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The Reversibility of Effects of Combination Inhaled Corticosteroids on Sustained Phonation Pressure and Flow in Ex Vivo Rabbit Larynges

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A thesis submitted to the faculty of
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Master of Science

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ABSTRACT

The Reversibility of Effects of Combination Inhaled Corticosteroids
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The purpose of this thesis is to investigate the reversibility of the adverse effects of combination inhaled corticosteroids (ICs) on vocal fold health as part of a five-year study. This pilot study tested the hypothesis that the adverse effects of ICs may be induced and then reversed, using a benchtop model and rabbit larynges measuring sustained subglottic pressure and airflow. Eighteen rabbits were assigned randomly to 5 subgroups, including baseline, induction experimental, induction control, reversibility experimental, and reversibility control. Baseline rabbits did not receive any treatment. Both experimental groups, induction and reversibility, were administered Advair until visual-perceptual ratings were noted. Their paired control groups were administered nebulized saline in the same dosage levels. Induction groups were immediately sacrificed, while reversibility groups entered a withdrawal phase until visual-perceptual ratings showed a return to baseline. Larynges were dissected and mounted on a benchtop model for phonation trials. Dependent variables included sustained phonatory pressure (cm/H_2O) and sustained phonatory airflow (L/min). The results of phonation trials indicate that sustained pressure and flow were higher for both induction and reversibility experimental groups when compared to baseline and control groups. The reversibility experimental group had lower sustained pressure and flow than the induction experimental group. These results indicate a reduction of signs after a period of rest. These findings suggest that voice symptoms may be reversible to a degree after combination IC use. These are important preliminary data that support future studies with larger sample sizes to confirm findings.

Keywords: asthma, combination inhaled corticosteroids, subglottic pressure, laryngeal flow, excised larynx, rabbit larynx

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DESCRIPTION OF THESIS STRUCTURE AND CONTENT

This thesis, *The Reversibility of Effects of Combination Inhaled Corticosteroids on Sustained Phonation Pressure and Flow in Ex Vivo Rabbit Larynges*, was funded by the David O. McKay School of Education at Brigham Young University and through the National Institute of Deafness and Other Communication Disorders, National Institutes of Health (1R01DC01629-01A1). Dr. Kristine Tanner, the primary investigator of the long-term research, obtained funding for the five-year project in collaboration with Brigham Young University and the University of Utah.

The work presented in this thesis was submitted for presentation at the 2023 annual convention of the American Speech-Language-Hearing Association in Boston, Massachusetts. It is written in a hybrid format that follows both traditional thesis requirements and journal publication formats.

Two reference lists are included in this thesis. The references cited in the main body of the text are just below the conclusion. The literature review is included in Appendix A. Appendices B, C, D, and E include protocols for materials, computer set-up, pressure calibration, and airflow calibration, respectively. Appendix F contains protocols for rabbit tissue dissection and data collection. Appendix G includes the data acquisition protocol, while Appendix H outlines the protocol for data segmentation and analysis. Appendix I outlines the timeline of the thesis, spanning from January 2022 through May 2023.

Introduction

Voice is an essential component of verbal communication. The voice is produced by the vocal folds, which are located in the larynx above the trachea. When the vocal folds vibrate, they produce sound that is filtered in the pharynx, oral cavity, and nasal cavity. The result is the auditory signal that we perceive as voice. Just like the other oropharyngeal and velopharyngeal structures, the larynx is an articulator. When an articulator—such as the tongue—is not functioning properly, there can be significant adverse effects on speech. Similarly, when the vocal folds are injured, communication is impaired. Vocal fold injury falls under the category of voice disorders. Much research has established that voice disorders can have substantial negative effects on quality of life, job performance, and psychosocial health (Colton et al., 2011; Stemple et al., 2010; Verdolini & Ramig, 2001).

Voice disorders may be characterized by a variety of patient-reported and auditory-perceptual features. Reported symptoms can include vocal effort, fatigue, strain, dryness, and weakness, to name a few. Clinicians also explain the voice qualities they hear using descriptive terms including but not limited to hoarseness, roughness, and breathiness. The severity of the voice may also be quantified using patient-based and clinician judgment scales. However, these qualitative descriptions should be combined with quantitative, objective measures and laryngeal imaging to represent all vocal attributes more fully. This complimentary approach to voice assessment is important to document voice disorders and track voice change with treatment (Deary et al., 2003; Hogikyan & Sethuraman, 1999; Jacobson et al., 1997; Roy et al., 2013).

Two measures of voice function involve quantifying aerodynamic changes in and below the larynx during phonation. These measures include subglottic air pressure and laryngeal airflow, and they are the driving mechanism for vocal fold vibration. Traditionally, air pressure

is quantified in cmH_2O and airflow in L/min; together, these measures correlate with vocal effort. More specifically, voice onset and sustained phonation measures may be used to quantify aberrant voice production and to compare with normative values for typical phonation. These measures are used routinely in the clinical care of voice patients. Additionally, they have a long history of use in research involving health and disordered voice, as well as translational work involving other methodologies such as animal models. Research topics span many disorder diagnoses, including structural, functional, and neurological laryngeal pathologies.

Asthma and Voice Disorders

One area of study involving voice disorders and the adverse effect on communication includes inhaler-related voice problems in asthma. There is evidence to suggest that asthma itself may adversely affect the health of the vocal folds. Dogan et al. (2007) implemented several quantitative and qualitative methods of measuring vocal fold change due to asthma, including stroboscopy, acoustic measurements, aerodynamics, and perceptual evaluations. The results of these various forms of observation show that patients with asthma have overall higher allergy symptoms, posterior laryngitis, turbinate hypertrophy, and chronic pharyngitis, than patients without asthma. This indicates that inflammation of the larynx and pharynx may already be more present in people with asthma. Further research indicates the additional adverse effects asthma treatment, primarily combination inhaled corticosteroids (ICs), have on the health of the vocal folds.

Asthma Inhalers

ICs can have different effects on various tissues in the upper and lower airways. While they are beneficial to lung health, they may be detrimental to vocal fold health. Studies show that ICs, primarily used to treat adverse symptoms of asthma, contribute to increased hoarseness,

throat irritation, sore throat, and cough (Bhalla et al., 2008; Erickson & Sivasankar, 2010; O'Byrne et al., 2019). These adverse effects may lead to increased levels of subglottic pressure and flow necessary to phonate, potentially causing increased phonotrauma over time (Bhalla et al., 2008). Therefore, it is necessary to determine what level of IC use creates this irritation which can lead to phonotrauma.

The specific adverse effects of ICs that have thus far been determined by research will be outlined as they pertain to vocal fold health and voice disorders. O'Byrne et al. (2019) discuss the positive effect of ICs on the lungs and their health. They note that IC usage improves asthma control, including respiratory symptoms and overall physiological function. However, they further acknowledge that there is not sufficient evidence to claim that ICs can entirely prevent poorer lung functioning long-term.

Sahrawat et al. (2014) studied the effect of ICs on the health of the vocal folds by treating 30 healthy adults with ICs for six days. They found that IC use caused negative effects on naturalistic speech in vocal quality and acoustic parameters. However, these negative changes reversed within one day of discontinuation of IC use. This is also supported by a study by Erickson and Sivasankar (2010) on 14 healthy adults. Split into two groups, these adults were exposed to either IC treatment or a sham treatment to determine the effect of ICs on vocal fold viscosity by measuring phonatory threshold pressure (PTP). After just one day of IC use, results show that the decreased viscosity of the vocal folds from IC treatment increased phonatory threshold pressure significantly for 2 hours before returning to baseline, while the sham treatments did not. Further testing is warranted to confirm these findings to determine the overall effect of ICs on aerodynamic measures of phonation and reversibility of effects.

One major barrier to studying these adverse effects of ICs is test subjects. While human studies are appropriate at times, it is not ethical to withhold or remove treatment from persons who need it; therefore, a different method is necessary to study certain aspects of voice disorders related to IC use. The different approach used in the current research is described below.

Laryngeal Specimen

As mentioned earlier, animal models are a common translational research methodology for determining potential effects on human subjects due to similar anatomical or physiological properties. Within voice research, different vocal fold specimens have been utilized to compare to human vocal folds. For example, both canine and porcine vocal folds have been popular choices for studies due to the physiological similarities to human vocal folds (Durkes & Sivasankar, 2017; Mau et al., 2011; Mills et al., 2017). However, there are some challenges associated with larger animals as applied to studies that require longer-term housing. For this reason, rabbits have emerged as a viable option for voice research. Keir and Page (2008) provided an extensive justification for why rabbit vocal folds are a suitable model for human vocal folds through a qualitative study demonstrating the similar histological properties of rabbit vocal folds to human folds. They found that there are significant correlations between the histologies and how these are impacted by asthma treatment. Further, rabbit models were ideal because they can be followed from birth and are appropriate for studying physiological and anatomical characteristics such as airway responsiveness, nerves, inflammation, and pulmonary function. A study by Dahlqvist et al. (2004) showed changes in the viscosity of rabbit vocal folds after use of ICs and compared inhaler types that had the greatest effects on PTP.

Aerodynamic Measures With a Benchtop Model

Laryngeal specimens used for voice research are ideally suited for traditional benchtop model research (Jiang & Titze, 1993; Maytag et al., 2013). In benchtop modeling, larynges are positioned on insulated tubing and phonation induced via subglottic airflow. This type of model facilitates experimental control, allowing researchers to assess several aerodynamic properties of vocal fold function. For example, PTP, phonation threshold flow (PTF), sustained subglottic pressure, and sustained airflow. Many studies have been performed to determine the minimum subglottic pressure necessary to initiate vibration, while research is limited regarding the sustained pressure and flow required for maintaining phonation in benchtop models. One study that used sustained aerodynamic measures was done by Lucero (1995), which examined the minimum lung pressure necessary to sustain vocal fold oscillation. By implementing a body-cover model analysis, they determined that the minimum amount of pressure necessary to sustain vocal fold pressure is less than the pressure necessary to initiate oscillation, due to damping caused by glottal pressure on the vocal folds. Therefore, sustained phonation pressure is likely to be lower than PTP.

Sustained flow is simply the amount of airflow necessary to sustain phonation. DeJonckere and Lebacq (2020) performed a study in which they examined the intraglottal pressure of modal phonation at voice onset. They found that the driving force of oscillation during sustained voicing must involve a more positive intraglottal pressure during the opening phase as compared to the closing phase. Furthermore, intraglottal pressure and airflow interactions differ between onset and sustained phonation. This indicates that measuring sustained pressure and flow can be an important and reliable measurement for examining voice function changes in benchtop models.

Current Problem and Purpose

To summarize, the negative consequences of ICs used to treat asthma need to be studied, to determine the potential reversibility of voice disorders related to such use. This can be effectively accomplished through the use of a rabbit laryngeal specimen with a benchtop model to assess the aerodynamic properties of vocal fold vibration, which reflect histological changes related to the voice disorders resulting from IC use.

According to a structured questionnaire completed by 190 individuals who used ICs to manage mild-moderate asthma, voice symptoms are prevalent and may include hoarseness, sore throat, throat irritation, and longstanding cough (Bhalla et al., 2008). Erickson and Sivasankar (2010) further explored the idea that ICs affect the health of the vocal folds, measured by monitoring PTP. They found that subjects using ICs had increased PTP, which can cause increased vocal effort and potential phonotrauma over time.

Preliminarily, Sahrawat et al. (2014) show that these adverse effects are reversible within a short time frame for non-users of ICs. The purpose of the current work is to further increase knowledge of these adverse effects by measuring the aerodynamic properties of phonation and how they change according to use of ICs, saline, or no use. It is part of a larger study to determine the degree of reversibility possible compared to overall length of time using ICs. This will aid in determining effectiveness of asthma treatments and may open doors to research further options for safe and healthy medication options.

Research Questions

The research questions for this thesis were as follows:

1. What is the effect of ICs on sustained pressure for the following group comparisons:
    a. Induction experimental versus matched control

b. Reversible experimental versus matched control

c. Reversible experimental versus baseline control

2. What is the effect of ICs on sustained flow for the following group comparisons:

a. Induction experimental versus matched control

b. Reversible experimental versus matched control

c. Reversible experimental versus baseline control

Method

Research Design

The purpose of this study was to examine the effects of ICs on the physiological components of rabbit vocal fold phonation. It was completed in the laboratory of Kristine Tanner, Ph.D. Her parent project, a longitudinal study investigating the reversibility of adverse effects of IC use, has been funded by the National Institute on Deafness and other Communication Disorders through grant number 1R01DC019269.

This between-subjects experimental design study was approved by the Risk Management and the Institutional Animal Care and Use Committee (IACUC) boards at Brigham Young University and the University of Utah, protocol #21-03008. All laryngeal specimen used in this study were housed in, treated at, and obtained from The University of Utah. Larynges were then transported to Brigham Young University for purposes of this thesis work.

Larynx Samples

The specimen utilized in this study were larynges harvested from 18 healthy male adult New Zealand white rabbits that were randomly assigned to one of five groups, which assignments examiners were blinded to, including (a) experimental induction, (b) control

induction, (c) experimental reversibility, (d) control reversibility, and (e) baseline control. The distribution of rabbits to each treatment group is displayed in Figure 1 below.

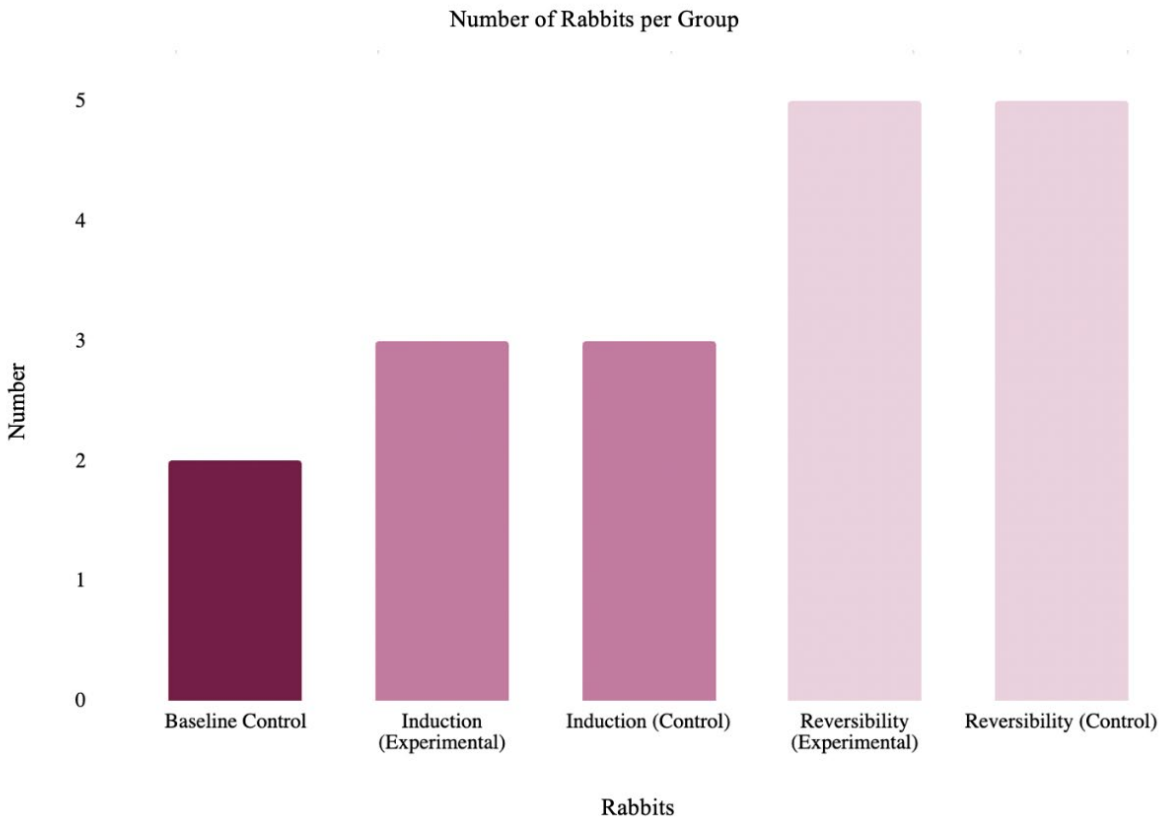
Pre-Study Treatment Administration

Each of these groups received treatment of either saline solution, ICs, or no treatment. The experimental induction group received a single puff of Advair (Fluticasone propionate – 45 mcg, salmeterol – 21 mcg) twice daily, inhaled for 18 breaths at each administration. This treatment continued until blinded raters observed visible perceptual changes with nasoendoscopy. At this point, treatment ceased, and rabbits were humanely sacrificed immediately. The control induction group was matched with the experimental induction group and received administration of nebulized saline each time their counterpart received IC treatment. They were sacrificed at the same time as their experimental induction counterparts to ensure the same amount of treatment.

The experimental reversibility group received the same administration of Advair as the experimental induction group. However, once visible changes were noted in the experimental reversibility group, treatment ceased until laryngeal and arytenoid tissues returned to their visual baseline before treatment began. This progress of tissue reversibility to baseline was assessed through nasoendoscopy every two weeks. Once visual baseline was obtained, the experimental reversibility group was sacrificed. The control reversibility rabbits were matched with the experimental reversibility rabbits. They received a control treatment of nebulized saline each time their counterparts received IC treatment. Control reversibility rabbits were humanely sacrificed once their paired experimental reversibility counterpart reached visual baseline after IC treatment.

Figure 1

Distribution of Rabbits in Treatment Groups



The baseline control group did not receive Advair or saline solution. They were sacrificed immediately following quarantine and larynges were stored for comparison. The humane method of euthanization was performed via ear vein with sodium pentobarbital (390 mg/mL) and phenytoin (50 mg/mL) IV injection (1mL/4.5 kg) to each rabbit at the times specified.

Procurement

All 18 rabbit larynges were grossly dissected immediately following euthanization. The specimens were then submerged in 50 mL test tubes in a phosphate buffered saline solution. These test tubes containing the larynges were then flash frozen in liquid nitrogen for preservation and immediately stored in a -80 C ThermoScientific freezer at the University of Utah. They were

then transported to Brigham Young University in a foam cooler filled with dry ice and stored in room 105 of the Taylor Building Annex until fine dissection and data collection could be performed.

Fine Dissection

Fine dissection was performed on the day each larynx was to be mounted and oscillated. The larynges were placed in a water bath (35-40 C) to thaw for 30 minutes prior to fine dissection. For fine dissection, a removal of the excess tissue superior to the vocal folds and arytenoid cartilage was performed. This is displayed below in Figure 2. The thyroid cartilage was removed superior to the thyroid notch and anterior commissure, as well as the epiglottis and false vocal folds to reveal the true vocal folds. Finally, a suture was placed superior to the anterior commissure for mounting purposes using a hook needle. All larynges were intermittently sprayed during the dissection process to maintain tissue hydration. Ultimately, all larynges were returned to their test tubes in saline solution for a minimum of five minutes before mounting them on the benchtop. All dissection was completed utilizing the following dissection tools: surgical scissors, hemostats, medical scalpels (sizes 10, 11, and 15), tweezers, and misting spray bottles filled with 0.9% $Na^{+}Cl^{-}$ saline solution for hydration purposes.

Data Collection Setup

To accurately assess the selected aspects of phonation in the rabbit larynx, several measures were collected. These include subglottic pressure, airflow, and acoustic measures. Each signal was recorded with LabChart, version 8 (Powerlab, AD Instruments, Colorado Springs, CO), a software that displayed and recorded audio and visual graphs of pressure, flow, and acoustics (displayed in Figure 3). Subglottic pressure was collected with a pressure transducer callibrated in units of mmHg in LabChart. Airflow was measured by a pneumotachograph that

recorded air flow in L/min in LabChart. The microphone signal was recorded in units of mV in LabChart.

Figure 2

Removal of Excess Tissue From Rabbit Larynx With Surgical Scissors

Benchtop Setup and Mounting Larynx

The benchtop model, as utilized by Jiang and Titze (1993) and Maytag et al. (2013), was implemented to phonate larynx subjects in Room 106 of the Taylor Building Annex at Brigham Young University. This is displayed in Figure 3. Rabbit larynges were mounted on an artificial trachea composed of silicone tubing that was tailored to produce sufficient airflow for rabbit

phonation. A pressure transducer (Model MLT844, AD Instruments, Sydney, Australia) was attached to this artificial trachea for measurement of subglottic pressure. Each larynx was secured above the artificial trachea with Teflon tape and a plastic zip tie. The larynx was stabilized at three points – each arytenoid cartilage, and the anterior portion of the thyroid cartilage. Two single-pronged micropositioners were inserted on either side of the arytenoid cartilages of the larynx, while a suture fastened to the thyroid cartilage was tied to an anterior micropositioner to stabilize the vocal folds and provide appropriate tension. A mounted larynx is pictured in Figure 4 and Figure 5 below. Respiratory tubing was attached inferiorly to the artificial larynx to provide air to oscillate the vocal folds. Air was passed through a metered compressed air canister to provide appropriate pressure, then humidified and warmed to 37 C by a TheraHeat heated humidifier (Model RC7000, Smiths Medical, Dublin, OH) before reaching the larynx. The pneumotachograph, as mentioned above for measurement of airflow, was attached in series with this same respiratory tubing. Finally, a microphone was placed between 5-10 cm superior to the vocal folds to record the acoustic signal generated by the air oscillating the vocal folds.

Phonation Trials

Once the benchtop set up was complete, phonation trials began. A digital thermometer and hygrometer were placed next to the benchtop so environmental temperature and humidity could be recorded. This was performed before and after each larynx was mounted and dismounted for phonation trials. Larynges were mounted immediately following fine dissection and sprayed with saline solution periodically throughout phonation trials to ensure proper hydration. Each larynx was phonated to produce 15 trials before dismounting. An average of 3-5

larynges were mounted for oscillation per data collection day, and the same process was repeated for each.

Figure 3

Benchtop Set Up With LabChart Software and Humidifier

Figure 4

Mounted Larynx on Benchtop Model

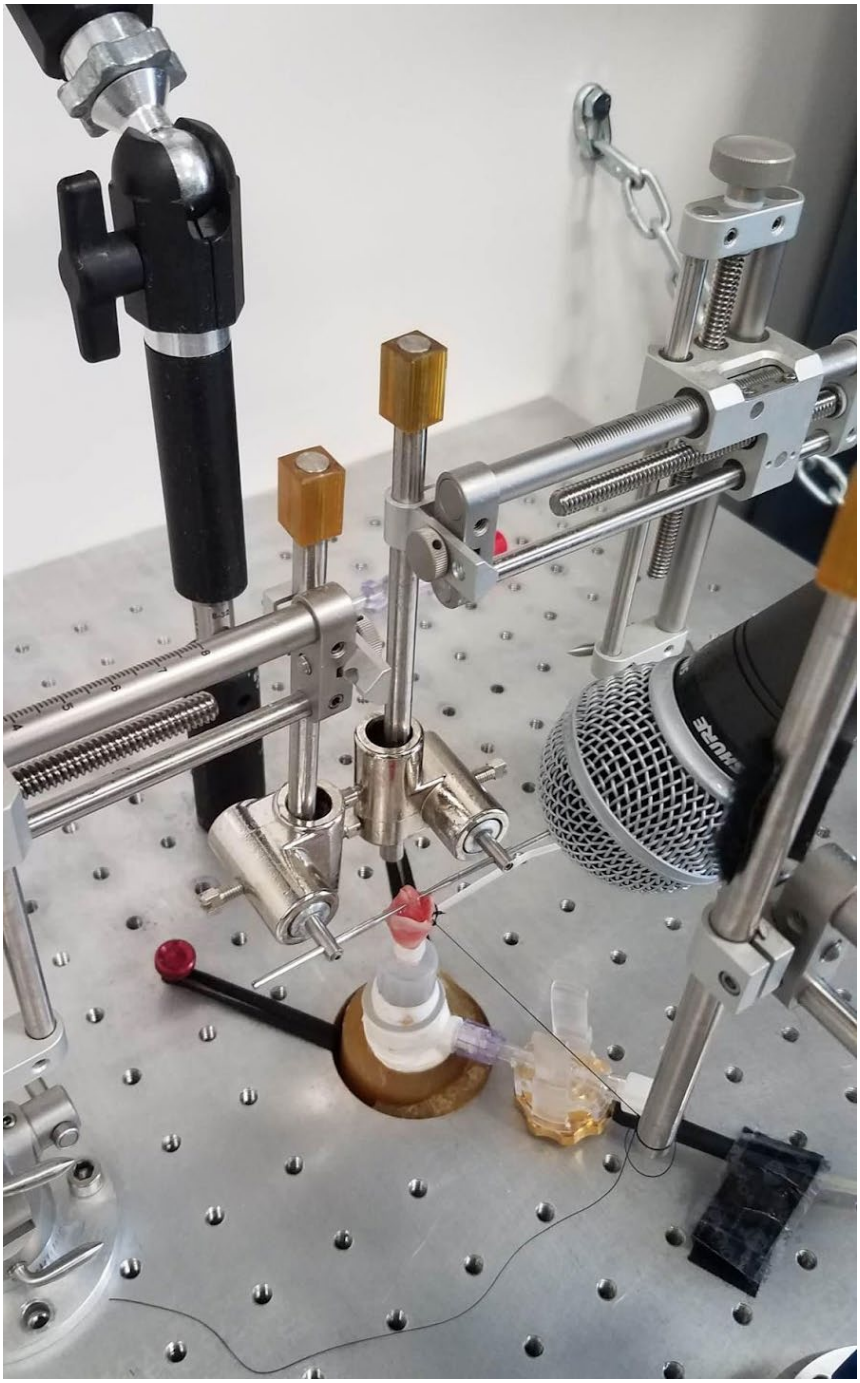
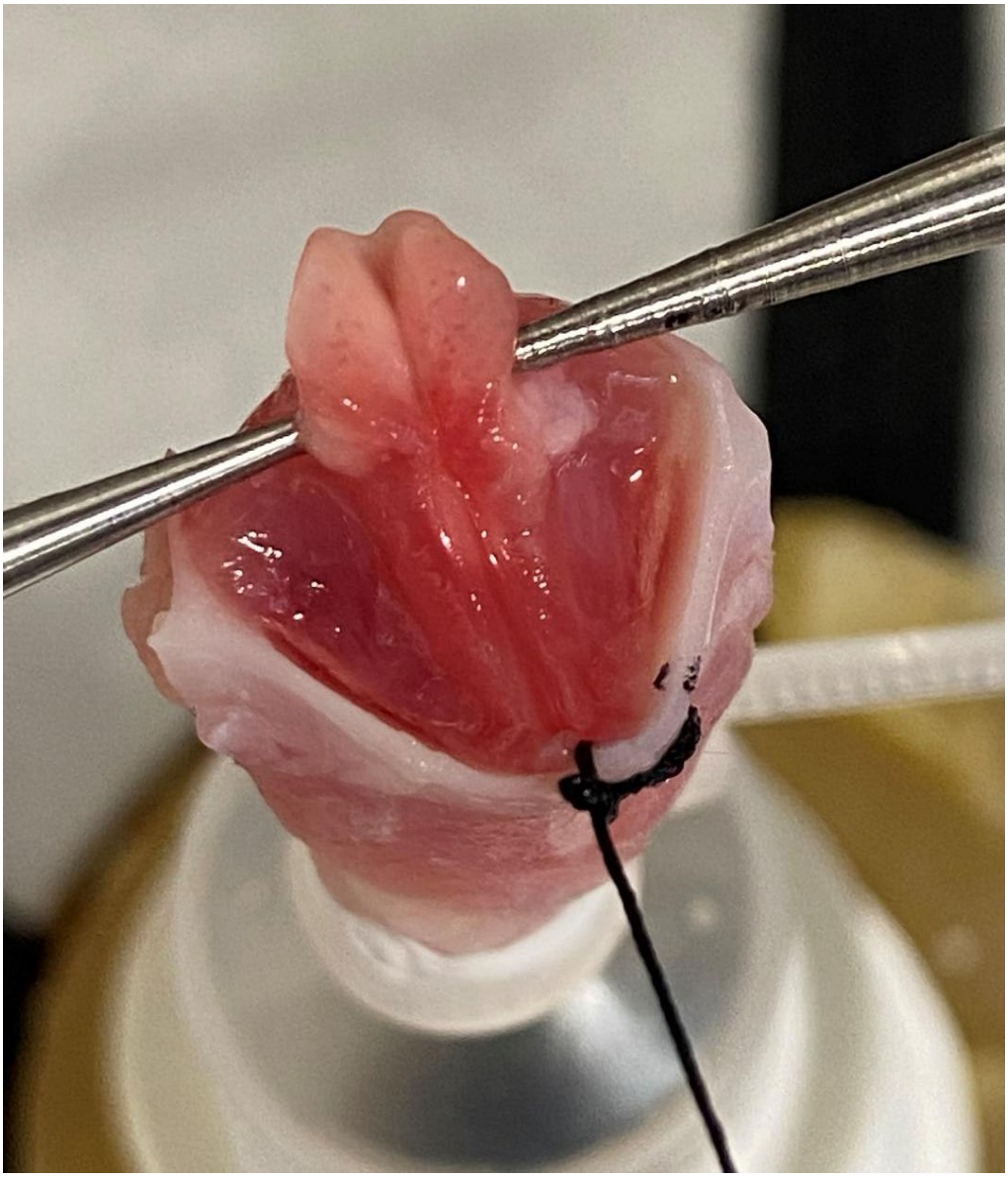


Figure 5

Mounted Larynx With Anterior Thyroid Suture and Micropositioners



Data Analysis

After the 18 larynges had been oscillated over 15 phonation trials, the data were analyzed by two researchers from the lab of Kristine Tanner, Ph.D., including this author and another

thesis student. Each segmented 50% of the data to mark the exact onset and offset of phonation in LabChart (ADInstruments, 2015). An example of LabChart segmentation is displayed in Figure 6 and Figure 7 below, displaying microphone signal, pressure, and flow in descending order. Segmentation was done with visual aids of waveforms and well as listening to microphone signals when necessary.

Figure 6

Segmented Data on LabChart Showing All Phonation Trials of One Rabbit

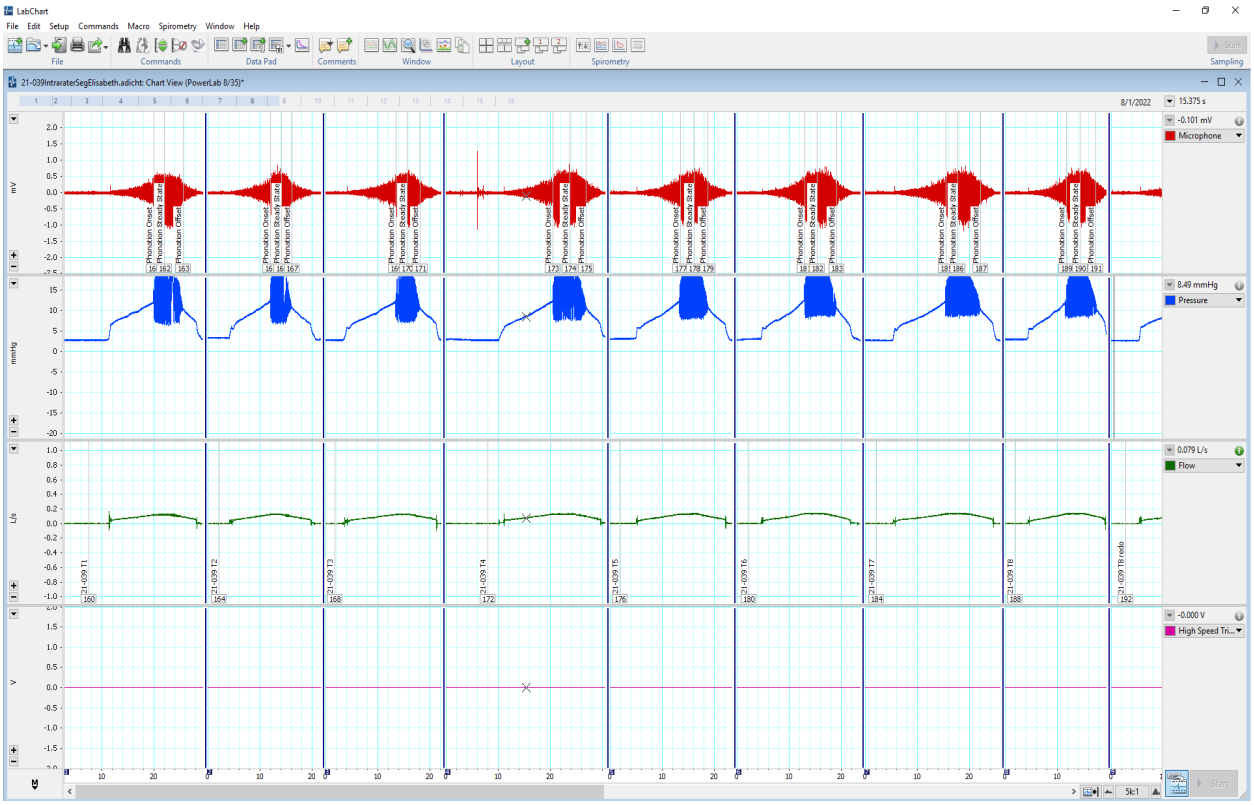
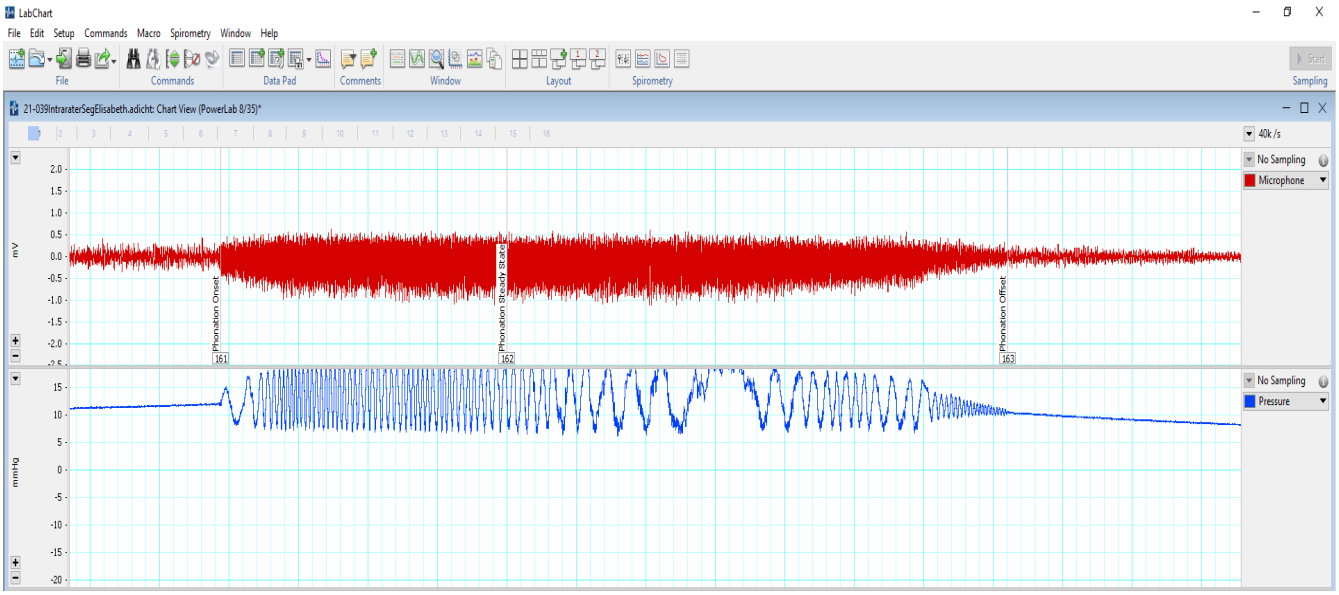


Figure 7

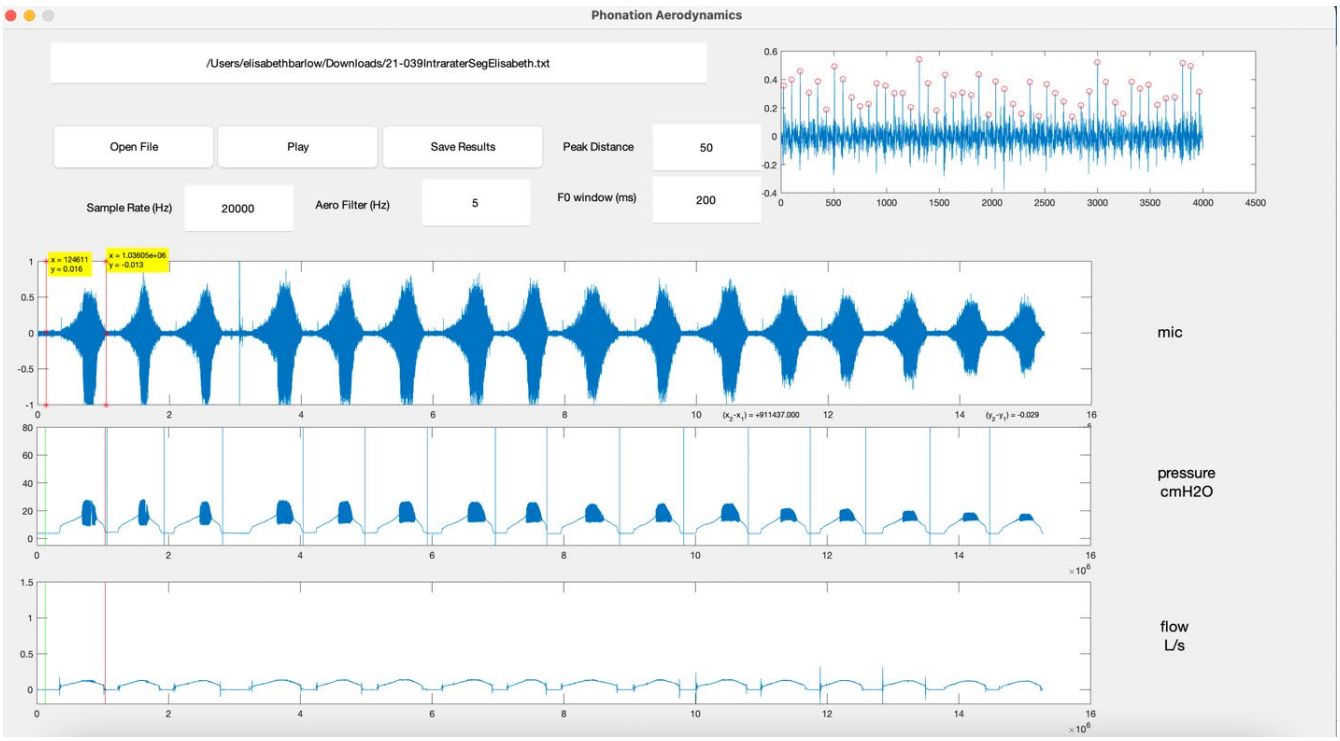
Segmented Data on LabChart Showing One Phonation Trial of One Rabbit



To ensure interrater reliability, the researchers segmented 10% of the other student's data for purposes of interjudge reliability; additionally, each student resegmented 10% of her own data. Pearson correlations of .99 indicated acceptable segmentation interjudge and intrajudge segmentation reliability. After this segmentation of phonatory onset and offset was completed, the data files (including microphone signal, pressure, and flow) were converted to .txt files and uploaded into a custom Matlab (MathWorks, 2010) application, designed by Christopher Dromey, Ph.D., for data analysis. This application was designed to examine the data from subglottic pressure and airflow for both sustained pressure and flow. It is pictured in Figure 8.

Figure 8

Analysis of Phonation Trials With Trial 1 Analyzed



Statistical Analysis

Measures of central tendency, including median, and range values for each rabbit subgroup were computed using Statistical Analysis Software (SAS) analysis (version 9.4, 2013). The data were examined from each subgroup for sustained pressure and sustained flow using descriptive statistics. Findings were interpreted as preliminary indications of differences due to the small sample size.

Results

This section gives a detailed outline of the results of the aerodynamic measures taken from phonation trials with rabbit larynges. Descriptive characteristics of laryngeal specimen and environmental conditions are provided. Additionally, descriptive statistics for sustained pressure

and flow are included. The purpose of obtaining these results is to support the hypothesis of increased sustained phonatory pressure and sustained phonatory flow after combination IC use. As mentioned previously, baseline and any changes in temperature were tracked during data collection. Additionally, percent relative humidity was documented at baseline and at the conclusion of data collection for each laryngeal specimen. These results are reported in Table 1 below.

Table 1

Ambient Temperature and Humidity During Data Collection

Group	Session Date	Initial Humidity	Final Humidity	Initial Temperature (°F)	Final Temperature (°F)
Baseline					
22-019	8/5/2022	50%	50%	72	73
22-024	8/9/2022	40%	41%	72	73
Induction					
21-041	7/29/2022	45%	44%	70	71
22-011	7/25/2022	46%	47%	69	69
22-022	7/25/2022	47%	48%	68	69
Induction Control					
21-042	7/29/2022	50%	50%	70	70
22-012	7/25/2022	48%	47%	67	68
22-023	8/9/2022	40%	40%	75	75
Reversibility					
21-037	8/1/2022	44%	45%	75	76
21-039	8/1/2022	49%	50%	73	74
22-013	7/29/2022	49%	49%	70	70
22-017	8/1/2022	50%	50%	72	75
22-020	8/5/2022	49%	48%	75	74
Reversibility Control					
21-038	7/25/2022	44%	45%	70	71
21-040	8/5/2022	54%	53%	71	72
22-014	7/29/2022	41%	41%	71	72
22-018	8/1/2022	45%	46%	73	74
22-021	8/9/2022	44%	42%	72	74

Specimen and Environment Data

Exact measurement of tracheal, laryngeal, and vocal fold dimensions was done upon completion of the fine dissection of the rabbit larynges with a digital caliper to accurately portray the precise dimensions of each rabbit larynx used in the study in millimeters. These measurements are displayed in Table 2 and Table 3. Description of measurements as shown in the tables is provided in the list below.

- Trachea width: inner diameter between lateral edge of trachea
- Trachea length: bottom edge of trachea to inferior edge of anterior thyroid cartilage
- Thyroid cartilage width: distance between edges of outer thyroid cartilage at widest portion
- Vocal fold length: distance from internal portion of anterior thyroid cartilage at anterior commissure to vocal process of arytenoid cartilages (with vocal folds adducted)
- Vocal fold width: width of one fold at widest point from medial to lateral edge
- Width from vocal fold to thyroid cartilage: distance from lateral edge of one vocal fold to inside edge of thyroid cartilage at its widest point

Table 2

Tracheal and Laryngeal Dimensions by Rabbit Number

Group	Tracheal Length (mm)	Tracheal Width (mm)	Thyroid Cartilage Width (mm)
Baseline			
22-019	14.14	6.41	13.52
22-024	19.65	6.27	13.23
Induction			
21-041	13.89	5.30	12.97
22-011	24.09	6.30	14.46
22-022	19.14	6.76	14.35
Induction Control			
21-042	11.57	6.70	13.51
22-012	22.42	6.20	15.98
22-023	17.09	6.85	15.20
Reversibility			
21-037	17.82	5.84	14.04
21-039	16.74	5.83	14.26
22-013	11.67	4.85	12.39
22-017	8.92	5.62	14.45
22-020	15.28	6.40	13.20
Reversibility			
Control			
21-038	22.94	7.55	16.16
21-040	13.48	5.65	14.54
22-014	25.17	6.64	12.05
22-018	17.34	6.45	14.79
22-021	16.34	6.07	14.05

Table 3

Vocal Fold Dimensions by Rabbit Number

Group	Vocal fold Length (mm)	Vocal fold Width (mm)	Width from vocal fold to thyroid cartilage (mm)
Baseline			
22-019	4.71	1.24	3.82
22-024	5.14	1.68	3.72
Induction			
21-041	5.26	1.33	5.34
22-011	6.48	1.84	4.60
22-022	4.43	2.15	4.64
Induction Control			
21-042	5.31	1.23	5.36
22-012	6.58	2.56	5.93
22-023	4.99	1.45	3.35
Reversibility			
21-037	3.01	1.50	3.60
21-039	3.86	1.79	4.13
22-013	4.01	1.56	3.47
22-017	3.20	1.43	3.70
22-020	4.38	1.72	4.49
Reversibility Control			
21-038	6.04	2.40	5.72
21-040	3.81	1.39	4.18
22-014	4.23	1.64	4.38
22-018	4.08	1.70	3.14
22-021	4.55	1.18	2.83

Aerodynamic Measures

Data for each of the following groups (baseline, induction, induction control, reversibility, reversibility control) were taken over 15 phonation trials per excised rabbit larynx. These measures included PTP, phonatory threshold flow (PTF), sustained phonatory pressure, and sustained phonatory flow. Offset pressure and flow were also noted. Table 4 includes sustained phonatory pressure and sustained phonatory flow data for each specimen. These data were obtained by two researchers who segmented, analyzed, and averaged data to for each phonation trial for each rabbit. The results for each group are shown in Table 4 below.

Table 4

Average Aerodynamic Measures by Rabbit Number (n = 15 trials)

Group	Sustained Phonatory Pressure (cm H_2O)	Sustained Phonatory Flow (L/min)
Baseline		
22-019	4.19	0.03
22-024	10.77	0.08
Induction		
21-041	11.97	0.16
22-011	12.47	0.05
22-022	7.91	0.05
Induction Control		
21-042	6.06	0.06
22-012	4.99	0.03
22-023	14.40	0.09
Reversibility		
21-037	6.56	0.08
21-039	14.80	0.13
22-013	7.01	0.05
22-017	11.97	0.10
22-020	5.09	0.05
Reversibility Control		
21-038	5.25	0.04
21-040	5.27	0.04
22-014	6.90	0.08
22-018	6.37	0.05
22-021	10.80	0.08

Descriptive Statistics

A one-way repeated measures analysis of variance (ANOVA) was undertaken to examine the effect of inhaler use on sustained phonation pressure. The results did not indicate significant differences for sustained pressure across groups [$F(4, 13) = .59, p = 0.67$]. Similarly for sustained flow, results did not indicate significant differences across groups [$F(4, 13) = .61, p = 0.66$]. Box and whisker plots for sustained pressure for all five are groups is shown in Figure 9, and for sustained flow in Figure 10.

Figure 9

Descriptive Statistics of Sustained Phonation Pressure for all Treatment Groups

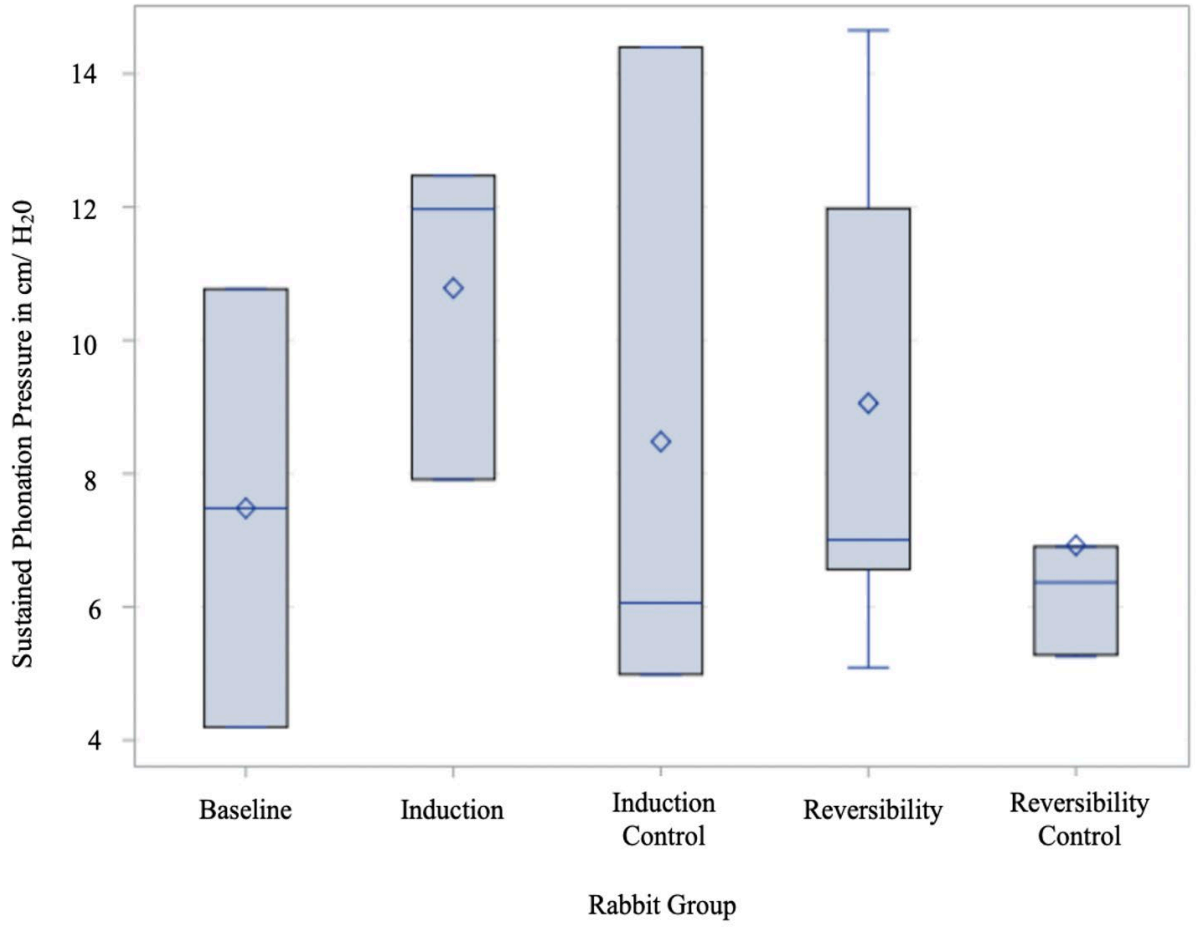
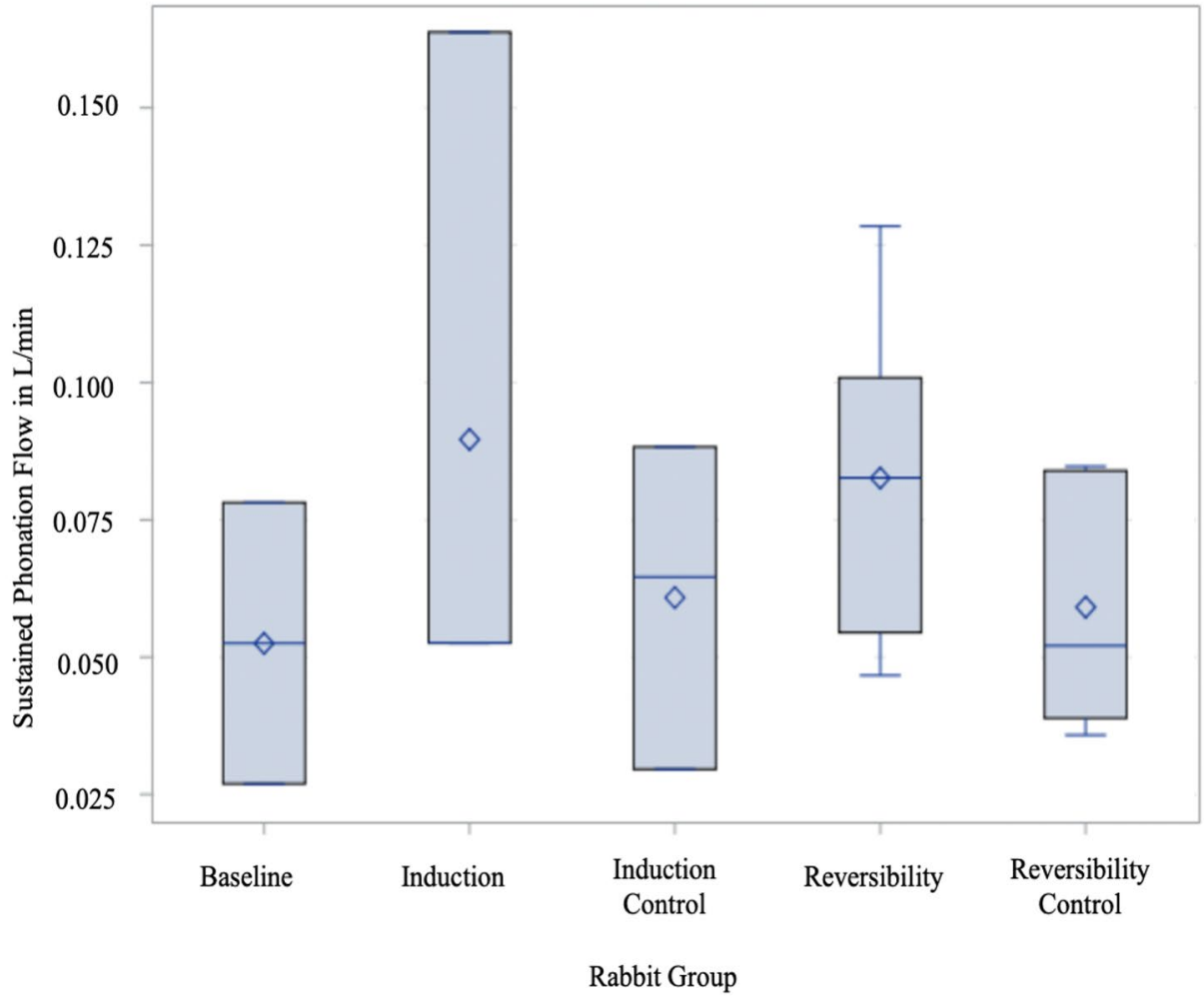


Figure 10

Descriptive Statistics of Sustained Phonation Flow for all Treatment Groups



Discussion

This thesis is part of a longitudinal, five-year study investigating the adverse effects of ICs on the health of the vocal folds and the potential reversibility of those effects. This portion of the study focused primarily on the reversibility of effects that result from using ICs. This was done by assessing subgroups of rabbits exposed to IC until visual-perceptual ratings indicated irritation to and erythema of the vocal folds caused by IC use. This was quantified by measuring

pressure and airflow during sustained phonation in rabbit larynges, which have a similar histology to the human vocal folds. Studies have shown that combination IC use has negatively affected quality of life, both through personal reports in questionnaires (Jacobson et al., 1997) and from objective data (DeJonckere & Lebacq, 2020; Finkelhor et al., 1988; Gallivan et al., 2007). It is increasingly important, therefore, to understand the reversibility of these effects is and how they can be mitigated as asthma prevalence continues to increase (O'Byrne et al., 2019).

Aerodynamic Data Results

Due to the small sample size, results of the study were not statistically significant; however, the results have clinical implications. The mean sustained phonatory pressure and sustained phonatory flow were higher for the induction experimental group than the reversibility experimental group. Additionally, the reversibility and induction experimental groups had higher sustained pressure and flow than the baseline and control groups. These findings support two hypotheses. First, the results indicate that greater pressure and airflow are necessary to create sustained airflow in rabbit larynges that have received IC treatment. Second, rabbits that are given time without treatment are able to recover to a degree, as shown by the lower pressure and flow necessary to phonate for the reversibility experimental group as compared to the induction experimental group. Data collection occurred in a highly controlled environment; the room temperature, dissection, mounting, and setup were all performed by the same researchers. Therefore, this difference between the five groups can be attributed to the independent variable (i.e., IC administration). IC use increased sustained phonatory pressure and sustained phonatory flow in all rabbits that were treated with it, and only decreased once treatment ceased. Again,

although these findings are not statistically significant, descriptively the results warrant discussion within the context of a small pilot study.

Rationale for Measurement of Sustained Pressure and Flow

Traditionally, the dependent variables in quantifying necessary airflow and pressure are phonation threshold pressure (PTP) and phonation threshold flow (PTF; Mau et al., 2011). These are important to measure the difference in effort to initiate phonation before and after IC use. In a study reported by Tanner et al. (2023), exploration of the effects of IC use on PTP and PTF using white New Zealand rabbits showed a significant increase in both measures after combination IC use. Thus, PTP and PTF are important measures for quantifying the adverse effects of IC use.

The vocal fold vibratory process of opening and closing can be attributed to the Bernoulli effect, which states that as air passes through a narrow opening, velocity increases, and pressure decreases. In the glottis, a vacuum is created that pulls the vocal folds together repeatedly. Therefore, an increase in pressure must be related to the histological and muscular portion of this theory. This further explains why pressure is important to measure, as a higher level of swelling and mass to the vocal folds, or lesions, would require a higher level of pressure to create these pulses (DeJonckere & Lebacq, 2021; Švec et al., 2021). Therefore, increased erythema and edema resulting from IC use would require greater pressure and airflow to sustain phonation.

It is equally important to measure sustained phonatory pressure and sustained phonatory flow, which are the dependent variables chosen for this portion of the study. Though they are not frequently measured in ex vivo benchtop studies (Lieberman et al., 1969; Lucero, 1995), they are still necessary. An important study by Pang (2021) investigating sustained pressure and airflow after combination IC use with rabbits showed a significant increase in sustained pressure and airflow, as well as visual-perceptual ratings. This was an essential finding to indicate the need for

examining reversibility effects while measuring sustained pressure and flow. The measurement of these variables is necessary because there is an irregularity in pressure and flow throughout the phonation sample (DeJonckere & Lebacq, 2020), and it is beneficial to measure at various points during phonation to have a comprehensive and accurate understanding of aerodynamic measures.

Reversibility

This was a pilot study exploring the implications of reversibility of IC use on the health of the vocal folds within a longitudinal study. One group of rabbits was given a period of time after IC use to recover from the erythema and edema caused by combination IC use, as indicated by visual-perceptual ratings. The aerodynamic data from this study show that erythema and edema, as well as pressure and flow, decreased for all rabbits in the reversibility group. This finding is indicative of the potential reversibility of adverse voice effects associated with IC use. The current findings complement those of Sahrawat et al. (2014), suggesting that the potential reversibility of the acoustic effects after IC use are likely confirmatory evidence for the preliminary findings in this work; that aerodynamic measures are reversible as well after cession of IC treatment. Though Sahrawat et al. (2014) studied healthy human subjects, the current work extends those findings using a rabbit model. For example, rabbits can phonate, but this vocalization is minimal compared to voice demands in humans. Therefore, this study demonstrated a trend toward adverse vocal fold effects associated with ICs in the absence of regular phonation. As such, it may be that the current results are even more valuable, given the observed changes in the absence of cumulative phonotrauma.

Limitations

As with all research, this study is subject to limitations. Firstly, the small sample size did not provide enough data to quantify potential findings statistically. Also, although extensive training was given, there is always the potential for minor human variation in procedure such as dissection and mounting of larynges. The materials used also may have introduced some limitations. Benchtop models are inherently different from those using an organic air source, though measures were taken to decrease differences as much as possible, including humidification and warming of air, altering mounting size to fit the trachea, and frequent hydration of tissue. Additionally, rabbit larynges are certainly not identical to humans, though they have similar histology and macrostructure in the vocal folds. Finally, it should be noted that only male rabbit larynges were used due to the fat deposits in the upper airway of female rabbits that make it difficult to scope for visual-perceptual ratings.

Despite these limitations, and the lack of statistical significance, the results are clinically important and provide a basis for future studies. Each rabbit was subjected to the same potential procedural limitations, and the results were as hypothesized and consistent between each specimen. This suggests that it was the independent variable that caused the changes in the dependent variable. Furthermore, the smaller sample size showed trends supporting the experimental hypothesis, indicating that the adequately powered parent project is likely to show significant differences.

Recommendations for Future Studies

It is recommended that future studies consider the following. First, as mentioned previously, sample size should be increased to improve the potential overall contribution of the study. Additionally, it was beneficial in this study to have training on fine dissection of larynges

by first practicing on porcine larynges to better understand laryngeal structure which may be helpful to continue. This study also benefitted from consistency in researchers who were performing the experiment and taking measurements. This thesis was a foundational, initial exploration of reversibility and how that affects quantitative measures of phonation.

Conclusion

This study yielded preliminary results which may have potential clinical implications. IC administration in rabbits was used to trigger visual-perceptual and aerodynamic differences in vocal fold structure and function. This was also a pilot study of the potential reversibility of any observed changes. These adverse effects included vocal fold erythema and edema, which are important indicators of negative effects on vocal fold health. Given the prevalence of asthma and heavy reliance on ICs as the treatment of choice, this study offers important preliminary findings to guide asthma management with ICs.

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APPENDIX A

Annotated Bibliography

This appendix includes a review of relevant literature that helped focus the research questions of this study and provide a sound basis for methodology and measurement rationale. It includes literature regarding benchtop models, rationale for use of different animal larynges, aerodynamic measures and properties, and qualitative and quantitative measurements of voice disorders. Each article reviewed was summarized below by categorization into purpose of the work, methodology, results, conclusions, and relevance to the current work.

Bake, M. A. C. (2021). *Variability of the aerodynamic measures of leporine larynges exposed to inhaled corticosteroids* [Master's thesis, Brigham Young University]. BYU ScholarsArchive. https://scholarsarchive.byu.edu/etd/9124/

Purpose of the study: This study was part of a larger, longitudinal study, of which the current thesis is also a part. The purpose of this individual portion was to examine the variability in aerodynamic measures, including PTP, PTF, sustained pressure, and sustained flow, of rabbit larynges that have been treated with combination IC and saline. The two groups were chosen so a comparison between them could also be performed.

Method: Twenty-two white, male New Zealand rabbits were divided into two groups, one that was treated with saline solution and one that was treated with combination ICs. They received 18 puffs twice daily for 8 weeks of their respective treatments and were then humanely euthanized and dissected. They were mounted on a benchtop model for 15 phonation trials each, with LabChart and Matlab for analysis. One was excluded from the study due to laryngeal damage prior to euthanization.

Results: The coefficient of variation was calculated for each aerodynamic measure. It was found that there was no significant difference in coefficient of variation between groups with PTP, but an independent sample showed significant differences between groups, and higher means for combination IC groups. Similar results were found for PTF. Sustained pressure and flow showed similar results, with some tests showing significance with others not, but all with higher averages in the combination IC group.

Conclusions: Significant results with aerodynamic measures indicate that combination IC use does affect aerodynamic measures in the voice. This study also helped display how there is some normalcy of variance between subjects that are within the same groups. This is significant because it can help when outliers exist for future studies.

Relevance to the current work: The current work continued the work of this thesis and this helped guide the research at hand. It is important to understand previous studies to know how to proceed for future studies,

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

Purpose of the study: Because of the vast amount of side effects that can be attributed to inhaled corticosteroid use, it was necessary to determine the prevalence of each side effect that can be attributed to corticosteroid use; particularly, localized side effects.

Method: One hundred-ninety patient volunteers, using inhaled corticosteroids with peak flow and reversibility-proven asthma, from two general practices, were chosen

with the help of asthma nurse specialists. The majority of patients were mild to moderate asthmatics that had been using inhaled corticosteroids for 1-5 years. A structured questionnaire about patient respiratory system was sent out by mail to the patient volunteers. Patients were asked to fill out the questionnaire about their symptoms caused by their inhaled corticosteroids. The symptoms inquired in the questionnaire included: hoarseness, voice weakness and voice loss, sore throat, throat irritation, and longstanding cough. The results were analyzed using Kruskal–Wallis analysis of variance, the Mann–Whitney U test, and Spearman's rank correlation.

Results: Patients reported having increased symptoms with increased time of use of inhaled corticosteroids. The most prevalent symptoms reported as irritants included hoarseness, throat irritation, sore throat, and cough. Voice weakness was also shown to be correlated to inhaled corticosteroid use. Almost all symptoms worsened with time and severity of asthma. Management of such side effects is very unsatisfactory, as there are not many methods to overcome these side effects that are almost certainly attributable to inhaled corticosteroid use. Additionally, use of multiple inhalers and spacers increased prevalence of side effects.

Conclusions: Patients are greatly impacted by the side effects that result from inhaled corticosteroid use. Side effects can be related to dose amounts and devices used. While they are usually only minor, they still alarm users. More trials are necessary to determine why local side effects occur, but it is clear that inhaled corticosteroids do cause adverse effects to the oropharynx and laryngopharynx.

Relevance to the current work: Inhaled corticosteroids have adverse effects on patients including hoarseness, sore throat, and cough. These side effects can alarm users

and increase discomfort. This may be related to the adverse effects that inhaled corticosteroids have on the physiology of the vocal folds, which is what is being studied in the current work.

Dahlqvist, Ake, Garskog, O., Laurent, C., Hertegard, S., Ambrosio, L., & Borzacchiello, A. (2004). Viscoelasticity of rabbit vocal folds after injection augmentation. *The Laryngoscope*, *114*(1), 138–142. https://doi.org/10.1097/00005537-200401000-00025

Purpose of the study: To compare the impact of different substances on the viscoelasticity of vocal folds. This aids in determining the effect on PTP and which substances least increase the viscoelasticity of the vocal folds. This was performed on rabbits six months after their vocal folds were injected with each substance.

Method: Participants included 14 New Zealand white rabbits. Cross-linked collagen, double cross-linked hyaluronan, and polytetrafluorethylene were injected into the lamina propria of the vocal folds of three different groups of rabbits, while the fourth group was injected with Teflon and Ethicon on the left side vocal fold. Six months after the injection, the animals were sacrificed, and the vocal folds were immediately dissected and frozen. A rotational rheometer was used to perform oscillation on the excised larynges. The dynamic viscosity of the vocal folds was then measured.

Results: The substances with the highest dynamic viscosity were Teflon, followed by collagen, DiHA, and hylan B gel at all frequencies. The hylan B and DiHA showed similar viscosity to noninjected control samples, so they did not have as much of an impact. However, the Teflon injected vocal folds had a large difference in viscosity between the control vocal folds.

Conclusions: Phonation threshold pressure (PTP) is directly related to how

viscous the vocal fold is; therefore, as the dynamic viscosity of the vocal fold decreases, so does the PTP. Because hylan B gel and DiHA were similar to typical vocal folds, they are more favorable than Teflon. They are more appropriate for use because they alter the vocal folds less.

Relevance to the current work: Rabbit larynges may be studied to determine the effectiveness of certain medications on the vocal folds. This could indicate that they may be studied to determine the damage caused by certain medications.

DeJonckere, P. H., & Lebacq, J. (2020). In vivo quantification of the intraglottal pressure: Modal phonation and voice onset. *Journal of Voice*, *34*(4), 19–39. https://doi.org/10.1016/j.jvoice.2019.01.001

Purpose of the study: To measure the course of intraglottal pressure during the open phase of vocal fold phonation. Both onset and sustained voice are observed during this study. They observed that the driving force of oscillation during sustained voicing must be a more positive intraglottal pressure than during the closing phase. This study provides an in vivo analysis of the opening, sustained, and closing phases of the vocal folds regarding glottal pressure, glottal area, and airflow.

Method: Participant was one healthy adult male with vocal training whose subglottic pressure values had already been observed as they relate to intensity. The subject's glottal area, transglottal flow, and acoustic signal were recorded. A corpus of voice recordings was taken within the 95-125 Hz and 70-90 dB range with a soft or moderately breathy onset. From these recordings, 28 were chosen that displayed clear glottal area, intraglottal pressure, and first VF contact. A second selection (of 24), overlapping the first 28 chosen, included four consecutive cycles in the rising phase of

VF contact in which intraglottal pressure was calculable for opening and closing phases. The degree of skewing of flow trace was also measured.

Results: They found that modeling phonation gave accurate data for onset and flow of glottal pressure, flow, and area. For the vocal folds to stay in motion, they only need to have a more positive pressure during opening than in closing (it does not need to be negative). They also found that average pressure was greater during closing than opening and was a net positive during sustained phonation.

Conclusions: The models of phonation were supported by in vivo analysis of glottal pressure and flow during onset, sustained, and offset VF phonation. Their results showed that the relationship between the downward trend of intraglottal pressure relates to the tissue displacement of the vocal folds. Additionally, the difference between pressure of onset and offset increases as onset progresses, as does glottal opening.

Relevance to the current work: The current work is observing the effect of corticosteroids on the sustained flow and pressure of the vocal folds. This article effectively describes and confirms the models of sustained phonation, which can serve as a control comparison for the control and experimental groups in the work at hand.

DeJonckere, P., & Lebacq, J. (2021). Intraglottal aerodynamics at vocal fold vibration onset. *Journal of Voice*, *35*(1), 156.e23-156.e32. https://doi.org/10.1016/j.jvoice.2019.08.002

Purpose of the study: The purpose of this study was to investigate what the aerodynamic measures were within the glottis at the time of vocal onset. Specifically, they looked at pressure, flow, viscosity, musculature, and factors such as the Bernoulli effect to determine what is the primary cause of onset.

Method: The experimental variables were glottal area, airflow, and pressure. About 100 recordings were played of the /er/ sound into the Rothenberg mask, to measure flow and pressure. An electroglottogram was also used for measurement of Photoglottography. They calculated results with the Reynold's number, used to predict onset of turbulence.

Results: Results show that the onset of turbulence is co-occurrent with the very first oscillation of phonation. The result, due to damping, mass, Bernoulli effect, is a sinusoidal movement for continuation of phonation.

Conclusions: The most common onset is a soft onset. Rising lung pressure is balanced by decreasing pressure from Bernoulli effect. Mechanical properties determine oscillation after this initiation, which is followed by free oscillation before mechanics begin.

Relevance to the current work: The current work observes the aerodynamic measures of the vocal folds and takes into account factors such as pressures, Bernoulli effect, etc. Additionally, PTP and PTF is important to understand though the current work is more concerned with sustained pressure and flow.

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

Purpose of the study: Because the primary focus of study on the cause of dysphonia concomitant with asthma is on inhaled corticosteroids, this paper focused instead on acoustic analyses of voice, including stroboscopy, acoustics, aerodynamics, and perceptual evaluations.

Method: Participants: forty adult patients with mild to moderate asthma; nonsmokers, no respiratory tract infection, nor other extraneous factors that could cause inflammation or dysphonia. Thirty-four were women and six were men. Procedure: Each patient underwent a lung examination, respiratory function tests, ENT examination, aerodynamic measures (including maximum phonation time, vital capacity, phonation quotient, and statistical analyses), and videolaryngostroboscopic examination. Additionally, perceptual assessments (including self-assessment of dysphonia and clinician assessment) and acoustic examinations (including voice recordings analyzed for jitter and shimmer perturbations) were performed.

Results: Allergy symptoms, posterior laryngitis, turbinate hypertrophy, and chronic pharyngitis were much higher in patients with asthma compared to a control group of the same age and gender demographic. Half of patients had higher voice handicap index than typical speakers. Shimmer was significantly higher in both men and women with asthma, but jitter was only significant for women. The aerodynamic measure that showed a significant difference for patients with asthma was the phonation quotient.

Conclusions: Objective measurements of the effects of asthma on phonation are important, as observation is not as obvious to the standard listener. This study may indicate that respiratory exercises and voice therapy could be useful in improving symptoms of dysphonia in patients with asthma.

Relevance to the current work: Though the current work is going to be primarily focusing on the adverse effects of inhaled corticosteroids on the voice, it is important to understand other factors that can contribute to dysphonia concomitant with asthma to ensure validity in the current work and understand the symptoms present.

Additionally, it is helpful to be aware of other options for treatment of dysphonia that may be available to patients with asthma.

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

Purpose of the study: This study was designed to produce data using rabbit larynges to discover glottal flow, vocal fold dynamics, and acoustic output due to a lack of qualitative data re: ex vivo rabbit larynges. Additionally, this may help increase understanding of swallowing and breathing problems.

Method: Twelve ex vivo New Zealand White female rabbit larynges were harvested right after sacrifice. The larynges were mounted on an artificial trachea and set up with controls for length, tension, etc. They were assessed for glottal area, glottal closure insufficiency, tissue characteristics, opening/closing behavior, and symmetry. Additionally, fundamental frequency, airflow, subglottic pressure, and speed were assessed.

Results: Glottal gap index (GGI) was significantly impacted by airflow. GGI also influenced phonatory parameters. The histology of rabbits was found to be lacking in elastin. PTP was lower than expected, but flow rate and subglottic pressure were higher than previously thought. This study also confirms that increasing subglottic pressure increases flow, fundamental frequency, and SPL.

Conclusions: Glottal gap index significantly impacted glottal, acoustic, and aerodynamic parameters. Additionally, studying rabbits appears to be more effective than studying other mammals.

Relevance to the current work: Understanding the relationship between human and rabbit larynges is essential to the current work, as the experiment will be comparing the effect of corticosteroids on rabbit larynges to what we might expect in humans.

Durkes, A., & Sivasankar, M. P. (2017). A method to administer agents to the larynx in an awake large animal. *Journal of Speech, Language, and Hearing Research*, *60*(11), 3171–3176. https://doi.org/10.1044/2017_JSLHR-S-17-0040

Purpose of the study: This study was performed to determine whether animals could be restrained without sedation to receive inhaled saline.

Method: Participants: Six female adult Sinclair pigs. Procedure: Pigs were trained with positive reinforcement to willingly enter a sling that was then lifted of the ground. They were then exposed to a saline solution 3 times a day, 5 days a week for 4 weeks.

Results: All participants willingly participated in receiving the saline solution without anesthesia. They did not show any adverse effects of being restrained and were behaviorally and physically healthy at the time of sacrifice (as shown by an examination of tissues in the upper airway).

Conclusions: Pigs are trainable and willingly received the saline solution. They were chosen because of their similarities to the human vocal folds, but results may have been skewed by the length of the nasal passage of the pigs. Training the animals is an effective alternative to anesthesia.

Relevance to the current work: Saline solution will be used on the control group of rabbits used in the study at hand. In this study, pigs received a saline solution without any adverse effects on the gross or fine histology of the vocal tract. Additionally, a humane method of administering saline without anesthesia was possible with pig subjects.

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

Purpose of the study: To determine whether treatments containing corticosteroids and LABAs negatively affect phonation through increasing the viscosity of the vocal folds, with the hypothesis being that presentation of these substances would increase PTP and PPE.

Method: Participants were14 adults of nine women and five men. Each met specific healthy voice criteria and had general good health. All participants were taking IC treatment for asthma, which contains corticosteroids and LABAs. Procedure: Participants were presented with their typical asthma medication containing corticosteroids and LABAs one day, and a sham treatment the following day (over 2 consecutive days). The vocal range of each participant was taken at the beginning of the session. Baseline measure of PTP, PPE, and FVC were taken, and then the same measures were performed immediately after treatment at one and two hours after treatment. This was taken during reading tasks, rest breathing tasks, and was measured with pneumotachographs and self-rating scales and analyzed with the SPSS software.

Results: IC treatment increased PTP over time, and the sham treatments did not. Neither the IC nor the sham treatment affected PPE.

Conclusions: IC treatments have significant negative effects on phonatory threshold pressure (PTP). It was an immediate effect of treatment and endured for up to two hours following treatment. Although this is preliminary, ruling out the effects of LABAs and corticosteroids on the vocal folds is not currently advised. The exact cause of the decrease of PTP is yet to be determined.

Relevance to the current work: The current work is to assess the adverse effects of inhaler medication on the vocal folds, which is exactly what is being reviewed in this study. It is known that IC treatment does negatively impact PTP, and our study will further determine effects on specific aspects of phonation.

Finkelhor, B. K., Titze, I. R., & Durham, P. L. (1988). The effect of viscosity changes in the vocal folds on the range of oscillation. *Journal of Voice*, *1*(4), 320–325. https://doi.org/10.1016/s0892-1997(88)80005-5

Purpose of the study: This study was performed to determine whether different liquid mediums affect vocal fold viscosity in phonation, as well as what effect vocal strain and pressure does to change phonation.

Method: Participants: Four excised canine larynges. Procedures: Canine larynges were mounted on a custom scaffolding and phonation trials were performed with humidified air in a similar fashion to pseudolungs. Mounting of larynges required stabilizers. Larynges were bathed in normal saline, distilled water, and hypertonic saline sequently for 15 minutes each before phonation trials began.

Results: Results indicated that increased pressures and reduced vocal strain (length) improved phonation. Additionally, phonation threshold was higher for saline solutions than for water solutions.

Conclusions: Measuring the viscosity of the vocal folds and how that affects phonation threshold pressure is important for understanding clinical implications. It is clear that phonation threshold pressure increases with increased vocal fold viscosity, and that phonation is easier when there is less strain on the vocal folds.

Relevance to the current work: This study helps relate to the study at hand in several ways; first, using animal larynges and a benchtop model is relevant to the methodology of this thesis; additionally, discussing viscosity of the vocal folds and how edema affects phonation pressure is directly related to the question at hand, as edema is a symptom of inhaled corticosteroid use.

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

Purpose of the study: Previous research shows the adverse effects of ICs on the health of the vocal folds, as seen in symptoms such as dysphonia, hoarseness, vocal fold bowing, and other mucosal abnormalities. This study aimed to implement video strobovideolaryngoscopy (SVL) with patients with asthma who have reported symptoms of hoarseness that are being treated with Ics. Specifically, they analyzed the mucosal wave with several different measures.

Method: Participants: 38 patients treated with Ics for bronchial asthma (28 women, 9 men, average age 56.9 years). Symptoms reported were primarily hoarseness

and dysphonia. Procedure: A total of 79 SVL examinations were performed among the 38 patients. These were analyzed by the author and shared with the patients.

Results: The recorded primary symptoms among participants were hoarseness and cough, followed by aphonia, dry throat, diplophonia, and throat clearing. The SVL findings showed that: 58% of patients used Advair Diskus ICS, 29% used Flovent, making 87% in total that used fluticasone ICS. Twenty-four patients had used ICS between 2 wks-6 months, 9 for 6 months-1 year, and the rest (five patients) for 1-5 years. Fifty of the 79 examinations performed showed abnormal open phase closures. Glottic closure was complete in 32/79 examinations, with 35 showing a posterior glottal gap, 10 with an anterior glottal gap, and nine each with hourglass glottis and incomplete closure. The mucosal wave amplitudes were normal for over half of patients, while some showed slightly decreased to moderately decreased amplitude. Mucosal quality was described as pearly white with a sharp free edge for the majority of patients, while some had pink, edemic, or rough qualities.

Conclusions: The results show that hoarseness and dysphonia amounts depend on the type of ICS implemented, dosage, frequency of use, etc. Fluticasone was shown to be the ICS that caused the most hoarseness, SVL abnormalities, and laryngitis. The most frequent findings included mucosal wave asymmetry, abnormal phase closure, and decreased mucosal wave amplitude. After reduction or cessation of fluticasone use, these symptoms decreased or ceased. They recommend that using lower doses of ICS, rinsing after use, and proper oral hygiene should decrease symptoms.

Relevance to the current work: The current work analyzes the effect of ICS on the health of the vocal folds in an effort to eventually understand reversibility. This study

further aids in providing information about previous findings regarding the damage caused by ICS and the side effects that occur, as well as giving some information regarding reversibility.

Ge, P. J., French, L. C., Ohno, T., Zealear, D. L., Rousseau, B. (2009). Model of evoked rabbit phonation. *Annals of Otology, Rhinology, & Laryngology, 118*(1), 51-55. https://doi.org/10.1177/000348940911800109

Purpose of the study: The purpose of this study is to demonstrate a model of evoking rabbit phonation in a manner that was previously done with canines. This is likely more effective than canine vocal folds, as rabbits are quieter and have a more similar histology to humans in their vocal fold make up.

Method: Participants were 10 white New Zealand breeder rabbits. Procedures included anesthetizing rabbits because this is an in vivo study. Rabbits were monitored and placed supine on a table. An excision was made from hyoid to sternum, and electrodes inserted into cricothyroid muscles. Pulses were delivered every 5 seconds and movement was observed. An endotracheal tube provided humidified air for airflow and was also measured.

Results: Various phonation intensity, airflow, and current levels were recorded and observed. Current required to initiate a vocal twitch was found to be about 1 mA. These results are similar to canine findings. Results indicate that rabbits are a useful animal for in vivo study for comparison to human vocal folds.

Conclusions: An effective method for in vivo studies was described and performed. It is feasible with rabbit subjects and was proven to be similar to canine but is more related to the human histology.

Relevance to the current work: The current work undertook phonation trials with ex vivo rabbit larynges; it was helpful to understand results of an in vivo study and understand its validity and implications on research.

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

Purpose of the study: Inhaled corticosteroids are useful in treating asthma, but have been linked to dysphonia. However, due to few studies performed that provide visual evidence of these effects, it was necessary to perform objective voice analysis and laryngoscopy to investigate voice changes. This study assessed voice changes and laryngeal abnormalities found in asthma patients using inhaled corticosteroid (ICS) treatment.

Method: Participants: Thirty patients (15 female, 15 male, mean ages of 20/21 years) with bronchial asthma treated with ICS. Patients using additional steroidal medication were excluded. Procedure: Patients were observed for a minimum of four months as they took their prescribed ICS medication. They were instructed to rinse with water after use to minimize risk of oral candidiasis. Patients were assessed for length of ICS use, severity of asthma, and degree of dysphonia. Each was assessed with videolaryngoscopy, using a rigid scope when possible, and a transnasal flexible fiberoptic scope when necessary. Phoniatricians observed the following seven areas after laryngoscopy: vocal fold edema, erythema, bowing, atrophy, irregular edges, interarytenoid thickening, and supraglottic hyperfunction. Additionally, acoustic analysis was performed by recording patients phonating at their natural pitch range.

Results: Fifty-three percent of patients presented with dysphonia. Out of the seven observed areas, the most prevalent in patients was interarytenoid thickening and vocal fold edema. Jitter was also significantly higher in men and women who used ICS. Local side effects do differ from patient to patient.

Conclusions: Over half of patients presented with dysphonia and had physical side effects, the most prevalent being interarytenoid thickening and vocal fold edema. Correlation between dysphonia and ICS was not significant, but was the most common side effect of ICS. The changes in vocal folds were independent of ICS, and suggests that other factors may be more relevant.

Relevance to the current work: The current work is to observe the adverse effects of ICs on the physiological functioning of the vocal folds, which is directly related to this study. The presence of dysphonia, as well as other physical side effects such as interarytenoid thickening and vocal fold edema is likely to directly affect the PTP necessary to phonate.

Hogikyan, N. D., & Sethuraman, G. (1999). Validation of an instrument to measure voice-related quality of life (V-RQOL). *Journal of Voice, 13*(4), 557–569. https://doi.org/10.1016/S0892-1997(99)80010-1

Purpose of the study: This article explains the impact of voice disorders on persons' quality of life and presents a standardized method of measuring such quality of life in voice patients, essentially attempting to objectively outline the degree of dysphonia presented by voice patients and the adverse effects of such voice disorders. They call this standardized method the Voice-Related Quality of Life index (V-RQOL).

Method: Participants: 109 new patients presenting with a voice complaint to the University of Michigan vocal health program in 1997, and 22 non-voice patients. Procedure: The 12-item V-RQOL and a general voice rating (poor to excellent) were administered to each patient. The V-RQOL was administered twice to determine test-retest reproducibility via mail prior to their voice treatment at the University of Michigan.

Results: The V-RQOL showed good reliability, for all but 3 questions that were dropped. Validity was found to correlate well to the general voice rating provided. Sensitivity to change was higher in the emotional category than the physical category.

Conclusions: The V-RQOL is a valid and reliable method to measure consequences of a voice disorder that is responsive to change.

Relevance to the current work: The current work analyzes the effects of corticosteroids on the health of the vocal folds, which in turn affects the quality of life of those who use them. Additionally, it may be important to further the research by obtaining ratings from users of corticosteroids regarding their vocal quality of life. Therefore, it is helpful to have a basis of information about an effective index that measures such quality of life.

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

Purpose of the study: To examine the prevalence of voice problems resulting from inhaled corticosteroids in Swedish patients, including how frequently they appear, what voice problems are most prevalent, and what the incidence of such voice problems are.

Method: Participants were 280 patients in the asthma and allergy departments of three hospitals in Stockholm, Sweden. Procedures: A 25 question questionnaire was distributed over a six-week period to patients who were generally aware of the purpose of the study (being the influence of medication on the voice) but did not know specifics about its relation to inhaled corticosteroids. A larger number of women than men answered the questionnaire. Most patients were between 30-39 and 50-59 years of age.

Results: The results of the study show a significant positive correlation between voice problems and the use of inhaled corticosteroids that was equally strong in women and men. Hoarseness and throat clearing were the most prevalent voice problems and pain was the least frequent voice problem. Throat clearing and a lump in the throat were correlated problems, as were hoarseness and loss of voice. Voice-demanding professions also played a factor in the presence of patient voice problems.

Conclusions: This study further confirmed the strong positive correlation between application of corticosteroids and voice problems, including the most prevalent issues of hoarseness and throat clearing. Prescription of such medication should be adjusted to each patient's needs and dosages should be clearly defined to decrease presence of voice problems.

Relevance to the current work: Voice problems related to corticosteroid application has a direct correlation to the effect of such medication on the physiological use of the vocal folds.

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

Purpose of the study: The purpose of this paper was to systematically identify and review the reasons why the rabbit is a good model to use for studying asthma and lung diseases in humans, due to the correlation between the rabbit vocal fold physiology to that of humans.

Method: Due to the qualitative nature of this paper, no specific methods were outlined. However, the following categories were analyzed in outlining the benefits of using rabbits in studying asthma and other lung disease: immunization, pulmonary function, airway hyperresponsiveness, antigen-induced airway responses (i.e., early and late airway obstruction, antigen-induced airway hyperresponsiveness), airway inflammation, airway nerves, the effect of drugs on allergen-induced airway responses in the rabbit airways, airway hyperresponsiveness and airway wall remodeling, airway smooth muscle in vitro, and other lung conditions.

Results: The above-mentioned categories in the methods section are all ways in which rabbits can be useful and comparable to humans for research purposes. They are easy to work with, have similar histology to humans, respond in the same way to asthma treatments, as well as other treatments.

Conclusions: Rabbit larynges are very helpful to studying lung physiology and pathophysiology as compared to smaller species. Additionally, their ability to be followed at birth, as well as their similarity to humans, makes them excellent candidates for asthma research.

Relevance to the current work: The current work will be utilizing rabbit larynges to study the effect of asthma inhalers (corticosteroids) on the health of the vocal folds. This study provides rationale for the relation between rabbit and human tissue on

the vocal folds and the subsequent study of their systems to compare to humans.

Lieberman, P., Knudson, R., & Mead, J. (1969). Determination of the rate of change of fundamental frequency with respect to subglottic air pressure during sustained phonation. *The Journal of the Acoustical Society of America*, *45*(6), 1537–1543. https://doi.org/10.1121/1.1911635

Purpose of the study: The purpose of this study is to determine how much the fundamental frequency, produced by the vocal folds, varies during sustained phonation depending on subglottic air pressure.

Method: One male speaker participated in the study. He was required to sustain sounds at different fundamental frequencies across two sessions over three months. His subglottic, transglottic and buccal air pressure were measured and recorded.

Results: The transglottic air pressure was greater at the initiation of each phonatory segment. The minimum transglottic air pressure necessary to sustain phonation in this subject was between 2.2-3.0 cm H2O. For higher fundamental frequencies, the transglottic pressure was higher, between 5-8 cm H2O.

Conclusions: The fundamental frequency of the voice is affected by both the tension in laryngeal musculature and the changes in air pressure of the glottis. This study proves that it is not just the change in musculature tension that alters the fundamental frequency, but that subglottic air pressure also has an effect.

Relevance to the current work: The current work will be analyzing the effect of inhaled corticosteroids on the health of the vocal folds by mounting and phonating excised rabbit larynges. Understanding that subglottic pressure factors into frequency and

is different from transglottal and buccal air pressure is important to know when taking measurements.

Lucero, J. C. (1995). The minimum lung pressure to sustain vocal fold oscillation. *The Journal of the Acoustical Society of America*, *98*(2), 779–784. https://doi.org/10.1121/1.414354

Purpose of the study: Previous studies had been performed to determine what the minimum PTP was; this study analyzes the minimum pressure necessary to sustain phonation utilizing the body-cover model.

Method: The body-cover model created by Titze (1988) was used to calculate the minimum sustained phonation pressure necessary as compared to PTP. Understanding the relationship between lung pressure and oscillation amplitude is important.

Results: Through many calculations, the ratio of phonatory threshold pressure to sustained pressure threshold is 0.04, 0.87, and 0.98.

Conclusions: The minimum amount of pressure necessary to sustain vocal fold pressure is less than the pressure necessary to initiate oscillation, due to damping caused by glottal pressure on the vocal folds.

Relevance to the current work: The current work will be written in conjunction with a study analyzing the phonatory threshold pressure, and it is important to understand how the relationship between PTP and SP should look in a typical vocal fold when comparing vocal folds that may be damaged by other factors.

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. https://doi.org/10.1002/lary.21880

Purpose of the study: Previous measures of phonatory threshold pressure (PTP) and phonatory threshold flow (PTF) have been done on canines, which differ from humans. This study implemented ex vivo human larynges to assess glottic closure and verify presence of hysteresis (energy loss found in canine larynges).

Method: Participants: Nine excised human larynges, all nonsmokers, that were dissected to reveal the thyroid lamina and used in the study within 24 hours post-mortem. Procedure: Bench apparatus setup was used to mount larynges. Larynges were set atop a pipe with airflow connected to assess for airflow measures, as well as instruments to measure the EGG signal and sound level. Five phonation trials were performed for each larynx; subglottic pressure was gradually increased and decreased to determine phonation onset and offset. ANOVA analysis was used to determine significant differences between aerodynamic measures at different glottal widths.

Results: Results with onset and offset of PTP and PTF show evidence of hysteresis, or the idea that onset thresholds are different from offset thresholds. Additionally, PTP and PTF fluctuated more within trials than offset measures. PTF onset and offset had a positive correlation with glottal area, while PTP onset and offset did not have a correlation. The PTP range was slightly higher than expected, but this could be due to the aged population of the larynges. A positive relationship of PTP was not able to be determined with posterior glottal width.

Conclusions: Some of the results of this study were potentially skewed due to the bowing of the vocal folds in the larynges. Thus, it may be more beneficial to implement animal larynges (they specifically mentioned canine larynges) to experiment and understand PTP and PTF. There was great variability between subjects in PTP and PTF

compared to previous animal models, indicating that human larynx subjects may not be as reliable as previously believed.

Relevance to the current work: The current work is also implementing a bench apparatus setup with airflow to test laryngeal PTP, PTF, SP (sustained pressure), and SF (sustained flow). Additionally, this information about the necessity to implement animal models into research, due to difficult access to healthy, younger larynges confirms the use of rabbit larynges.

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. https://doi.org/10.1016/j.jvoice.2016.12.018

Purpose of the study: To assess the similarities between rabbit vocal folds and human vocal folds and determine if they are an appropriate larynx to study in research for comparison to human vocal folds.

Method: Participants: Seven rabbit larynges harvested from white New Zealand rabbits that were dissected and mounted on a modified excised laryngeal apparatus. Procedures: The complete phonation pressure range (PPR), which is the range from PTP to PIP, was used for data collection. Flow was increased little by little from PTP until PIP was reached. Each airflow level was sustained for 5 seconds, allowing for measures of aerodynamics, acoustic recordings, and use of high-speed videos. These were analyzed using a one-way repeated measures analysis of variance.

Results: Increasing airflow and elongation have an effect on phonation parameters. PTF does not differ much across these conditions, but as elongation increased, PTP increased, PIP decreased, VA at both PTP and PIP decreased, and PPR

decreased significantly. Rabbit larynges also appear to be more sensitive than canine larynges to changes in pressure.

Conclusions: This method for studying phonation with rabbit larynges was proved reliable. Increasing airflow and elongation in the rabbit larynges affected all factors, including subglottic pressure, fundamental frequency, SPL, and VA.

Relevance to the current work: The purpose and validity of studying rabbit larynxes is explained. The vocal fold mechanics of excised rabbit larynges and their relation to human vocal fold vibration is studied. Rabbit vocal folds are ideal for inflammation studies due to ease of setup and closer relation to human vocal folds. The measured mechanisms were airflow, subglottic pressure, fundamental frequency, sound pressure level, and vibratory amplitude.

Novaleski, C. K., Kojima, T., Chang, S., Luo, H., Valenzuela, C. V., & Rousseau, B. (2016). Nonstimulated rabbit phonation model: Cricothyroid approximation. *The Laryngoscope*, *126*(7), 1589–1594. https://doi.org/10.1002/lary.25559

Purpose of the study: Many previous studies implementing variations of thyroplasty with both animal and human subjects to assess phonation. This study implemented thyroplasty with in vivo rabbits to understand phonatory output and realistic simulations of voicing.

Method: Participants: Six male white New Zealand breeder rabbits that were anesthetized while being monitored for vitals. Procedure: Each rabbit received a tracheostomy and endotracheal tubes were placed. The thyroid and cricoid cartilages were sutured together to approximate each other to allow for lengthening and tensing of the vocal folds. Phonation was recorded for 10-20 seconds and used for analysis of sound

pressure and frequency values. High speed imaging was implemented to analyze for one glottal cycle. The rabbits were sacrificed and larynges were harvested.

Results: The average sound pressure among the rabbit larynges was 61.39 dB. The average airflow rate was 85.91 mL/s. Subglottic pressure average was 9.00 cm H2O. This information received from the in vivo laryngeal models can serve as a comparison to computer simulations of phonation.

Conclusions: The results were consistent with modal intensity phonation that has been previously recorded. This provides support for experimentation with in vivo larynges, as healthy thyroplasticized rabbit larynges have been proven to respond similarly to those stimulated neuromuscularly.

Relevance to the current work: The current study will be utilizing ex vivo rabbit larynges to assess voicing elements. Understanding different experimentations using the rabbit larynges allows for further understanding and confirmation of the usefulness of implementing subglottic phonation to experiment with rabbit larynges.

O'Byrne, P., Fabbri, L. M., Pavord, I. D., Papi, A., Petruzzelli, S., & Lange, P. (2019). Asthma progression and mortality: The role of inhaled corticosteroids. *European Respiratory Journal*, *54*(1), Article 1900491. https://doi.org/10.1183/13993003.00491-2019

Purpose of the study: Research has shown that asthma mortality rates have recently decreased, partially due to treatment and the introduction of inhaled corticosteroids. This paper is a systematic review of the effect of inhaled corticosteroids (ICS) on asthma progression and mortality.

Method: This paper outlined the following topics: the natural history of asthma lung function in childhood asthma; lung function in adult-onset asthma; mortality in

asthma; impact of asthma diagnosis, assessment, and treatment; and the effect of current medications (including ICS) on asthma progression, mortality, and lung function. Additionally, they focus on the specific role of ICS in different asthma types.

Results: Asthma in children has been shown to decrease forced expiratory volume and forced vital capacity, but did not seem to have a significant impact on decreasing lung function over time as compared to controls. Adults with asthma also have lower forced expiratory volume and forced vital capacity. In contrast to childhood-onset asthma, adult-onset asthma has been shown to have a higher risk of disease progression. Treatment of asthma has changed over time, with a primary focus that is now on controlling and preventing symptoms. This led to increased use of ICS in treatment. Most patients with asthma that are treated with ICS have improved symptoms and lung function.

Conclusions: ICS improve asthma control, including in symptoms and physiological function. There is less evidence to support that it prevents worsening lung function in some patients with asthma. Though ICS may decrease exacerbations, which worsen lung function, there isn't sufficient evidence to claim that ICS can entirely prevent poorer lung functioning as time passes.

Relevance to the current work: This study relates the impact of ICS on asthma symptoms. The current work is analyzing the adverse effects of such inhaled corticosteroids on the health of the vocal folds and understanding why ICS is necessary and relevant to patients with asthma. The information learned from this study can help guide recommendations after the current work has been performed.

Pang, C. (2021). *Effects of inhaled combination corticosteroid drugs on aerodynamic measures of phonation and visual-perceptual measures of vocal fold and arytenoid tissue in excised rabbit larynges* [Master's thesis, Brigham Young University]. BYU ScholarsArchive. https://scholarsarchive.byu.edu/etd/8934

Purpose of the study: To determine how combination IC use affects rabbit larynges by measuring sustained pressure and flow and using visual-perceptual ratings.

Method: Participants: 22 white New Zealand rabbits. Procedures: 11 of the 22 were exposed to combination ICs for 8 weeks, 18 breaths twice daily via nasal mask. The other 11 were exposed to nebulized saline as a control group.

Results: Sustained pressure and sustained airflow necessary to phonate were significantly increased by combination IC use. Visual-perceptual ratings showed significantly higher erythema and edema in vocal folds.

Conclusions: This was a foundational study that showed that sustained pressure and flow also are good measures of changes in vocal folds, as significant differences were shown. Visual-perceptual ratings are also necessary.

Relevance to the current work: Sets the groundwork showing that sustained pressure and flow are relevant measures to changes in the vocal fold health. Also shows that visual-perceptual ratings are effective and significant, which is used in this study.

Prigmore, A. C. (2020). *A comparison of phonation threshold pressure and phonation threshold flow between pig and rabbit benchtop-mounted larynges* [Master's thesis, Brigham Young University]. BYU ScholarsArchive. https://scholarsarchive.byu.edu/etd/8404/

Purpose of the study: The purpose of this study was to compare aerodynamic measures between two different subjects of larynges that have been commonly used in voice

research due to their histological properties, which are porcine and leporine larynges. A review of the various histological properties was performed. This was because little research had been done using rabbit larynges for voice disorders, and it was important to determine their effectiveness before using them for further research.

Method: Participants: Excised larynges from 15 white New Zealand male rabbits and 15 pigs from a local butcher shop. Procedures: Larynges were immediately dissected following euthanization of animals and frozen. They were later finely dissected and mounted on a benchtop model for phonation trials. Each rabbit and pig underwent analysis for PTP and PTF.

Results: There were significant differences between PTP and PTF between the groups (pigs and rabbits). This may be due to size, histology, structure. It is recommended to use rabbit vocal folds in the future when related to human histology.

Conclusions: Rabbits are an effective subject for research when examining aerodynamic measures of PTP and PTF.

Relevance to the current work: The current work implemented rabbit larynges in research for diagnosis of voice disorders, so this thesis was foundational to the work performed. Without this thesis, it would not have been possible to do effective research using the rabbit larynges due to lack of previous knowledge of their effectiveness.

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. https://doi.org/10.3109/14015439.2013.777110

Purpose of the study: Many different qualitative studies show the vocal difficulties of persons with asthma who use inhaled corticosteroids (ICS). However, there has yet to be

a study that examines the isolated effects of ICS, which is necessary as concomitant factors such as cough, sinus issues, etc. can also affect vocal fold health.

Method: Fifteen healthy females and fifteen healthy males with an average age of 24 years were recruited. They had no history of speech, language, voice disorders, asthma, respiratory illness, or inhaler use, nor did they smoke. Participants first gave a voice recording pretest, then inhaled corticosteroids a total of six times over 6 days with voice recordings between almost all administrations of the steroids. Analysis of variance was used to determine vocal difference between the sessions.

Results: No effect was found on fundamental frequency, F1, F2, F3, except for F1 for /i/, which was significantly lower after ICS implementation. FSP, or estimated F0 during a speech sample, changed over ICS intervals, which may be due to the irritation of the vocal folds caused by ICS.

Conclusions: The results of ICS were most prevalent in naturalistic speech as compared to sustained vowel phonation in both males and females. These negative changes reversed within one day of discontinuation of ICS use.

Relevance to the current work: The current work is studying the adverse effects of ICS on the health of the vocal folds, which is directly relevant to this study. The findings show that a study such as the current one is necessary, including one with a control group on healthy vocal folds over more time, to allow for better measures of long-term effects and eventually reversibility.

Sivasankar, M., & Leydon, C. (2010). The role of hydration in vocal fold physiology. *Current Opinion in Otolaryngology & Head and Neck Surgery*, *18*(3), 171–175. https://doi.org/10.1097/MOO.0b013e3283393784

Purpose of the study: To review literature on the importance of vocal fold hydration. Specifically, the article focuses on literatures surrounding how hydration affects the way vocal folds function and their biomechanical properties. Another purpose was to describe how hydration can be useful in prevention and management of voice problems. Hydration should decrease viscosity of the vocal folds, decreasing overall PTP and improving vocal fold health.

Method: An article review was performed to identify the various effects of hydration levels on the vocal folds.

Results: Because vocal fold oscillation is dependent on its viscosity, PTP is proposed to decrease as hydration increases. Additionally, a systemic hydration of the body regulates the biomechanical properties of the vocal folds, and superficial hydration does not appear to make a difference. Superficial hydration may even adversely affect the phonation of vocal folds.

Conclusions: This review of literature shows how both systemic and superficial dehydration are damaging to both animal and human vocal folds. Hydration intervention is tentatively recommended, but more research needs to be performed to confirm findings.

Relevance to the current work: This outlines the necessity to maintain vocal fold health through hydration and touches on its influence on pressure (PTP) in the vocal folds. The current work is analyzing the effect of corticosteroids on the health of the vocal folds, so understanding what can cause physiologic damage is important.

Story, B. H., & Titze, I. R. (1995). Voice simulation with a body-cover model of the vocal folds. *Journal of the Acoustical Society of America, 97*(2), 1249-1260.

https://doi.org/10.1121/1.412234

Purpose of the study: To present a three-part cover/body model of the vocal folds, heavily based on the two-part cover/body model, as well as present how pressure, airflow, and other measurements play into effective vocal fold models/theories.

Method: A systematic review of previous models and the research related to it is performed, as well as a description of the new cover/body model.

Results: The three-part model is an effective method of describing vocal fold motion and histology.

Conclusions: This model may help describe vocal fold movement, specifically when there is decreased stiffness, but may be reduced to two-part model when increased stiffness is present. An increased understanding of physiological elements is presented.

Relevance to the current work: The physiological and histological principles explored in this article directly related to the quantitative measures taken in the study at hand. A deeper understanding of different vocal fold models and theories is helpful in aiding to form hypotheses and questions that relate to aerodynamic measures and how they are affected by external factors.

Švec, J. G., Schutte, H. K., Chen, C. J., & Titze, I. R. (2021). Integrative insights into the myoelastic-aerodynamic theory and acoustics of phonation. Scientific tribute to Donald G. Miller. *Journal of Voice*, *37*(3), 305–313. https://doi.org/10.1016/j.jvoice.2021.01.023

Purpose of the study: The purpose of this study was to describe the myoelastic-aerodynamic theory (MEAD) and the acoustics of phonation by describing previous research and explaining its implications.

Methods: A systematic explanation, separated by principle of MEAD and the

acoustics of speech, was performed. For example, one section was dedicated to measurements, while another was dedicated to mechanisms of oscillation. Within these sections, various studies were explained.

Results: The MEAD is widely accepted for humans and most mammals and birds. However, it is sometimes misunderstood. A greater explanation and understanding of glottal shape, airflow, pressure, and the Bernoulli effect aid in greater understanding of vocal fold function.

Conclusions: A summary of the MEAD is as follows: Subglottic pressure is higher than supraglottal pressure prior to phonation. This allows for air to push up and create a wave in the folds, which causes closure and reopening of the folds.

Relevance to the current work: The current work observes the more quantitative data of aerodynamics of the vocal folds, and this paper describes these efficiently and expansively.

Tanner, K., Fujiki, R. B., Dromey, C., Merrill, R. M., Robb, W., Kendall, K. A., Hopkin, J. A., Channell, R. W., & Sivasankar, M. P. (2016). Laryngeal desiccation challenge and nebulized isotonic saline in healthy male singers and nonsingers: Effects on acoustic, aerodynamic, and self-perceived effort and dryness measures. *Journal of Voice: Official Journal of the Voice Foundation*, *30*(6), 670–676. https://doi.org/10.1016/j.jvoice.2015.08.016

Purpose of the study: Due to lack of research performed regarding vocal fold hydration in males, this study was performed to investigate the issue of laryngeal dryness and the effect of isotonic saline on the voice production of males with and without vocal training.

Method: Participants were 20 males around 21 years of age. Half were trained vocalist university students while the other half were general students who had not had formal voice training previously. Procedure: Participants breathed in medical grade dry air via an oral-nasal mask, then saline treatment with either 3 or 9 mL of isotonic saline (double-blind administration). The PTP, CSID (cepstral and spectral index of dysphonia) while reading or sustaining a vowel, and participant ratings of effort and dryness were taken at baseline, after dehydration, and after the saline treatment.

Results: After desiccation, effort, dryness, and CSID increased significantly in all participants, and decreased after saline treatment. Singers showed lower values for vocal effort, CSID for sustained vowels, and lower dryness and PTP.

Conclusions: The study is the first of its kind but may suggest that male physiologic and acoustic measures do not show a difference in hydration after desiccation and saline treatment, but their self-perceived dryness does change.

Relevance to the current work: The current study examines the effect of inhaled corticosteroids on the vocal folds, which relates to a nebulized saline treatment. It is important to understand the importance of hydration to the vocal folds and the effect this has on their health.

Tanner, K., Robison, H. J., Stevens, M. E., Merrill, R. M., Dromey, C., Barkmeier-Kraemer, J., & Christensen, M. B. (2023). Corticosteroid-laba inhalers increase phonation threshold pressure (PTP) and flow (PTF) in rabbits. *The Laryngoscope*. Advanced Online Publication. https://doi.org/10.1002/lary.30585

Purpose of the study: This study investigated the quantitative effects of inhaled corticosteroids on the health of the vocal folds by measuring PTP and PTF on experimental and control groups with excised rabbit larynges.

Method: Participants: 22 white male New Zealand rabbits. Procedures: Over a period of 8 weeks, an experimental group of 11 rabbits were exposed to 18 breaths of inhaled corticosteroids via a nasal mask twice daily. A control group consisting of 11 rabbits were exposed to 18 breaths of nebulized saline via a nasal mask. Rabbits were then euthanized and larynges excised, and analysis of the PTP and PTF was performed via a benchtop model and analyzed in LabChart and Matlab.

Results: IC exposure significantly increased PTP and PTF.

Conclusions: Exposure to inhaled corticosteroids significantly affects aerodynamic measures, and therefore indicates strong correlation to voice disorders resulting from IC use.

Relevance to the current work: This was the first quantitative study with significant results using PTP and PTF and sets a baseline for reversibility studies with ex vivo rabbit larynges.

APPENDIX B

Materials

Materials for Dissection

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

Materials for data acquisition

- Dell computer
- Dell computer monitor
- PowerLab™ data acquisition hardward (AD Instruments)
- LabChart™ data acquisition softward (AD Instruments
- 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 (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. On the upper right corner, select “start”
        i. Allow LabChart to run for 15 minutes—the program requires sufficient time to warm up
3. Input channel settings
    a. On the upper left corner of LabChart window, select “Setup” tab --> channel settings
    b. Verify that the following settings are applied:
        i. Microphone: sampling rate 40 k/s; range 10 mV; units mV
        ii. Pressure: sampling rate 1 k/s; range 20 mV; units mmHg
        iii. Flow: sampling rate 1 k/s; range 200 mV; units mV
        iv. High speed trigger: sampling rate 1 k/s; range 2 V; units V
    c. Units will be set during specific pressure and flow calibration
    d. Press “OK” in the bottom right corner when settings are accurate
4. Add a comment that settings were double- checked
    a. See a word box on the upper right part of the screen
        i. Type in “settings”
        ii. In the drop-down box to the left of the text box, make sure it is set to “All”
        iii. Press the “Add” button to the right of the text box
            1. You can drag the comment to be closer to the actual moment of change by hovering the mouse over the small black box at the bottom of the screen, directly below the comment. When a white left/right arrow pops up, you can drag the comment
5. To return to the live recording of data, press the button in the bottom right corner entitled “Show latest data”

APPENDIX D

Pressure Calibration, LabChart Protocol

1. Zero the pressure transducer before collecting data
    a. Attach the pressure transducer to the clear piece with the white cap
        i. Pinch clear prongs together and fit circle around the golden piece's rim
    b. Attach the pressure transducer to a small wooden block for stability.
    c. Fasten the transducer wire between Velcro on the benchtop.
    d. Attach the manometer (sphygmomanometer dial piece) via the blue stop cock
        i. The air-tight screw end should attach to the outlet on the stop cock that is 180 degrees from the tube that attaches the manometer
        ii. Remove the white stop cock on the pressure transducer to open it to atmospheric pressure
        iii. The hand within the manometer dial should be within the small rectangle at the bottom when zeroing
    e. Make sure that the pressure transducer is stable
    f. On LabChart, press the start button to collect data for approximately 3 seconds
        i. Press stop
        ii. Highlight most recent section of blue data
            1. Click on "Pressure" drop down box on right side of screen
            2. Select "Bridge Amp"
            3. Set range to 20 mV
            4. Do not set a low pass value
            5. Do not check "Mains filter" box
            6. Press "zero" button
            7. Click "OK"
        iii. Leave a comment noting that pressure has been zeroed
            1. Alt+ p (pre-set comment)
            2. Add the white cap back to the clear piece
2. Take the syringe (25 cc/ml) and pull the plunger out to the end of the syringe
3. Add the syringe to the open outlet on the stop cock
4. Press "start" on LabChart
5. Insert plunger into syringe until the manometer dial reads 40 mmHg—hold this for 5 seconds
    a. Add a comment: Alt+ 4 (pre-set comment indicating 40 mmHg)
6. Press stop
7. At the bottom of the screen, adjust the horizontal scaling to approximately 50:, or until the 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. All animal tissues obtained for this thesis were obtained from the University of Utah
2. All in vivo animal procedures were completed at the University of Utah
3. Researchers at the University of Utah administered twice-daily doses of either inhaled combination corticosteroids (salmeterol fluticasone propionate) or nebulized isotonic saline to in vivo experimental and control rabbits, respectively
4. Researchers at the University of Utah sacrificed animals and flash froze rabbit larynges in phosphate buffered solution
5. Researchers from Brigham Young University transported 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
6. Rabbit larynges procured from the University of Utah are stored in a commercial ThermoScientific freezer at –80° Celsius

Thaw frozen larynges

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

Fine dissection

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

Suture

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

Storage

1. Temporary storage prior to data collection for no more than four hours
    b. Place completed larynges in coded vials of fresh phosphate buffered solution
    c. Store vials in food-grade refrigerator to maintain tissue hydration

APPENDIX G

Data Acquisition Protocol

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

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

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

APPENDIX H

Data Segmentation and Analysis Protocol

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

8. Open excel file to see pressure, flow, and resistance values for phonation onset, steady phonation, and offset phonation
9. Repeat steps with each trial

APPENDIX I

Thesis Timeline

5/22

- 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.

6/22

- Training in fine dissection of rabbit larynges and benchtop setup. Training in collecting acoustic and aerodynamic data.
- Training for data segmentation of raw data on LabChart™ to prepare for upload to MatLab™ program for analysis.

7/22-8/22

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

8/22-10/22

- Segmentation of phonation trials using LabChart software performed by Melanie Blauer and Elisabeth Barlow.

10/22-12/22

- Complete analysis of phonation pressure and flow using Matlab software by Melanie Blauer and Elisabeth Barlow.

11/22-12/22

- Write and edit prospectus.

1/23

- 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.

2/23

- Prospectus meeting and approval by thesis committee, discussing specific thesis questions and importance of current study.
- Edit prospectus documents to align with feedback received from thesis committee.

3/23-4/23

- Complete results, discussion, and conclusion with feedback from thesis chair.

5/23

- Circulate completed thesis to committee and department faculty.
- Complete oral thesis defense.