic measurements taken were compared to similar *ex vivo* larynx experiments to evaluate the effectiveness and accuracy of the measurements. The automated onset and offset measurements results were constant for the phonation position of the larynx. Overall, the results of this study validated the use of the modified setup.

Conclusion: The setup described and researched in this study provides and automated, standardized method to studying *ex vivo* larynges. *Ex vivo* larynx experiments are in general limited by tissue degeneration and dehydration, therefore, establishing a mechanism to automate the experimental process with each larynx has proven to be beneficial and more time effective.

Relevance to the Current Work: This study relates to the current work as it gives an overview of different factors an *ex vivo* experiment must control when studying the glottis. The current study uses *ex vivo* rabbit larynges, and while it is not an automated setup as described and researched in this study, arytenoid rotation, cricothyroid contraction, and airflow are all manually manipulated in the experiment and

must be maintained constant. This study provides resources for how to maintain stable measurements.

Campagnolo, A. M., Tsuji, D. H., Sennes, L. U., Imamura, R., & Saldiva, P. H. N. (2010).

Histologic study of acute vocal fold wound healing after corticosteroid injection in a rabbit model. *Annals of Otolaryngology, Rhinology, & Laryngology*, 119(2), 133–139.

<https://doi.org/10.1177/000348941011900211>

Purpose of the Study: The purpose of this study was to evaluate the histological effects of injecting dexamethasone sodium phosphate (corticosteroid) into the vocal folds and the effects it had on acute vocal fold wound healing in rabbit larynges after surgically induced injury.

Method: This study involved 12 New Zealand albino rabbits. Each rabbit underwent laryngeal microsurgery where a puncture wound was made with a knife in the upper surface at midpoint of each vocal fold. After the wound was made, 0.1 mL of dexamethasone sodium phosphate was injected into the left vocal fold and the right vocal fold served as a control and was not injected. Three days after the injection six of the 12 rabbits were sacrificed, with the remaining six were sacrificed 7 days after the injection. The histological features of the excised larynges were then studied.

Results: The mean numerical density of cells in the cortico-steroid treated group was 3.16, the mean in the control group was 2.48. There was no significant difference between the amount of collagen in the treated vs. the control group vocal folds. The concentration of collagen fibers in each vocal fold did show significant increased at 7 days in both the treated and the control group. There was a statistically significant difference in collagen deposition between the treated and the control group, showing

corticosteroid treatment to reduce quantity of accumulated collagen during wound healing.

Conclusion: Corticosteroid injection after vocal fold injury reduces collagen deposits at the site of injury while in the acute state of recovery.

Relevance to Current Work: This study examined vocal fold healing in response to corticosteroid injection in rabbits. Details regarding changes in the mucosal-wave and how that impacts phonation were discussed in this study. The current study also uses rabbit vocal folds to study vocal fold health and impacts of corticosteroids. Corticosteroids will be inhaled, however it is hypothesized that changes to the mucosal wave will occur, leading to changes in phonation.

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. doi:10.1044/1092-4388(2009/09-0024

Purpose of the Study: Dysphonia is a common side effect of ICs treatment for asthma. The purpose of this study was to examine the adverse effects of ICs treatment on PTP and PPE (perceived phonatory effort). The researchers tested the hypothesis that ICs treatment would elevate PTP and PPE, but that the sham treatment would not. They also examined the relationship between PTP and PPE in individuals receiving ICs treatment.

Method: Participants for this study included 14 adults (9 women and 5 men) with no reported voice or laryngeal problems. Each participant completed the VHI and scored a mild voice handicap. All participants were taking Advair diskus IC to treatment of their asthma once or twice daily for at least 4 months. Data collection occurred at the time participants habitually took their medication to ensure methodological consistency. Each

participant completed two experimental sessions on consecutive days to get an IC and sham treatment. Data was collected during both sessions by calculating vocal pitch range, PTP, PPE, and FVC. Measurements were recorded both before treatment, immediately after treatment, 1 hr. and 2 hrs. after treatment.

Results: Results indicated that IC treatment did significantly increase PTP while the sham treatment did not. The increase in PTP lasted for 2 hours following IC administration. PPE was not significantly affected by either treatment. Weak correlations appeared between PTP and PPE.

Conclusion: This study concluded that IC treatments can have a direct effect on phonation by increasing PTP in individuals with asthma. The study examined the effectiveness of different levels of pitch and determined that when evaluating voice functions after IC treatment, it is most useful to evaluate pitch at the 80th percent pitch. The increase in PTP is suspected to be caused by the deposit of up to 75% of the IC on the vocal fold mucosa.

Relevance to the Current Work: This study provides background information regarding voice disorders associated with IC treatment. In addition, it lays a foundation for the reliability of using PTP as a determinant of vocal health differences due to IC treatment. The current study uses PTP as a primary aerodynamic measure to study the effects of IC treatment on the voice.

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.

doi:10.1016/j.jvoice.2005.09.003

Purpose of the Study: The purpose of this study was to provide updates to previously found results of stroboscopy (SVL) in individuals who complain of dysphonia due to ICS treatment. The report includes information from the SVL including mucosal wave symmetry/periodicity, amplitude and magnitude, phase closure, glottic closure, supraglottic activity, mucosal quality, and closure on/off glottic plane.

Method: Participants of this study included 38 patients diagnosed with bronchial asthma being treated with ICS and included symptoms of hoarseness and dysphonia. Each individual completed a comprehensive history and a physical exam prior to being assessed by SVL. Throughout the study, a total of 81 videolaryngoscopies were performed, including 73 transoral SVL, and 6 transnasal SVL. Each examination was then analyzed for phase closure, amplitude, glottic closure, supraglottic activity, mucosal quality, and closure on/off glottic plane.

Results: Abnormal periodicity of the mucosal wave occurred in 29/38 patients taking ICS while 63% (50/79) of all SVL examinations were abnormal. Phase closure varied in the participants and was open to differentiating degrees in 74% (28/38) patients and 63% (50/79) in all SVL. Examination of glottic closure often revealed more than one type of abnormal glottic closure (i.e., bowing, anterior glottal gap, hourglass glottis). Abnormal glottic closure occurred in 63% (24/38) patients using ICS and 59% (47/79) of all SVL examinations. Abnormalities in the amplitude and magnitude of the mucosal wave occurred in 50% (19/38) of patients using ICS and 35% (28/79) in all SVL examinations. Supraglottic hyperactivity occurred in 39% (15/38) patients and 25% (20/79) of all SVL. Mucosal quality and vocal fold edge abnormalities was recorded in 34% (13/38) of patients using ICS and 34% (27/79) SVL examinations.

Conclusion: Side effects of ICS are dependent on the type of ICs used, inhalation device, dosage, frequency of use, particle size, and local effects. This study showed the fluticasone containing ICS were the most frequently prescribed ICS that leads to voice hoarseness, SVL abnormalities, and laryngitis.

Relevance to the Current Work: This study highlighted the differences in side effects of different ICs, with fluticasone containing ICs being the most commonly prescribed ICs, as well as the most common cause of dysphonia. The current study uses fluticasone propionate and salmeterol in treatment. This study helps to make clear the differences between different ICs, and which ICs have the largest effect on the voice. This validates the administration of fluticasone in the current study.

Galván, C. A., & Guarderas, J. C. (2012). Practical considerations for dysphonia caused by inhaled corticosteroids. *Mayo Clinic Proceedings*, 87(9), 901-904

Review Article: The purpose of this article was to review the pertinent literature on the incidence of dysphonia associated with ICs, the mechanisms of dysphonia, and the relationship between ICS and dysphonia. It was reported that 5%-58% of individuals who use ICs experience dysphonia. ICs are associated with a higher risk of dysphonia and the method of delivering the ICs affects the likelihood of developing dysphonia, therefore it was concluded that with an individual with dysphonia, clinicians and researchers should take into consideration if they are using ICS and which method (MDI or powder) they are using.

Relevance to the Current Work: This article emphasizes the fact that a high percentage of individuals who use ICs develop some form of dysphonia. This validates the necessity of the current study.

Hassen, H. E., & Hasseba, A. M. (2016). Voice evaluation in asthma patients using inhaled corticosteroids. *The Turkish Journal of Ear Nose and Throat*, 26(2), 101-108.

Doi:10.5606/kbbihtisas.2016.79740

Purpose of the Study: Dysphonia caused by ICs usage has been studied by many researchers, however the specific laryngoscopic and vocal findings of individuals using ICs have not been studied extensively. The purpose of this study was to assess the voice changes and laryngeal abnormalities in asthmatic patients using ICS.

Method: Participants were recruited from the Phoniatic Clinic of the King Fahd Hospital Jeddah with a total of 30 patients using ICS treatment for bronchial asthma recruited. The minimal duration of treatment for inclusion in the study was 4 months. A protocol of voice evaluations that included an elementary diagnostic procedure, clinical diagnostic aids, and additional instrumental measurements was completed for each participant. In the elementary diagnostic procedure, a comprehensive history and otorhinolaryngological examination was performed. The severity of an individual's asthma was assigned using the Global Initiative for Asthma criteria. Additionally, a speech sample including conversational speech and dialogue were recorded and assessed by two phoniaticians. Next, a modified GRABS scaled was used to rank the degree of dysphonia in each subject experienced. Clinical diagnostic aids were performed using a Videolaryngoscopy, which was analyzed by two phoniaticians examining vocal fold edema, erythema, bowing, atrophy, irregular edges, interarytenoid thickening, and supraglottic hyperfunction. Lastly, acoustic analysis was performed where participants phonated at their natural pitch and loudness levels.

Results: It was reported that 53% of ICS users experienced dysphonia to some degree, with 36.7% having mild dysphonia and 16.7% having moderate dysphonia. Videolaryngoscopy revealed the most typical finding was interarytenoid thickening and erythema, as both appeared in 56.7% of participants. Other common findings were vocal fold edema (36.7%), supraglottic hyperfunction (53.3%), and irregular vocal fold edges (53.3%). Researchers also found that jitter percentages were significantly higher in these individuals as compared to normal individuals.

Conclusion: Dysphonia was found in 53% of patients. Most of the voice acoustic parameters were higher than standard values. The most common side effect of inhaled steroids is dysphonia.

Relevance to the Current Work: This study reports prevalence of dysphonia in individuals who use ICS. The current work is researching the aerodynamic effects of ICS usage on the voice and its contribution to dysphonia caused by ICS usage.

Hoffman, M. R., Rieves, A. L., Budde, A. J., Surender, K., Zhang, Y., & Jiang, J. J. (2012).

Phonation instability flow in excised canine larynges. *Journal of Voice*, 26(3), 280–284.

<https://doi.org/10.1016/j.jvoice.2011.03.007>

Purpose of the Study: Aerodynamic measures of subglottal pressure, mean flow rate, and vocal efficiency provide valuable information regarding the functions of the voice. The purpose of this study was to examine aerodynamic measurements in an atypical, chaotic voice by measuring phonation instability flow (PIF) and phonation flow range (PFR). Phonation flow was chosen as the measurement as phonation instability pressure as it has been shown to be clinically useful in indicating at what point phonation becomes

unstable. Researchers wanted to provide an additional measurement with clinical validity to diagnose disordered voices therefore PFR was also included in this study.

Method: Seven canine larynges were excised using protocol established by Jiang and Titze (1993). Before experimentation, dissection of unnecessary tissue was performed, and the true vocal folds were exposed. Once properly dissected, the larynx was mounted on a bench apparatus as specified by Jiang and Titze (1993). In an effort to simulate the human respiratory system a pseudolung attached to the benchtop was used to initiate and sustain phonation throughout the trials designed to simulate the human respiratory system. For each trial, airflow was gradually increased until phonation began, at which point onset time, pressure, and flow were recorded. Airflow was then increased to the point where the stable harmonic frequency of the voice was lost and appeared to be aperiodic and noise-like; this point was marked as the PIP and PIF. Airflow was then decreased until phonation ceased. Phonation floor range was calculated by subtracting PTF from PIF. This process was repeated 5 times in three different glottal configurations (0% elongation and 0 mm posterior glottal gap; 20% elongation and 0 mm posterior glottal gap; and 20% elongation with 3 mm posterior glottal gap).

Results: Results of the study show that an increase in vocal fold elongation does not affect PTF, PIF, or PIP, however there is a nearly significant increase in PTP following elongation. It was shown that increasing glottal abduction significantly impacted PTP, PIF, and PFR and had near significant impacts on PTF.

Conclusion: Phonation instability flow was a new aerodynamic measurement presented in this study and it was shown to be sensitive to glottal abduction as predicted.

It is suggested that using PIF instead of PIP to measure vocal instability may be a simpler means to quantify aerodynamic functions of the voice.

Relevance to the Current Work: The current study uses PTP and PTF as primary aerodynamic measures to evaluate the function of the voice. This study relates to the current work in its use of a similar methodology and benchtop set up for experiments and data collection.

Hong, S. J., Lee, S. H., Jin, S. M., Kwon, S. Y., Jung, K. Y., Kim, M. K., Park, H., & Lee, K. W. (2011). Vocal fold wound healing after injection of human adipose-derived stem cells in a rabbit model. *Acta Oto-Laryngologica*, *131*(11), 1198-1204.
doi:10.3109/00016489.2011.599816

Purpose of the Study: The purpose of this study was to examine the phonological and morphological qualities of scarred rabbit vocal folds after injection of human adipose-derived stem cells, as well as to examine the survival degree of hADSC.

Method: This study involved 12 white New Zealand rabbits that were anesthetized and injured with a CO2 laser. They were then injected with hADSC in the left vocal fold and phosphate-buffered saline (PBS) in the right vocal fold. Every 4 weeks a rigid endoscopic exam was performed to document any morphological changes including surface irregularities, granulation tissue formation, and atrophic changes. After 12 weeks of injection, each rabbit was euthanized and histological changes were documented including intra-epithelial inflammation, stromal inflammation, perichondral fibrosis, and collagen deposition. Finally, the tissue was examined for evidence of human cells.

Results: There was a variety of surface irregularities found in both the left and right vocal folds, however there were no significant differences between the two. It was reported that 2/12 hADSC treatment rabbits and 10/12 control PBS rabbits had granulations tissue formation 12 weeks after injection. Significantly less granulation tissue formatted in the treated vocal folds compared to the control group. There were no significant differences found between the groups in histological changes regarding intra-epithelial inflammation and stromal inflammation; however, the right vocal folds injected with PBS were found to have significantly more collagen deposits and perichondral fibrosis than hADSC.

Conclusion: The hADSC remained viable after 12 weeks and may prove to be a more cost-effective alternative to MSC treatment for vocal fold scarring. Furthermore, it was shown that hADSC improved vocal fold healing and produced less signs of vocal fold scarring.

Relevance to the Current Work: Both this study and the current study use white New Zealand rabbits to study the effects of treatments on the vocal folds. This study looked at the effects of hADSC on vocal fold healing while the current study is looking at the effects of ICS on vocal fold health. This study adds to the growing body of research that validates the use of rabbit vocal folds as reliable models for the study of histological and morphological changes in the vocal folds due to treatment.

Hottinger, D. G., Tao, C., & Jiang, J. J. (2007). Comparing phonation threshold flow and pressure by abducting excised larynges. *The Laryngoscope*, 117(9), 1695-1699.

doi:10.1097/MLG.0b013e3180959e38

Purpose of the Study: Phonation threshold flow has been proposed as an additional aerodynamic voice measure to PTP. The purpose of this study was to examine the effects of different glottal widths and abduction on PTP and PTF. Furthermore, this study looked at the response of PTF to changes in posterior glottal width.

Method: This study examined 10 excised canine larynges that were harvested immediately post-mortem from animals that had died of natural causes. The larynges were stored and frozen in a 0.9% saline solution prior to the dissection of the false folds and portions of the posterior thyroid cartilage. Larynges were then mounted on a pseudo-lung benchtop apparatus and stabilized by micropositioners. Glottal width was adjusted from 0.0-4.0 mm by metal shims. Subglottal pressure and airflow were measured for each glottal width. Data was then analyzed using LabView.

Results: The average airflow for each glottal width was reported. The average air flow for 0.0 mm was 376 mL/s, 1.0 mm was 415 mL/s, 2.0 mm was 580 mL/s, 3.0 was 867 mL/s, and 4.0 mm was 1,199 mL/s. PTP was also recorded for each glottal width. 0.0 mm was 9.89, 1.0 mm was 9.26, 2.0 mm was 8.75, 3.0 mm was 9.02, and 4.0 mm was 9.71 cm H₂O. These results indicate that PTF is sensitive to changes in glottal width and has a linear relationship with glottal width while PTP is not sensitive to changes in glottal width.

Conclusion: Phonation Threshold Flow is more indicative of vocal fold pathologies that affect glottal width than PTP, therefore PTF should be used diagnostically.

Relevance to the Current Work: This study is relevant to the current work as it uses both PTP and PTF as aerodynamic measures to examine the health of the vocal

folds. Both measures have indicative potential for different pathologies and this study confirms that PTF changes indicate a vocal fold pathology that affects glottal width. This means that an increase in PTF would merit an examination of glottal width.

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. doi:10.1016/j.jvoice.2003.05.003

Purpose of the Study: The purpose of this study was to examine the prevalence of voice disorders in individuals who use inhaled corticosteroids in Swedish asthma patients. The three primary questions answered in this study were 1) to what extent do asthma patients experience voice problems? 2) do certain voice problems appear more frequently than others? and 3) are voice problems related to age or gender, the dose, certain substances, the type of asthma, voice-demanding professions, or acid regurgitation?

Method: A pilot study was performed to develop a questionnaire to be given to patients. Once the questionnaire was developed, it was administered to 3 different hospitals and the study was administered over a 6-week period. All participants were diagnosed with asthma. Overall, 80% (280/350) of participants responded and the results were analyzed.

Results: Results of the questionnaire indicated that there is a significant positive relationship between inhalation of cortisone and voice problems. The reported problems were equally significant for both women and men. The degree of asthma, cortisone dose, and prevalence of acid regurgitation was significantly positively correlated with voice problems. Among voice problems, throat clearing, and hoarseness were the most commonly reported problems. Further results indicated that younger individuals with

asthma had a higher prevalence of voice demanding professions. These individuals reported a greater extent of voice problems. A greater number of voice problems were reported in patients with voice demanding professions and who used inhaled corticosteroids.

Conclusion: Overall, this study agrees with other studies that show that indicate a positive correlation between voice problems and inhaled corticosteroids. Individuals who experienced voice disturbances at a greater degree compared to patients with mild asthma symptoms appeared to suffer more from their asthma. Voice problems increased with cortison dosage, therefore individuals with more severe asthma typically reported more voice problems.

Relevance to the Current Work: This study relates to the current work as it further examines the effects inhaled corticosteroids have on the voice and on quality of life. The current study is aiming to better diagnose and assess voice problems due to ICS usage, and this study is helpful in pinpointing areas that are of main concern.

Jiang, J., O'mara, T., Conley, D., & Hanson, D. (1999). Phonation threshold pressure measurements during phonation by airflow interruption. *The Laryngoscope*, 109(3), 425–432. <https://doi.org/10.1097/00005537-199903000-00016>

Purpose of the Study: The purpose of this study was to determine the range of values obtainable by noninvasive subglottal pressure measurements. The study also sought to estimate the differences in PTP between normal vocal folds and individuals with vocal fold polyps.

Method: Participants in this study included 11 control participants and 13 participants with polyps. Each individual had an airtight mask affixed over their mouth

and nose and were instructed to produce a sustained /a/ while airflow was directed into a section of a pipe. Airflow, intramask pressure, and intensity (in dB) was recorded for each phonation. PTP was then predicted by calculating the difference between an estimate of subglottic pressure and the vocal tract pressure at the point phonation ceased. The 11 control participants sustained /a/ at 75, 80, and 85 dB without difficulty. The 13 individuals with polyps sustained phonation at 65, 70, and 75 dB.

Results: The mean PTP measurements for normal voices were 2.38, 2.67, and 2.98 at each dB level. The mean PTP measurement for individuals with vocal fold polyps was 4.79, 5.85, and 7.37 at each dB level. The differences between means are statistically significant.

Conclusion: This study attempts to use noninvasive means to measure and estimate PTP showed statistical significance. The PTP measurements for control subjects correlate well with additional similar studies measuring PTP. The difference between polyp PTP and normal subjects PTP was statistically significant with PTP in patients with vocal fold polyps being significantly higher than that of control subjects.

Relevance to the Current Work: PTP is a main aerodynamic measurement used in the current study. This study evaluated different ways to measure PTP and showed that it is a reliable way to quantify vocal fold health, which is a main reason why PTP is being evaluated in the current study.

Jiang, J. J., Regner, M. F., Tao, C., & Pauls, S. (2008). Phonation threshold flow in elongated excised larynges. *Annals of Otolaryngology, Rhinology & Laryngology*, 117(7), 548–553.

<https://doi.org/10.1177/000348940811700714>

Purpose of the Study: The purpose of this study is to further examine the aerodynamic measure PTF proposed by Jiang and Chao (2007). Researchers investigated the reliability and range of PTF measurements on excised canine larynges with controlled elongation. PTF measurements were compared to PTP measurements to find the similarities and relationship between the two aerodynamic measures. The physical parameters of the vocal folds were manipulated to examine how PTF varies with different pathological conditions. It was hypothesized that PTF and PTP are directly related in ranges during phonation initiation.

Method: This study included 10 excised canine larynges that were collected and mounted to a benchtop pseudo-lung apparatus. Three-pronged devices were inserted into the arytenoid cartilages to stabilize the larynx, and the anterior edge of the thyroid cartilage was attached to a micrometer via sutures as to manually elongate the vocal folds. Five trials were performed for each elongation level at increments of +5% elongation. An Omega airflow meter was connected to the pseudo-respiratory tract to record airflow and a digital pressure meter was connected below the vocal folds to record subglottal pressure. During each trial, airflow was gradually increased from 0 L/min up until phonation was audible and visible on Lab View. Statistical analysis was performed using SigmaStata 3.0 to determine PTP and PTF.

Results: The mean PTF values were found to be between 101 mL/s and 217 mL/s. At physiologic length (0% elongation), mean PTF was 162 mL/s. Statistically significant differences in PTF were found at each elongation level. At +5% elongation, mean PTF was 181.14 mL/s, at +10% elongation it was 233.65 mL/s, and at +15% elongation it was 313.33 mL/s.

Conclusion: This study indicates that PTF is dependent on the biomechanical and physiological properties of the vocal folds. For example, it is made clear in this study that PTF increases with elongation. PTF is also dependent upon vocal fold tension and therefore can be considered an accurate aerodynamic diagnostic tool for assessing laryngeal function.

Relevance to the Current Work: PTF is a primary aerodynamic measure used in the current study to evaluate vocal fold health and laryngeal function, and this study makes clear the effectiveness of PTF as a measure

Jiang, J. J., & Tao, C. (2007). The minimum glottal airflow to initiate vocal fold oscillation. *The Journal of the Acoustical Society of America*, 121(5), 2873-2881. doi:10.1121/1.2710961

Purpose of the Study: It has been shown that aerodynamic measures can indicate speech system dysfunction. Subglottal pressure is one parameter that has been extensively researched. The purpose of this article was to propose PTF as a new aerodynamic parameter to be used in the assessment of laryngeal pathologies. PTF is defined as "the minimum glottal airflow required to initiate phonation".

Method: Researchers analyzed the body-cover vocal fold model to research the relationship between PTF and phonatory system properties. Using a variety of mathematical equations, researchers investigated properties such as the influence of glottal configuration, tissue properties, vocal tract loading, and tissue stiffness on PTF.

Results: The results of this study indicate that PTF can be reduced by reducing glottal area, mucosal wave velocity, tissue viscosity, increasing vertical length of the glottal duct, reducing the prephonatory convergent angle, or by increasing the

prephonatory divergent angle. Additionally, it was found that PTF can be useful when studying vocal fold vibration and energy transformation in the speech system.

Conclusion: Researchers proposed PTF as a sensitive and effective indicator when assessing the functional efficiency of the laryngeal system. It is concluded that PTF may be more practical to use in clinical settings than PTP as it is noninvasive and changes when vocal fold biomechanics change.

Relevance to the Current Work: PTF is a key aerodynamic parameter used in the current studies research. This study lays the framework for the use of PTF and the validity of using PTF clinically and in 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.

doi:10.1016/j.pupt.2008.01.005

Purpose of the Study: Using the rabbit to study asthma as a model for the human voice is a relatively new area of research. The purpose of this article was to compare features of asthma in rabbits to humans.

Method: This study used rabbits immunized with asthma neonatally for the purpose of comparison with human asthma symptoms. In total, 8 specific areas were examined in asthmatic rabbits immunized with asthma. These areas included pulmonary function, airway hyperresponsiveness, antigen-induced airway responses, airway inflammation, airway nerves, the effects of drugs on allergen-induced airway responses, airway hyperresponsiveness and airway wall remodeling, and airway smooth muscle in vitro were compared for human asthma and asthmatic rabbits.

Results: Asthmatic rabbits exhibit enhanced airway hyperresponsiveness similar to that of humans. It was found that rabbits had heightened responses to stimuli in the airway including histamine, methacholine, and adenosine 5' monophosphate. It was also found that asthmatic, allergic rabbits were sensitive to and responded to similar drugs as humans with asthma.

Conclusion: The rabbit is a valid and useful model for use in studying asthma treatment and lung physiology. The many similarities between human and rabbit larynges make them ideal for use in studying asthma and lung disease.

Relevance to the Current Work: This study compared asthmatic rabbits to humans with asthma to evaluate the effectiveness of using rabbits as a model to study asthma and other lung diseases. The current work utilizes rabbits to examine the effects of inhaled corticosteroids on aerodynamic measures of the voice, and thus the study provides a framework for understanding the effectiveness of using rabbits as a model for studying asthma. Furthermore, this study provides evidence that using the rabbit as a model to study the effectiveness of different treatments for asthma is appropriate, which is the basis of the current study.

Mau, T., Muhlestein, J., Callahan, S., Weinheimer, K. T., & Chan, R. W. (2011). Phonation threshold pressure and flow in excised human larynges. *The Laryngoscope*, *121*(8), 1743-1751. doi:10.1002/lary.21880

Purpose of the Study: The purpose of this study was to evaluate and report PTP and PTF in excised human larynges so as to determine whether they are sensitive changes to posterior glottal width. A secondary purpose was to verify the presence of hysteresis in human vocal fold oscillation *ex vivo*.

Method: This study included 9 excised human larynges that were harvested within 24 hours postmortem. Prior to phonation trials, excess tissue was dissected and the larynges were mounted on a benchtop setup. 5 phonation trials were administered for each posterior glottal width (0.5, 1, 2, and 3 mm) in each larynx. Subglottal pressure was slowly increased by adjusting the pressure regulator until phonation onset was detected. Effects of glottal area and posterior glottal width were documented.

Results: It was found that PTP and PTF varied more than offset PTP and PTF. PTP and PTF offset fluctuated significantly less than PTP and PTF onset measures. A positive correlation was found between PTF onset and posterior glottal width. Inter-subject variability was reported for both PTP and PTF. PTF was higher in *ex vivo* than *in vivo* models.

Conclusion: PTP and PTF aerodynamic measurements in excised human larynges were similar to those reported in excised canine larynges. Offset PTP and PTF were lower than onset values. Offset values were determined to be more valuable than onset values.

Relevance to the Current Work: This study provides an analysis of the aerodynamic measures PTP and PTF in phonation onset and offset. Likewise, the current study is examining PTP and PTF values in *ex vivo* rabbit larynges.

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: Excised larynges are frequently used for voice research. Canine larynges are the common model and the methodology setup necessary for canine larynges

use is well established. This study examined how researchers can modify benchtop setups so as to be used for rabbit larynges. Researchers aimed to develop a method of obtaining accurate and reproducible data from rabbit larynges, including protocol for dissection, excision, and mounting the larynx protocol. An additional purpose of this study was to develop a way to achieve reliable phonation and collected video and EGG data.

Method: Study subjects included 5 adult New Zealand white rabbit larynges whose larynges were harvested immediately postmortem for the study. Before use, each larynx was thawed, and excess tissues was dissected using microscissors. A custom-built mounting apparatus was designed for this study and each larynx was mounted on a Luer lock in the center of the apparatus. The arytenoids were adducted by rods, and humidified air was passed through the larynx to elicit vocal fold vibration. Five trials were collected for each larynx. A number of variables were measured during the trials including onset and offset pressure and flow, fundamental frequency, jitter, shimmer, and signal-to-noise ratio.

Results: Reliable phonation was achieved in each rabbit larynx. Phonation threshold pressure was recorded at 16.50 ± 1.24 cm H₂O. Phonation threshold flow was reported as 4.61 ± 0.41 L/min. The mucosal wave amplitude for the left vocal fold was 3.32 ± 2.35 pixels and 2.92 ± 2.88 pixels for the right vocal fold. The discrepancy's found in these values was similar to discrepancies found in canine larynges.

Conclusion: Researchers were able to obtain an accurate method to use rabbits as excised laryngeal models. The data found in this study was found to be reproduceable and repeatable in terms of dissection, excision, mounting the larynx, achieving reliable

phonation, and collecting data. Rabbit larynges were determined to be valuable in evaluating vocal fold tissue change.

Relevance to the Current Work: This study provided a reliable methodology for the use of rabbit larynges in *ex vivo* studies. The current work uses excised rabbit larynges to study aerodynamic effects of ICs on the voice and uses similar dissection techniques to those described in this study. Additionally, a similar benchtop model to the one in this study is used for mounting larynges in the current work.

Mills, R. D., Dodd, K., Ablavsky, A., Devine, E., & Jiang, J. J. (2017). Parameters from the complete phonatory range of an excised rabbit larynx. *Journal of Voice*, 31(4), 517.e9-517.e17. doi:10.1016/j.jvoice.2016.12.018

Purpose of the Study: Excised larynges are frequently used to study the vocal folds. Traditionally, canine larynges were used to study vocal fold mechanics and pathologies, however they are not an ideal model for studying vocal fold histology. Because of the similarities in histological makeup between human and rabbit vocal folds, it is proposed that the rabbit will be a more accurate model for the study of histological changes. The purpose of this study was to make adjustments to a canine excise booth to allow collection of a complete phonatory range. PTP and phonation instability pressure (PIP) were measured and aerodynamic, acoustic, and mucosal measurements were taken across the entire phonatory range using rabbit larynges.

Method: This study included 7 white New Zealand rabbits that were sacrificed and whose larynges were harvested and stored for use in this study. After dissection, each larynx was mounted on a modified benchtop setup as specified by Jiang and Titze (1993). Data was collected over the complete phonation pressure range (PPR), which is the range

from PTP to PIP. Flow was increased manually from PTP at increments of 0.25 L/min until PIP was achieved. PIP was estimated using acoustic waveforms and a real-time spectrogram.

Results: In total, 15 parameters were taken during the study and 12/15 were found to be significant. These include PFR, PTP, PPR, F0 at PTP, F0 at PIP, F0 range, SPL at PTP, SPL range, VA at PTP, VA at PIP, and VA range. It was found that PTP measurements increased as subglottal pressure, fundamental frequency, and sound pressure levels and elongation were increased.

Conclusion: This study provided a reliable methodology for the study of phonation parameters of excised rabbit larynges. The results of this study lead the researchers to believe that the rabbits is a reliable model for the study of tissue inflammation.

Relevance to the Current Work: The current work examines aerodynamic measures in excised rabbit larynges that have undergone ICS treatment, which is suspected to inflame the vocal folds. This study reported phonatory range of excised rabbit larynges, which we will also be reporting in the current study. In addition, the current work utilizes a similar benchtop setup that this study reported using.

Plant, R. L. (2005). Aerodynamics of the human larynx during vocal fold vibration. *The Laryngoscope*, 115(12), 2087–2100.

<https://doi.org/10.1097/01.mlg.0000184324.45040.17>

Purpose of the Study: The purpose of this study was to comprehensively examine the influence of aerodynamics (including sound intensity, fundamental frequency, and subglottic air pressure) on laryngeal function. This purpose was separated into 3 portions

1) a multidimensional comparison of the interaction of subglottic pressure, sound intensity, and fundamental frequency during speech; 2) examining changes during the glottic cycle in subglottic pressure; and 3) determining PTP and how it depends on other aerodynamic parameters.

Method: Study participants for this prospective study included 6 healthy individuals with no history of voice disorders. Each participant phonated /i/ with a vented face mask over their face. Each participant completed 5 different speaking tasks. The first task was to speak three tokens with a breathy onset. The second task was to speak three tokens with a low pitch while increasing sound intensity with each token. The third task was to speak three tokens at a mid-pitch level while increasing intensity with each token. The fourth task was to speak three tokens at a high pitch while increasing intensity each token. The last task was to speak three tokens while gliding from a low to high pitch. Airflow, electroglottography (EGG), subglottic pressure, and sound signal were measured during each task. Subglottic pressure was measured directly inserting a 21-gauge needle in the cricothyroid membrane. Electroglottography was recorded by placing contact electrodes on both sides of the neck over the thyroid cartilage.

Results: The relationships between different aerodynamic parameters were reported. It was found that there was a linear relationship between sound intensity and subglottic pressure for most participants. Rapid variations were found in subglottic pressuring during each glottic cycle, relating to the opening of the vocal folds at the beginning of each cycle. It was found that threshold pressure was primarily dependent on sound intensity and fundamental frequency. Additionally, it was found the PTP was higher at onset than at offset.

Conclusion: The researchers in this study conclude that the larynx responded predictably despite variability found in specific parameters. It was found that the behavior of the larynx is governed by the physical properties of air and the anatomy of the vocal tract.

Relevance to the Current Work: Phonation occurs as a result of interactions between aerodynamics and glottal properties, and researchers must have a thorough understanding of those parameters. This study provided instructions for measuring subglottic air pressure, a key aerodynamic parameter in the current work. Additionally, this study provided foundational information regarding the workings of the larynx and what to predictably expect when experimenting on aerodynamic properties.

Plant, R. L., Freed, G. L., & Plant, R. E. (2004). Direct measurement of onset and offset phonation threshold pressure in normal subjects. *The Journal of the Acoustical Society of America*, 116(6), 3640–3646. <https://doi.org/10.1121/1.1812309>

Purpose of the Study: The purpose of this study was to measure onset and offset pressure in normal subjects with a variety of voicing conditions. Fundamental frequency, vocal intensity, laryngeal airway resistance, and the maximum of the first derivative of the electroglottography signal were also measured. The goal of the study was to determine if there is a relationship between PTP and the four variables measured and to examine the differences in onset and offset PTP.

Method: Participants recruited for this study were 5 healthy adults age 26-47. Each individual participated in 5 different voicing segments which were repeated throughout the study. First, each individual said three tokens with a breathy onset. Second, three tokens at a low pitch with increasing lower intensities. Third, three tokens

at a midpitch level while increasing intensity with each token. Fourth, three tokens at a high pitch also increasing intensity with each token. Fifth, three tokens gliding from low to high pitch. To collect data, a Roothenbereg-type mask was placed on the participants face, EGG electrodes were placed on the neck, and a 21-gauge needle was inserted through the cricothyroid membrane to measure subglottic pressure. For analysis purposes, the onset of phonation was defined as the first periodically varying EGG signal and the point of phonation onset or offset was selected at the peak of the first derivative of the first or last EGG waveform.

Results: Univariate and multiple linear regression analysis of the relationship between variables and threshold pressure were performed. It was found that the relationships between intensity and fundamental frequency had the largest number of significant relationships. Significant relationships were found between fundamental frequency and pressure onset or offset. Intensity, airway resistance, and frequency predicted threshold pressure in 3 different combinations. Results indicated that offset pressure threshold is lower than onset pressure threshold.

Conclusion: Directly measuring subglottic pressure indicated that fundamental frequency influences PTP. It was also shown that vocal intensity and laryngeal airway resistance can influence PTP.

Relevance to the Current Work: This study examined the differences between onset and offset phonation threshold pressure and the different variables that influence both. In the current study, onset phonation threshold pressure is used as a primary aerodynamic measure. This study provides insight into the use of PTP and into the variables that impact PTP values.

Regner, M. F., Tao, C., Zhuang, P., & Jiang, J. J. (2008). Onset and offset phonation threshold flow in excised canine larynges. *The Laryngoscope*, *118*(7), 1313-1317.

doi:10.1097/mlg.0b013e31816e2ec7

Purpose of the Study: It has been found that PTF is a reliable aerodynamic parameter and has been compared to PTP in clinical usage. Studies have shown that PTF is more sensitive to posterior glottal width changes and may be more useful in diagnosing pathological conditions with an incomplete glottal gap. The purpose of this study was to further explore the usefulness of PTF. This study investigated onset and offset PTF, specifically examining the hysteresis effect in PTF. Researchers hypothesized that onset PTF is higher than offset PTF, and that the hysteresis effect of PTF is similar to that seen in PTP.

Method: An excised larynx benchtop setup developed by Jiang et al. was used with ten canine larynges. Prior to mounting on the benchtop, each larynx was dissected to reveal the true vocal folds and reduce dehydration during the trials. Pressure was measured for each trial directly below the larynx and acoustic data was collected. For each trial, airflow was slowly increased consistently until phonation was detected, after which airflow was decreased until phonation offset. Onset PTP and PTF and offset PTP and PTF were recorded. This process was repeated three times for each elongation level (5%, 10%, and 15%). Lastly, the mean onset and offset PTF values were calculated for each elongation length.

Results: The mean onset PTF at 0% was reported as 344.14 mL/s, while the mean offset PTF at 0% was 287.34 mL/s. The mean onset PTF at +5% was 222.78 mL/s, and at offset was 182.02 mL/s. The mean onset PTF at +10% 363.11 mL/s, and offset

was 276.57 mL/s. The mean onset PTF at +15% elongation was 505.24 mL/s and offset was 379.75 mL/s. The mean PTF hysteresis ration was 0.795 ± 0.116 . Additionally, mean onset PTP was found to be greater than mean offset PTP.

Conclusion: It was found that onset PTF was higher than offset PTF, evidence of the hysteresis effect in PTF. Additionally, it was concluded that offset PTF is sensitive to changes in posterior glottal width changes, just as onset PTF is. Therefore, offset PTF can be clinically effective as well.

Relevance to the Current Work: The current work utilizes PTP and PTF to determine vocal fold health. This study provided theoretical insight to the clinical usefulness of PTF and into changes PTF is sensitive to. The current work will primarily look at onset PTF, which makes information on hysteresis effect in PTF as well as PTP essential.

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

Purpose of the Study: Previous studies have reported the prevalence of voice disorders in the general population; however, teachers were excluded from the general population reports for comparison purposes. The purpose of this study was to report the prevalence of voice disorders and variables associated with populations who have an increased risk of voice disorders, including teachers in the general population.

Method: A sample of the general population was selected from Iowa and Utah to complete a phone interview for this study. The sample was obtained using a random digit dialing method allowing researchers to access residential telephone numbers. Participants

were asked a number of questions to assess symptoms or signs of voice disorders, consequences of voice disorders, and potential risk factors of voice disorders. Additional questions were asked regarding the presence and frequency of voice disorder symptoms and whether they were occupation-related, medical conditions related to voice disorders, medication use, chemical exposure, degrees of voice-related work disruption, and sociodemographic characteristics.

Results: A number of results associated with voice disorder prevalence were reported. Overall, 29.9% of participants reported a lifetime prevalence of voice disorders with 6.6% reporting current voice disorders. Women and individuals between the age 40-59 reported an increase in chronic voice disorders. Additional risk factors included voice use patterns, voice demands, esophageal reflux, chemical exposures, colds, and sinus infections. It was found that voice disorders adversely impacted occupation in 4.3% of participants and 7.2% of participants reported missing work 1 or more days due to voice problems.

Conclusion: This study provides valuable information regarding the prevalence of voice disorders and the factors that contribute to an increased risk of developing voice disorders. This information can be used in prevention of and education on about voice disorders in high risk populations.

Relevance to the Current Work: This study provides data on the prevalence of voice disorders in the general public. The current work looks at voice disorders due to ICs use for asthma treatment. This study provides data on the impact voice disorders have on one's occupation, and what individuals with ICS induced dysphonia also struggle with.

Roy, N., Merrill, R. M., Thibeault, S., Parsa, R. A., Gray, S. D., & Smith, E. M. (2004).

Prevalence of voice disorders in teachers and the general population. *Journal of Speech, Language, and Hearing Research*, 47(2), 281-293. doi:10.1044/1092-4388(2004/023)

Purpose of the Study: The purpose of this study was to determine the prevalence of voice disorders in age-specific groups of the general population, and more specifically in teachers. It also examined demographic variables that may contribute to a higher prevalence of voice disorders.

Method: In total, 2,351 Teachers and nonteachers were surveyed from Utah and Iowa for this study. Each participant completed a 30-minute phone survey answering a voice disorders questionnaire.

Results: It was reported that 43% of the total population reported having voice disorders and 18.6% reported having chronic voice problems, defined as longer than 4 weeks. Additionally, 81.4% reported experiencing acute voice problems, defined as less than 4 weeks. The prevalence of voice disorders increased with age and was most common in people 50-59 years old. Teachers reported voice problems significantly more frequently than nonteachers. Women had a higher prevalence of voice disorders than men.

Conclusion: The results of this study indicate that a large percentage of the population will experience acute voice problems at some point in their life, with teachers being more likely to experience voice disorders than other members of the general population. In addition, teachers are more likely to report voice disorders. One variable surveyed was frequency of voice disorders co-occurring with asthma and it was found

that prevalence of voice disorders was significantly higher in individuals who had asthma.

Relevance to the Current Work: This study provided information on the prevalence of voice disorders in the general public and in teachers who are at high risk. One of the aspects examined was the prevalence of voice disorders cooccurring with asthma. It was reported that there was a significantly higher likelihood that an individual with asthma would experience a voice disorder during their lifetime. The current study is part of a larger research project designed to evaluate the voice disorders that are a result of ICS use as a treatment for asthma, therefore this study will help to further diagnose and prevent voice disorders in the general public and in individuals who have high voice usage jobs.

Sahrawat, R., Robb, M. P., Kirk, R., & Beckert, L. (2014). Effects of inhaled corticosteroids on voice production in healthy adults. *Logopedics Phoniatrics Vocology*, 39(3), 108-116.

Purpose of the Study: The purpose of this study was to examine the effects of ICs on individuals with otherwise healthy voices. It has been noted that often individuals with asthma experience various comorbidities such as a constant cough, allergies, paranasal sinus disease, and gastroesophageal reflux; therefore, the exact effects of ICs on the voice in individuals with asthma has not been studied. This study intends to answer the questions 1) Does short-term repeated use of ICS result in change in acoustic parameters of voice production, and 2) is there a sex difference in the effects of ICS on voice production?

Method: This study included 30 participants (15 male and 15 female) who were recruited from the University of Canterbury with ages ranging from 18-30 (average age

of 24). Each participant had no history of speech, voice, or language disorders and was judged to have normal voice quality by an experienced speech-language pathologist. Furthermore, none of the participants had a history of asthma or respiratory illness. Over a period of 6 days, individuals recorded audio samples and received ICS treatment. On the first day of the study, the individual came in in the morning to audio record the baseline samples. The audio recording consisted of sustained /i/, /a/, and /u/ for 5 seconds three times each, as well as reading the 'Rainbow Passage' to obtain a connected speech sample. After the baseline audio recording, each participant inhaled 500 µg of corticosteroid, fluticasone propionate (FP). One hour after inhalation, each participant made a second audio recording of their voice. In the evening of the first day of the study the participants received a second round of ICS treatment and one hour after treatment participated in an additional audio recording. Days 2-4 of the study the participants received ICS treatment in both the morning and the evening. On day 5 of the study, the participants received ICS treatment in the morning and evening and recorded an audio sample in the evening. On day 6, the participants recorded their Pos-ICS audio recording in the morning. After all audio samples were recorded, acoustic analysis and long-term spectral analysis were performed to examine vocal fundamental frequency, formant frequency and formant bandwidth.

Results: Results indicate a short-term detrimental effect on a variety of acoustic properties of the voice with acoustic parameters returning to normal after ICs treatment had been discontinued. There was no statistically significant relationship between the effects of ICs on fundamental frequency. There was a significant difference in F1 between genders, and F1 was significantly lower for /i/ after Is usage. There was a

significant difference in F2 between genders, but no significant effect due to ICs treatment. Exposure to ICs had no significant effect of F2 for the three vowels sampled. The bandwidth analysis indicated no significant effects on BW from exposure to ICS. The use of ICS did not affect the average vocal fold vibration rate, as indicated by no significant difference in fundamental frequency. FCS and ST analysis of the connected speech sample indicate an increase in FSP during the audio recording on the 5th day.

Conclusion: The findings of this study indicate that researchers must have a tradeoff between using participants who have pre-existing asthma conditions and participants who have healthy, normal voice functioning. This study controlled for comorbid factors that appear with asthma. Effects of ICs use appeared within one week of treatment and was most evident in connected speech samples. The changes found in this study were relatively minor, however they show the immediate impact of ICS on healthy vocal folds.

Relevance to the Current Work: The effects of ICs is the key component of the current work. This study examined the effects of ICs on healthy human participants just as the current study will test the effects of ICs treatment on healthy rabbits. This study analyzed different acoustic parameters, whereas the current study is mainly focused on aerodynamic measures; however, it is important to understand all aspects and parameters to understand the effects ICs can have on the voice.

Swanson, E. R., Abdollahian, D., Ohno, T., Ge, P., Zeale, D. L., & Rousseau, B. (2009).

Characterization of raised phonation in an evoked rabbit phonation model. *The*

Laryngoscope, 119(7), 1439–1443. <https://doi.org/10.1002/lary.20532>

Purpose of the Study: The purpose of this study was to determine if an increase in airflow rate would change the glottal configuration in rabbit larynges and contribute to an increase in phonation intensity.

Method: Endoscopic glottal images were collected from 6 New Zealand white breeder rabbits to document glottal configuration. Acoustic data was also collected and analyzed for phonation intensity between modal and raised phonation. Each rabbit was anesthetized for data collection. The neck of the rabbit was shaved and prepped for surgery and the larynx and trachea were exposed via a midline incision. A cuffed endotracheal tube was inserted into the upper portion of the trachea with the cuff inflated to seal off the trachea and ensure flow of air through the glottis. Once the cuff was inflated, electrodes were inserted into the CT muscle to serve as cathodes and anodes for electrical stimulation. Video documentation of glottal positioning and closure was obtained during phonation and acoustic signals were recorded.

Results: The mean phonatory intensity was 54.19 dB SPL during modal phonation and 60.31 during raised phonation. Images during modal phonation show a convergent glottis with slightly separated vocal folds while raised phonation show a convergent glottis with a larger separation of the vocal folds.

Conclusion: This study revealed that during raised phonation there is a greater separation of the vocal folds and a convergent glottis. This change in configuration was found to be consistent with an increase in phonation intensity and the amplitude of sound.

Relevance to the Current Work: Both this study and the current study use rabbit larynges to examine properties of phonation. This study provides baseline information regarding using rabbit larynges to study the voice.

Williamson, I. J., Matusiewicz, S. P., Brown, P. H., Greening, A. P., Crompton, G. K. (1995).

Frequency of voice problems and cough in patients using pressurized aerosol inhaled steroid preparations. *European Respiratory Journal*, 8(4), 590-592.

doi:10.1183/09031936.95.08040590

Purpose of the Study: Local side effects of ICs treatment for asthma are well known and have been studied in various study designs. Due to variety of study designs, a large range in the prevalence of dysphonia and oral candidiasis has been reported. The purpose of this study is to document the prevalence of voice disorders and throat symptoms in individuals using inhaled steroids to treat asthma. Pressurized aerosol, metered dose, and inhaled corticosteroid preparations were compared in the study.

Method: Participants included 269 patients attending the chest clinic at Northern General Hospital in Edinburgh who completed an interview-based questionnaire to gather information related to inhaler usage, dose frequency, and treatment duration. Additional questions were asked concerning the presence of local oropharyngeal adverse effects and dysphonia. When symptoms were reported, frequency of occurrence was rated by most days or every day. Additionally, a control group of 100 patients were randomly selected from a diabetic out-patient clinic to complete the survey.

Results: Results indicate 58% (147) of participants using steroid inhalers reported having voice or throat symptoms while only 13% (12) of the control participants to voice or throat symptoms. Symptoms were more commonly reported by women than men however there were not correlations between symptoms and age, smoking status, type of steroid aerosol use, dose frequency, duration of treatment, or use of other medication. It was found that throat symptoms increased in prevalence in those prescribed higher doses

of inhaled steroids. As reported, 67% (172) of patients using steroid MDI had an inhaler induced cough, throat symptoms, or voice symptoms, however 6% (11) reported these symptoms preceded steroid treatment. Approximately 1/3 of participants reported throat clearing and huskiness of the voice as symptoms of the medication. Overall, approximately 2/3 of participants experienced at least one local side effect of inhaled steroid usage.

Conclusion: Inhaled steroids have been proven to be useful in managing asthma symptoms, however local side effects have been frequently reported. The researchers of this study conclude that there is a high prevalence of local adverse effects in use of pressurized metered dose steroid aerosols for asthma treatment.

Relevance to the Current Work: The current work studies adverse effects of ICS on aerodynamic parameters of the voice. This study provided data on the frequency of throat and voice problems due to asthma treatment, providing justification for completing further research to improve asthma treatment.

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 Otolology, Rhinology, & Laryngology*, 118(2), 154–159.

<https://doi.org/10.1177/000348940911800212>

Purpose of the Study: The purpose of this study was to experiment and investigate the effects of vocal fold surface dehydration on PTF in ex vivo canine larynges. The researchers used a benchtop setup similar to the setup developed by Jiang and Titze (1993). It was predicted that with an increase in surface dehydration, PTF would also increase.

Method: This study involved 11 canine larynges that were extracted after sacrifice for other experimental studies. The subjects were divided such that 8/11 were used as the experimental group which underwent dehydration, 2/11 were used as control larynges, and 1/11 larynx was used as a control and then underwent dehydration. Prior to experimental trials, excess tissue was dissected away to expose the true vocal folds and allow for insertion of micrometers in arytenoid cartilage. Each larynx was then mounted to the benchtop setup, as outlined by Jiang and Titze (1993). For the control trials, humidified air was passed through the pseudolung and the vocal folds were hydrated using 0.9% saline solution between trials. For the experimental group, the humidifier was removed from the pseudolung apparatus and no saline solution was applied to the surface of the vocal folds between trials. During the dehydration trials, each larynx underwent 23 series of 10-second periods of phonation with dry airflow through the vocal folds, then a 3-second rest period. PTP and PTF onset were recorded. The control group received a similar series of 23 trials, however the 10-second period of phonation consisted of humidified air passed through the vocal folds with saline solution applied during the 3-second rest period.

Results: Results showed that PTF significantly increased as dehydration increased. It was shown that there was a statistically significant difference between initial and final PTF in the dehydration group. It was also shown that PTF changed and was dependent on the level of dehydration from trial to trial. The initial PTF in dehydrated larynges ranged from 133.9 mL/s to 661.9 mL/s. The final PTF values ranged from 196.5 mL/s to 1,219.2 mL/s.

Conclusion: The results of this study indicate that an increase in vocal fold surface dehydration leads to a statistically significant PTF increase. This finding suggests that for vocal fold pathologies causing surface dehydration, PTF may be a reliable clinical tool to examine vocal function.

Relevance to the Current Work: This study used PTF as the main aerodynamic measurement to examine vocal fold function and health. Similarly, in the current study, PTF is used to examine the health of both treated and control group vocal folds. This study compared PTF in an experimental trial and in control groups, just as the current study does. Additionally, this study uses saline solution for the control group, similarly, the current study uses saline solution treatment in the control group. Saline solution is also used throughout trials to ensure hydration is maintained in the vocal folds during trials in both studies.

Zhuang, P., Sprecher, A. J., Hoffman, M. R., Zhang, Y., Fourakis, M., Jiang, J. J., & Wei, C. (2009). Phonation threshold flow measurements in normal and pathological phonation. *The Laryngoscope*, *119*(4). doi: 10.1002/lary.21537

Purpose of the Study: The purpose of this study was to examine PTF in human subjects. This study compared PTF in individuals with normal voices, vocal fold nodules, and vocal fold polyps. This study examined the effectiveness of using PTF as an aerodynamic measure to detect vocal fold pathologies.

Method: Participants included 40 individuals with healthy vocal folds, 21 individuals with vocal fold nodules, and 23 individuals with vocal fold polyps. Participants sustained /a/ to measure MFR and PTF. Subjects practiced until they felt comfortable with the procedure. Subjects phonated a sustained /a/ into a cardboard tube

placed rested above the tongue ~1 in. into the mouth (to standardize vocal tract). To obtain MFR recordings, subjects maintained intensity of 72 dB w/ visual feedback for 2-3 seconds. MFR trail repeated 5 times w/ subjects resting 3-5 seconds between recordings. PTF was recorded by initiating phonation at a soft intensity and decrease intensity over 3-5 seconds until no phonation was detected PTF was estimated as the airflow at the point where phonation ceased (50 dB). Each subject repeated the PTF trial 10 times, resting for 5-6 seconds between recordings

Results: Significant differences in PTF between normal voices and individuals with vocal fold polyps was detected. There was no statistical difference in individuals with nodules. Additionally, there was a statistically significant difference in males compared to females.

Conclusion: PTF is a significant aerodynamic measure that can be used to assess vocal fold pathologies like polyps. PTF was found to be sensitive to gender.

Relevance to the Current Work: The current study uses PTF to examine the health of excised vocal folds. This study demonstrates that PTF is an effective measure of different vocal fold pathologies, which validates the use of PTF in the current 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 Otolaryngology, Rhinology, & Laryngology, 122(9), 555–560.

<https://doi.org/10.1177/000348941312200904>

Purpose of the Study: Aerodynamic parameters are useful clinically as they provide objective, quantitative data to reflect vocal fold health. Both PTP and PTF are valuable and are sensitive to various laryngeal changes. The purpose of this study was to examine

Phonation Threshold Power (PTW) as an aerodynamic measure that combines PTP and PTF. Examiners evaluated PTW in normal subjects, and subjects with vocal fold disorders in order to determine its effectiveness as an aerodynamic measurement in distinguishing between disordered and normal voices.

Method: Participants included 100 subjects with normal voice function, 22 individuals with vocal fold cysts, 72 subjects with vocal fold polyps, and 19 subjects with vocal fold mobility disorders participated in this study. PTF was measured using methods established by Zhuang et al. (2013) where the subject sustained /a/ into a cardboard tube. Each subject started phonation at a soft intensity and over 3-5 seconds decreased intensity until phonation ceased. PTF measurements were repeated 3 times for each subject. PTP was measured by placing a nose clip and face mask on the participant with an oral tube placed 2 cm into their mouth. Participants were then asked to say /pi/ 6-7 times per breath gradually increasing their volume until /i/ was clearly produced. PTW was then calculated as a product of PTP and PTF.

Results: It was found that PTP, PTF, and PTW were different between each experimental group. In individuals with vocal fold polyps, all parameters decreased after excision. PTF in normal, mass lesion, and movement disorders were reported as 0.09 ± 0.04 , 0.18 ± 0.08 , and 0.22 ± 0.12 L/s. PTP was reported in normal, mass lesion, and movement disordered subjects as 4.22 ± 1.02 , 7.20 ± 1.84 , and 6.65 ± 2.09 cm H₂O. Lastly, PTW was reported for normal, mass lesion, and movement disorder groups as 0.38 ± 0.20 , 1.28 ± 0.68 , and 1.62 ± 0.77 cm H₂O x L/s.

Conclusion: It was found that PTW is sensitive to changes due to lesions and vocal fold mobility disorders and can be observed after mass lesion excision. It is

concluded that PTW could be a clinically useful parameter for evaluating aerodynamic inputs of the voice.

Relevance to the Current Work: This study relates to the current work as it examines aerodynamic parameters in the voice with different vocal fold pathologies. The current study utilizes PTP and PTF in combination to evaluate vocal fold health in excised rabbit larynges after ICS treatment. This study provides information and research on the value of using both PTP and PTF in aerodynamic research.

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 hardware (AD Instruments)
- LabChart™ data acquisition software (AD Instruments)
- Microphone (Model SM-48, Shure, Niles, IL)
- High-speed camera (KayPentax, Montvale, NJ)
- Medical-grade air tank (2) containing compressed, low-humidity air (30 psi, <1% relative humidity)
- Physiological pressure transducer (Model MLT844, AD Instruments)
- Sphygmomanometer (AD Instruments)
- Syringe (25 cc/ml)
- Pressure calibration block

- Gauze (to 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
 - 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. In 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. In 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 the 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 the Velcro pieces 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 two bumps are visible without needing to scroll
8. Highlight the two bumps 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 trachea 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 by researchers 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 the rabbits 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
 - a. Abduct false vocal folds using forceps
 - b. Resect false vocal folds with anterior to posterior incision, starting at anterior commissure
2. 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 visual-perceptual analysis
 - c. Turn air tank on using hand-dial until steady phonation is perceived. After approximately four 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 close 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#” 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

APPENDIX I

Thesis Timeline

5/19

- Training for lab use, including orientation to instruction manuals and videos in cloud storage, hard drive storage, lab computer and program usage, and pressure and flow calibration.

6/19

- Training in fine dissection of rabbit larynges and benchtop setup. Trained 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

- Prepare for thesis defense by completing first written draft of thesis document
- Schedule oral thesis defense

3/21

- Complete oral thesis defense