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

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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

Christina Lynn Pang

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

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Department of Communication Disorders
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ABSTRACT

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

Christina Lynn Pang
Department of Communication Disorders, BYU
Master of Science

The purpose of this thesis is to examine the effects of inhaled corticosteroid drugs (ICs) on the voice due to their frequent use in treating an increasing prevalence of asthma disorders. As part of a larger five-year study, the focus of this thesis is specifically on whether 8 weeks of in vivo exposure to ICs will cause changes in the sustained subglottal pressure, sustained airflow, and visual–perceptual ratings of edema and erythema in excised rabbit larynges. Researchers administered either ICs or a control nebulized isotonic saline solution to 22 rabbits in vivo, sacrificed them, and harvested their larynges for benchtop research. While ensuring proper tissue preservation, researchers then finely dissected the larynges to expose the true vocal folds and run phonation trials. Dependent variables included continuous acoustic signals (Hz), subglottal pressure (cm H2O), and airflow (L/min) data for 15 phonation trials per rabbit larynx. Researchers also collected still image photographs at this time and subsequently normalized them for use in the visual–perceptual portion of this thesis. For visual–perceptual ratings, raters used a 0–3 equal appearing interval scale to rate aspects of edema and erythema on left and right vocal fold and arytenoid tissues. Results indicate that, when compared to control larynges exposed to nebulized isotonic saline, experimental larynges treated with ICs require significantly higher subglottal pressure to maintain phonation, \( p < .05 \). Mean sustained phonation for experimental larynges is 11.24 cm H2O compared to 8.92 cm H2O for that of control larynges. Phonation trials for experimental larynges have significantly higher sustained airflow with a mean of 0.09 L/min compared to 0.07 L/min for that of control larynges, \( p < .05 \). Surprisingly, experimental larynges have higher average fundamental frequencies with less variability (mean: 519 Hz, standard deviation: 66 Hz) than that of control larynges (mean: 446 Hz, standard deviation: 130 Hz). On visual–perceptual ratings, experimental larynges have significantly higher severity ratings on all eight items rated, \( p < .0001 \) – \( p = .0305 \). Based on these results, it is concluded that ICs cause significant damage to rabbit vocal folds, as evidenced by higher sustained pressure, higher airflow, and higher severity ratings for experimental versus control larynges. The dependent variables in this thesis are novel in benchtop model research and demonstrate a unique perspective on this research question. Thus, this thesis informs future phonation, benchtop, and visual–perceptual research.

Keywords: combination inhaled corticosteroids, asthma, excised larynx, rabbit larynx, subglottic pressure, subglottic airflow, visual–perceptual assessment
ACKNOWLEDGMENTS

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DESCRIPTION OF THESIS STRUCTURE AND CONTENT

This thesis, entitled *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*, was funded by the David O. McKay School of Education at Brigham Young University and through the National Institute on Deafness and Other Communication Disorders, National Institutes of Health (1R01DC01629-01A1). Funding was obtained by the principal investigator, Dr. Kristine Tanner, as part of a larger 5-year research project in collaboration with various research labs at Brigham Young University and the University of Utah. The data in this thesis were submitted and accepted for presentation at the annual American Speech-Language-Hearing Association 2020 convention in San Diego, California. This information was not presented due to government restrictions on public gatherings during the COVID19 international pandemic. Information presented in this thesis will be published in a peer-reviewed journal as part of the parent project with the thesis author listed as one of many multidisciplinary authors. This thesis is written in a hybrid format following university and journal publication requirements.

References are listed following the main body of this thesis and within the literature review contained in the Appendix A. Specific protocols for materials, computer set-up, pressure calibration, and airflow calibration are contained in appendices B, C, D, and E respectively. Appendix F contains specific protocols for rabbit tissue dissection and preparation for data collection, while Appendix G contains the protocol for data acquisition. Appendix H contains protocols for raw acoustic and aerodynamic data segmentation and analysis. Samples of instructions and slides for visual–perceptual ratings are included in Appendix I. The timeline of this thesis, spanning from September 2019 through March 2021 is contained in Appendix J.
Introduction

Vocal folds vibrate when they adduct and subglottal pressure is sufficient to initiate and sustain oscillation. Different health conditions cause changes in either the adduction or myoelastic properties of the vocal folds, leading to voice disorders. To prevent and treat voice disorders, it is important to understand the aerodynamic and acoustic characteristics of phonation that are associated with different vocal registers, frequency ranges, and intensity levels. Keeping these characteristics in mind, the effects of specific health conditions, hydration, medications, and treatments on the voice are often studied. In the current thesis, aerodynamic, acoustic, and visual–perceptual data were collected in order to study the general effects of inhaled combination corticosteroid drugs (ICs) on the voice.

As a treatment for individuals with asthma, ICs have been studied extensively. While some inhalers are short-acting, ICs are a combination of a long-acting beta agonist and a steroid that work to reduce inflammation for extended periods of time. This combination elicits an anti-inflammatory effect on asthmatic inflammation in the airway (Uhlík et al., 2007). While ICs have proven to be effective in treating asthma, more recent studies have examined their effects on the voice. The use of ICs has been associated with damage to or inflammation of vocal fold tissue and the development of dysphonia (Erickson & Sivasankar, 2010; Hassen & Hasseba, 2016; Sahrawat et al., 2014). Additional research is needed to learn whether, when compared to a control treatment, ICs will cause damage to vocal fold tissue.

The effects of nebulized isotonic saline on the voice have been studied extensively and research justifies its use as a control treatment for voice research. Durkes and Sivasankar (2017) found that when administered to adult pigs three times a day for 20 days, nebulized isotonic saline had no histologically negative effect on the nasal passageways, the lungs, or the vocal
folds. When studied as a short–term treatment (i.e., 8–10 minutes of inhaled nebulized isotonic saline) after a desiccation challenge, no significant positive or negative effect was noted (Tanner et al., 2007). In these short–term conditions, nebulized isotonic saline had a neutral effect on the voice, thus supporting its use as a control treatment.

When nebulized isotonic saline was used as a long–term treatment for individuals with Primary Sjögren’s Syndrome, a positive effect on the voice was seen (Tanner et al., 2015). Individuals with Primary Sjögren’s Syndrome experience a dehydrated voice. After 2 weeks of twice daily doses of nebulized isotonic saline, improvements in self-ratings of the voice and in acoustic measures of reading and sustained vowel tasks were observed. Ultimately, nebulized isotonic saline has been shown to have a neutral to positive effect on the voice. While a positive effect on the voice might be expected after long–term use, the current thesis administers nebulized isotonic saline in very low doses. Current research indicates that nebulized isotonic saline as administered in low doses will have a neutral effect on the voice.

**Voice Research Models**

Many research models have been replicated and validated for use in better understanding the voice and aspects of voice disorders. Using in vivo laryngeal models in research designs is beneficial as characteristics of phonation can be observed without the possibly confounding effects of laryngeal excision, vocal fold fine dissection, and external manipulation of airflow (Novaleski et al., 2016). Additionally, real-time visual–perceptual, aerodynamic, and acoustic changes can be observed in in vivo subjects in conditions mirroring the real world. In vivo human subjects are ideal for easily translating findings to human populations. Further, research including specific clinical populations best translates to understanding the voice in those clinical populations. Among other things, voice research involving both healthy subjects and clinical
populations has described vocal characteristics in different populations, measured phonation threshold power in relation to phonation threshold pressure (PTP; i.e., the subglottal pressure necessary to initiate phonation) and phonation threshold flow (PTF; i.e., the subglottal airflow necessary to initiate phonation), evaluated the use of laryngoscopic images in the evaluation of laryngeal health, and determined the effects of IC drugs on the voice (Belafsky et al., 2001; Hassen & Hasseba, 2016; Heller et al., 2014; Mau et al., 2011; Titze, 1988; Zhuang et al., 2013). In vivo human populations are ideal for translating research findings to best describe the effects of ICs on the human voice.

Due to difficulties associated with the approval process, recruiting, and carrying out research with living human subjects, other models are often sought in early stages of research. Some limitations to conducting research with human populations include difficulty with participant blinding, possibly limited sample sizes, and ethical considerations in withholding treatment from a control group (Erickson & Sivasankar, 2010). There is also limited control of extraneous variables in human research, such as levels of vocal use, daily systemic hydration, and vocally abusive or damaging behaviors. Some of these limitations can be overcome by using ex vivo human larynges in research models. Treatment trials and controls can be ethically administered to human larynges harvested post-mortem as there are no repercussions to withholding or administering treatment after death by natural causes. Participant blinding is also unnecessary for ex vivo larynges. Not all limitations can be overcome by using ex vivo human larynges, however. Levels of vocal use or vocal abuse and possible health conditions continue to affect the vocal folds and affect human larynges post-mortem. Some limitations of using human larynges that cannot be overcome either in vivo or ex vivo may be overcome by using animal models of phonation.
Vocal fold vibration research has used a wide variety of animal models, including tigers, lions, sheep, dogs, pigs, rabbits, cows, and deer (Alipour & Jaiswal, 2008; Jiang et al., 2001; Klemuk et al., 2011; Mills et al., 2017). Larynges are harvested, dissected, mounted on a benchtop, and caused to phonate via the method developed by Jiang and Titze (1993). This allows for the collection of acoustic and aerodynamic information about vocal fold vibration in a highly controlled environment. Dog and pig larynges are similar to human larynges in size, with similar length of vocal folds, size of cricothyroid muscle, and cricothyroid joint mobility (Jiang et al., 2001). Both dog and pig larynges have been used frequently in voice research, though pig larynges have more human-like tissue thickness and histology than dog larynges (Jiang et al., 2001; Hottinger et al., 2007; Regner et al., 2008; Regner & Jiang, 2011; Witt et al., 2009). Due to controversy over using domestic pets as animal models in research, pig larynges are more accessible than dog larynges in vocal fold research. Both dog and pig larynges are viable models for vocal fold research as they have been used extensively and there is a large research base on their tissue and vibratory characteristics. However, dog and pig larynges have limitations in vocal fold research. They are large animals that are difficult to maintain and house for the purposes of longitudinal research. It is also difficult to control for the level of vocal use and possible vocal abuse in these specific animals.

The rabbit larynx offers a convenient alternative to pig and dog larynges because rabbits are small and quiet in nature. Compared to dogs and pigs, rabbits are relatively easy to store and care for. Additionally, because rabbits do not typically use their voices, effects of vocally abusive behaviors on the vocal folds are not a concern. Rabbit larynges are very similar to human larynges in that they have a similar superficial vocal fold layer, consisting of loose gelatin-like substance, and all three vocal fold layers have similar histology to that of human
vocal folds (Maytag et al., 2013). Maytag et al. (2013) adopted the benchtop model traditionally used for dog and pig larynges for use with ex vivo rabbit larynges. This rabbit larynx model was additionally used by Mills et al. (2017) and other researchers. In inflammation studies of the vocal folds, the rabbit is a particularly well-suited animal model as its similar histology will more accurately reflect possible human vocal fold changes than other animal models. Rabbit vocal folds were shown to act similarly to human vocal folds under increased elongation conditions (Mills et al., 2017). As measured at PTP, increased elongation led to increased subglottal pressure; as measured at phonation instability pressure (the point at which phonatory signals become aperiodic noise rather than harmonic frequencies), increased elongation led to decreased airflow; and as measured at both PTP and phonation instability pressure, increased elongation led to increased fundamental frequency ($F_0$), decreased range of acoustic and aerodynamic parameters, and decreased vibratory amplitude (Mills et al., 2017). Ultimately, the rabbit model is ideal for the purposes of the current thesis as it is a small animal that is easy to maintain for the longer duration of the study. Using the rabbit larynx model also allows for strict control of experimental treatment versus control treatment administration, dosage, voice usage, age, and gender. By using ex vivo rabbit larynges, it is also possible to measure subglottal air pressure and airflow directly while collecting high-speed video and acoustic data.

**Aerodynamic Outcome Measures**

Common measures of vocal fold vibration in both clinical and research settings are subglottal pressure and airflow measured either orally or nasally. Elevated PTP and PTF may indicate possible vocal fold pathology, making them good measures for voice evaluations and comparisons. Specifically, PTP is sensitive to the presence of vocal fold lesions (e.g., such as in vocal fold polyps, nodules, and edema) while PTF is sensitive to changes in glottal width (e.g.,
as seen in vocal fold mobility disorders, paralysis, and arytenoid dislocation) (Tanner et al., 2016; Zhuang et al., 2013). PTF has been estimated in research studies as the airflow at the point of voicing offset. This is obtained as subjects sustain a vowel with their lips sealed around a cardboard tube and gradually decrease intensity until voicing stops (Zhuang et al., 2013). Airflow through the tube is measured, and the point at which voicing stops is considered PTF, or the point at which airflow is no longer sufficient to sustain phonation. This method is non-invasive, but it is difficult to directly relate PTF at offset to PTP at onset when they are measured at different points in the phonatory cycle. PTP is commonly used and well understood, but it can be difficult to measure in clinical and research populations. Hydration studies have shown that increased PTP does not necessarily correlate with increased perceived phonatory effort as rated by research subjects (Solomon & DiMattia, 2000; Tanner et al., 2007). Thus, self-ratings of perceived phonatory effort cannot be used as an estimate of subglottal pressure necessary to initiate phonation. Direct measurement of subglottal pressure is also invasive, involving insertion of an esophageal balloon or tracheal puncture (Lieberman et al., 1969; Sundberg et al., 2013). For use in clinical settings and some research settings, specific protocols for the indirect measurement of subglottal pressure have been verified. By measuring peak intraoral pressure during the closed /p/ phase of repetitions of the syllable /pi/, pressure at phonation onset can be estimated (Smitheran & Hixon, 1981). In the current thesis, many of these limitations can be overcome via the benchtop model. Subglottal pressure and airflow can both be measured directly at onset by placement of a subglottal pressure transducer and airflow meter beneath the vocal folds.

In different models of vocal fold vibration, subglottal air pressure and airflow may be measured during sustained phonation in addition to at phonation onset and offset. By comparing
these measures at different points in the vibratory cycle, specific relationships can be better understood. The air pressure and airflow needed to initiate phonation is typically greater than that needed to sustain phonation (Regner et al., 2008). Thus, the pressure and airflow measured at offset is lower than at onset. Sustained air pressure and airflow may be measured at the midpoint between onset and offset, or they may be measured as an average during a sustained phonation task. Subglottal air pressure during sustained phonation has been measured to describe in vivo rabbit phonation; to examine the relationship between subglottal pressure, F0, and vocal intensity; to quantify the difference in pressure between the opening and closing phases of vocal fold vibration; and to explore the relationship between pressure, airflow, glottal adduction, and vibratory patterns in excised human hemilarynges (DeJonckere & Lebacq, 2020; Dollinger et al., 2016; Novaleski et al., 2016; Plant & Younger, 2000). Subglottal pressure during sustained phonation (i.e., pitch glides or sustained vowel tasks) has been measured directly in in vivo human subjects via esophageal balloon and cricotracheal puncture (Lieberman et al., 1969; Sundberg et al., 2013). Among other things, subglottal airflow has been measured during sustained phonation to differentiate between human vocal registers, to differentiate between trained and untrained voices, and to describe ex vivo rabbit phonation (Blomgren et al., 1998; Dollinger et al., 2018; Master et al., 2015). Airflow during sustained phonation is typically measured through a pneumotachograph mask (Blomgren et al., 1998; Master et al., 2015; Sundberg et al., 2013). Novaleski et al. (2016) measured both sustained subglottal air pressure and airflow using in vivo rabbit models. Measuring subglottal air pressure and airflow at different points during vocal fold vibration contributes to more fully describing vocal fold vibration under different conditions.

Subglottal air pressure and airflow are often used to compute laryngeal resistance and
phonation threshold power. All these measures are related, and their relationship with each other and with other physical measures of the voice contribute to fully understanding the vocal mechanism (Zhuang et al., 2013). For example, as air pressure increases, sound pressure level, F₀, and airflow all typically increase (Dollinger et al., 2016; Dollinger et al., 2018). In a study by Regner and Jiang (2011), phonation threshold power was sensitive to changes in posterior glottal width and the presence of vocal fold lesions but did not significantly correlate with vocal fold elongation. Using a theoretical model of vocal fold vibration, Jiang and Tao (2007) found that PTF decreased as tissue viscosity, pre-phonatory glottal area, and the velocity of the mucosal wave decreased. These relationships are important in interpreting findings to know whether changes in airflow and air pressure are due to normal aerodynamic factors or due to vocal fold pathology.

**Visual–Perceptual Ratings**

Laryngeal imaging is often used to diagnose vocal fold pathology, rate severity, and track progress or change. The gold standard clinical assessment for voice is videolaryngostroboscopy (Sataloff et al., 2010). Using videolaryngostroboscopy, the vocal folds can be visualized directly both at rest and during vocal fold vibration. In videolaryngostroboscopy, the F₀ of vocal fold vibration is synchronized with a flashing strobe light in order to simulate either a still vocal fold image or slow-motion vocal fold vibration. This method is used widely but is difficult to implement when vocal fold pathology leads to inconsistent F₀. High-speed videoendoscopy is another method of laryngeal imaging that overcomes this limitation by taking up to 8000 frames per second to directly visualize vocal fold vibration (Poburka et al., 2017). Using high-speed videoendoscopy, dysphonic and irregular vocal fold vibration can be visualized through use of a constant light rather than strobe light. Despite its strengths, high-speed videoendoscopy may be
less accessible than videolaryngostroboscopy because it is expensive and requires a great deal of storage. Using either high-speed videoendoscopy or videolaryngostroboscopy, it is important to have a standardized method for laryngeal image evaluation.

Kreiman and Gerratt (1998) examined several studies that used either equal-appearing interval scales or visual analogue rating scales. They concluded that when using either method, it is important to use external representations (Kreiman & Gerratt, 1998). An external representation would be used as an anchor for the rater’s perception. Exposure to several exemplars is likely to sway the rater’s internal representation; using an external representation gives a point from which all items may be more objectively compared and subsequently rated. External representations can be referred to throughout the visual–perceptual rating task to ensure consistency. One scale used to evaluate the health of laryngeal tissue through visual–perceptual ratings is the Reflux Finding Score. The Reflux Finding Score evaluates still laryngeal images of individuals with laryngopharyngeal reflux by rating the following laryngeal characteristics: subglottic edema, ventricular obliteration, erythema/hyperemia, vocal fold edema, diffuse laryngeal edema, posterior commissure hypertrophy, granuloma/granulation, and thick endolaryngeal mucus (Belafsky et al., 2001; Fass et al., 2010). Still laryngeal images can be collected using videolaryngoscopy, a laryngeal imaging method that uses a constant light to clearly visualize the still structures of the pharynx and larynx. The Laryngopharyngeal Reflux Disease Index was also found to be a valid and reliable tool for classifying laryngopharyngeal reflux disease (Beaver et al., 2003). Researchers collected still laryngeal images using videolaryngoscopy, which were then rated for edema and erythema of supraglottal, glottal, and subglottal tissue on an equal-appearing interval scale with scores from 0–3. In examining signs of reflux laryngitis, edema and erythema of the larynx were significantly greater in the participants with reflux laryngitis than in
healthy participants (Pribuišienė et al., 2008). Edema and erythema may be sensitive diagnostic measures. These studies provide a foundation for the use of visual–perceptual ratings of vocal fold edema and erythema in addition to other outcome measures in examining vocal fold pathologies.

In examining the effects of IC treatment on the voice, Hassen and Hasseba (2016) collected acoustic, auditory–perceptual, and visual–perceptual measurements. Participants included individuals with asthma who were receiving IC treatment for at least 4 months prior to the beginning of the study. Dysphonia was rated on the GRBAS scale; a sustained vowel was analyzed acoustically; and videolaryngoscopic recordings of the vocal folds were examined for edema and erythema, irregular vocal fold edges, interarytenoid thickening, and supraglottic hyperfunction (Hassen & Hasseba, 2016). This study is particularly relevant to the current thesis as it examines the effects of ICs on the voice through visual–perceptual ratings of edema and erythema. While researchers found that participants had high levels of dysphonia, acoustic irregularity, and physical laryngeal changes, these factors could not be solely attributed to the use of ICs based on this study. The presence of asthma, for example, could have contributed to higher risk for vocal pathology. The current thesis overcame this limitation by using a between–groups case–control experimental research design with the only group difference being use of ICs.

**Current Problem and Purpose**

ICs are commonly associated with voice disorders, but research to establish their potential to cause voice disorders is limited (Erickson & Sivasankar, 2010; Hassen & Hasseba, 2016; Sahrawat et al., 2014). The current thesis studied the effects of IC drugs on the voice by comparing an experimental group of rabbits that received IC treatment to a control group of
rabbits that received a control nebulized isotonic saline treatment. As described, past research shows that ICs may have a negative effect on vocal fold tissue. The current study introduced greater levels of control than was seen in previous studies, thus contributing stronger research evidence toward understanding this hypothesis. Nebulized isotonic saline has been proven to have no negative effects on the voice and no positive effect when used in low doses, thus validating its use as a control treatment in this study. The rabbit model was used in this study partly due to the inexpensive and convenient nature of housing rabbits during treatment administration. More importantly, the rabbit model has recently been studied and validated as a reliable vocal fold model with similar histology to human vocal folds (Maytag et al., 2013). Rabbit vocal folds may react to different conditions similarly to human vocal folds, making them an ideal model for studying inflammation.

Phonation of the rabbit larynges was simulated via the benchtop model. Studies have shown the importance of measuring several factors of phonation in order to better understand the vocal mechanism. Using aerodynamic, acoustic, and visual signals in this thesis gave an adequate description of the effects of ICs on the voice. This thesis analyzed subglottal pressure and airflow during sustained phonation and visual–perceptual ratings of edema and erythema to compare the experimental and the control groups.

**Research Questions**

1. Do experimental rabbit larynges with eight-week exposure to ICs have higher sustained pressure and greater airflow when phonating compared to control rabbit larynges with eight-week exposure to an inhaled nebulized isotonic saline solution?
2. Do still images of experimental rabbit larynges with eight-week exposure to ICs show higher levels of edema and erythema in visual–perceptual ratings when compared to
photographs of control rabbit larynges with eight-week exposure to an inhaled nebulized isotonic saline solution?

Method

This thesis was conducted in conjunction with a parent project funded by the National Institutes of Health. The grant that funded portions of this research was provided by the National Institute on Deafness and other Communication Disorders through grant number 1R01DC019269. Kristine Tanner, Ph.D., was the principal investigator for the parent project; this thesis study was conducted in her laboratory. The human subjects protocol for this work was approved by the Institutional Review Board at Brigham Young University, X18007. Likewise, the animal portion of this project was approved by Risk Management and the Institutional Animal Care and Use Committee boards at Brigham Young University and The University of Utah, protocol 18-01001. For this thesis, all excised laryngeal tissue was obtained from The University of Utah. The thesis author is primarily responsible for the portions of the parent project that are reported in this document.

This work involved two primary methodologies. The first included an excised larynx benchtop study of the effects of ICs on aerodynamic measurements of voice function. The second methodology consisted of visual–perceptual judgments of the benchtop larynges. A between–groups case–control experimental research design was employed, with each group receiving twice-daily administration of ICs or a nebulized isotonic saline control during an eight-week period. The independent variable was group, experimental versus control. The dependent variables were sustained subglottal pressure during phonation (cm H2O), sustained airflow (L/min), and visual–perceptual ratings of arytenoid and vocal fold edema and erythema (0–3 equal appearing interval scale of severity).
Operational Procedure Overview

As part of the parent project, all in vivo animal procedures were performed at The University of Utah. The animals for this study included 22 New Zealand white adult male rabbits. They were all retired breeders, ages seven to eight months and weighing 3.1–4.8 kg. The rabbits were randomly assigned to the experimental and control groups (n = 11 per group). Experimental rabbits received twice-daily IC salmeterol fluticasone propionate administered via a metered dose inhaler (MDI) and using a facemask and spacer; rabbits inhaled transnasally for 20 breaths. Similarly, control group rabbits received twice-daily nebulized isotonic saline (0.9% NaCl) via a facemask for 20 breaths. Exceptions occurred on two holidays, when rabbits received one administration. Following euthanasia, larynges were surgically excised and stored in labeled and coded vials of phosphate–buffered solution (PBS). Using established methodology, vials were placed in an isopropyl alcohol bath and then flash frozen to minimize the formation of ice crystals; these vials were stored in a -80° Celsius freezer.

All procedures completed by the thesis author are detailed in a timeline in Appendix J. For the current study, larynges were retrieved from The University of Utah and transported in a foam cooler with dry ice to Brigham Young University, John Taylor Building Annex, room 105. The frozen vials were then placed in a Thermoscientific -80° Celsius freezer. Larynges were retrieved in this manner prior to each data collection session in four groups, consisting of five to six larynges each. All further tissue preparation, dissection, benchtop mounting, photography, data collection, and data segmentation procedures for this thesis were performed in room 106 of the John Taylor Building Annex. On the day of data collection, larynges were thawed in a lab sink in room temperature water for approximately 30 minutes, finely dissected, and mounted on benchtop for data collection. Before mounting, larynges were stored in fresh PBS in a food–
grade refrigerator. Larynges were sprayed liberally with isotonic saline throughout dissection and while mounted to maintain tissue hydration.

**Dissection Description**

After larynges were completely thawed, researchers finely dissected them following established protocol to expose the true vocal folds. Detailed dissection procedures are included in Appendix F. Dissection procedures were performed on a benchtop covered with dissection mats and using a #11 size X-acto™ knife, hemostatic forceps, and manicure scissors. Researchers wore white, nitrile, powder free gloves and had face masks, aprons, and safety glasses. A detailed description of materials used is included in Appendix B. The esophagus was resected inferiorly to superiorly to expose to the arytenoid cartilages. Extrinsic laryngeal tissue was resected, sparing the posterior cricoarytenoid, lateral cricoarytenoid, and cricothyroid muscles. Tissue superior to the false vocal folds was resected, including the epiglottis and the portion of the thyroid cartilage approximately 4 mm superior to the vocal folds. Figure 1 shows a rabbit larynx with the esophagus removed, the arytenoid cartilages exposed, and the epiglottis still intact. The anterior commissure was identified inferiorly and medially to the fat pads, which were resected along with the false vocal folds. To facilitate resection of the false vocal folds and protect the true vocal folds, the false vocal folds were abduced using forceps and resected with an anterior to posterior incision starting at the anterior commissure. Figure 2 shows a rabbit larynx with only the left ventricular fold resected. Excess tissue that could affect vocal fold vibration was resected, including the ventricular folds. A suture (item M-S418R19, AD Surgical Sunnyvale, CA) was made in the remaining portion of the thyroid cartilage for purposes of stabilization during mounting and data collection. The suture needle was inserted through the thyroid cartilage approximately 1 mm superior to the anterior commissure. A string was
threaded, two loops were made, and the needle was disposed of in a red hazardous waste box. As described, the larynges were stored in a coded vial of fresh PBS in a food–grade refrigerator to maintain tissue hydration until they were mounted on benchtop for data collection later that day. Sani-Cloth germicidal disposable wipes were used to disinfect equipment following all procedures involving laryngeal tissues.

**Benchtop Mount**

The benchtop model of excised larynx phonation, as described by Jiang and Titze (1993) and modified for rabbit models by Maytag et al. (2013), was used in this study. A custom tube for rabbit tracheal mounting was attached to a PVC pipe and emerged through the surface of a Thorlabs bench (Ann Arbor, MI). Three micropositioners (Model 1460, Kopf Industries) were connected to the benchtop via ¼-20 headless screws. Two of the micropositioners were positioned laterally and one anteriorly to the tracheal mount for vocal fold adduction and larynx stabilization, respectively. A mounted larynx is shown in Figure 3, with two lateral micropositioners and one anterior micropositioner.
Figure 1

*Rabbit Larynx With Intact Epiglottis and Exposed Arytenoid Cartilages*

![Rabbit Larynx With Intact Epiglottis and Exposed Arytenoid Cartilages](image1)

Figure 2

*Rabbit Larynx With Left True Vocal Fold Exposed*

![Rabbit Larynx With Left True Vocal Fold Exposed](image2)
Subglottal air for phonation was generated from compressed air tanks filled with medical–grade, low–humidity air (< 1% relative humidity). Airflow was controlled using an adjustable flow regulator standardized at 50 psi. Air tanks were secured to the wall next to the benchtop per the standards of the Joint Commission on Accreditation of Healthcare Organization.
and the Occupational Safety Health Administration. Air was directed through a 100 Liter respiratory flow head (Model MLT300L, AD Instruments, Sydney, Australia) that was secured beneath the benchtop with Velcro. Air then passed through a TheraHeat humidifier (Model RC70000, Smith Medical, Dublin, OH) with heated distilled water. Next, air flowed through a 20 cm, aluminum, foam–insulated custom pseudolung for purposes of reducing reverberation in the airflow. A PVC pipe was used to direct airflow from the pseudolung to the custom tracheal mount. A physiological pressure transducer (Model MLT844, AD Instruments, Sydney, Australia) was inserted into this PVC pipe to measure subglottal pressure. In Figure 3, the pressure transducer is on the benchtop covered by a piece of protective gauze. This benchtop setup is shown in Figure 4.

**Figure 4**

*Benchtop Setup*

Other measurement devices shown in Figure 4 included a microphone and a high–speed camera. The microphone (Model SM-48, Shure, Niles, IL) was mounted superior and posterior to the larynx approximately 6 inches from the mounted larynx to collect audio signals of
phonation with a 40,000 Hz sample rate. The high-speed video camera was also mounted directly superior to the tracheal mount to collect data relating to the parent project of this study. A permanent marker was used to mark each larynx on the thyroid cartilage approximately .5 cm posterior to the anterior commissure for purposes of high-speed video calibration.

**Signal Acquisition Procedures**

Data from the airflow meter, pressure transducer, and microphone were recorded on a Dell computer on LabChart data acquisition software (ADInstruments, 2015). Appendix C contains the specific protocol for LabChart computer use. Instruments were calibrated and zeroed prior to each data collection session per manufacturer instructions. Protocols for instrument calibration and settings checks were posted on lab computers and followed exactly. These protocols are contained in Appendix D and Appendix E. LabChart was opened and run for at least 15 minutes prior to calibration. Channel settings for the “official rabbit template” were checked for airflow (1k/s, range 200mV in L/min), pressure (1k/s, range 20mV in mmHg), and acoustic (1k/s, range 10 mV) signals. Airflow was calibrated using a one-liter Pneumotach Calibration Unit (MCU-4, Glottal Enterprises). Pressure was calibrated using a sphygmomanometer (AD instruments), a syringe (25 ml), a pressure calibration block, and gauze to reduce reverberation. Any instrumental drift that occurred throughout the data collection session was corrected in a custom Matlab program designed by Christopher Dromey, Ph.D (The MathWorks Inc, 2010).

Each rabbit larynx was mounted on the custom tracheal mount and data were collected from 15 phonation trials. As seen in Figure 3, a single prong attached to each lateral micropositioner gently punctured the lateral surface of the arytenoid cartilages to position and adduct the vocal folds. The suture string was tied to the anterior micropositioner to provide
stabilization. Researchers were careful to avoid vocal fold elongation when mounting. The trachea was secured using cable ties and Teflon tape. Air was passed to check for any air leakage except for through the vocal folds. Necessary adjustments to mounting were made until phonation was maintained and no air leakage was found, except as measured to pass between the adducted vocal folds. Temperature and humidity were recorded from an AcuRite™ hygrometer (Model 01083M) consistently both before and after 15 phonation trials were performed for each larynx. Three researchers managed separate instruments and performed set tasks to initiate phonation and collect data. Detailed descriptions of tasks for data acquisition are included in Appendix G. Researchers managed the same instrument and performed the same tasks across data collection sessions to maintain consistency between trials and between larynges. Conditions were not varied between phonation trials or between data collection sessions. One researcher was responsible for collecting high–speed video of phonation on the first, fifth, 10th, and 15th phonation trial for each larynx. To collect high–speed video, the room was dark, and a commercial light was used to illuminate the larynx (Genaray Monobright, Genaray LLC., China). A second researcher ran the LabChart program, starting and pausing data collection before and after each phonation trial, labeling each rabbit and number of phonation trials, and inserting preset comments for marking phonation onset, sustained phonation, and phonation offset. Markers for two phonation trials, along with acoustic, pressure, and airflow data, are shown in Figure 5. A third researcher controlled airflow, gradually increasing airflow until phonation was noted, sustaining airflow for approximately 3 seconds, and gradually reducing airflow to zero. This researcher also misted larynges with nebulized isotonic saline approximately once every three phonation trials to maintain proper tissue hydration.
Following 15 phonation trials, larynges were removed from the benchtop mount by loosening the lateral micropositioners and slipping the suture loop off of the anterior micropositioner. Further laryngeal measurements were taken using a digital scale (Ozeri Model Zk14-STM) and a digital caliper (UltraTECHTM no. 1433). Measurements included weight of the larynx, width and length of the trachea, width and length of the vocal folds (from arytenoid cartilages to anterior commissure), distance from the vocal folds to the lateral edge of the thyroid cartilage, outer width of the largest portion of the thyroid cartilage, and length of thyroid cartilage from prominence to bottom. Larynges were again stored in labeled vials of fresh PBS and were transported back to The University of Utah for further examinations related to the parent project connected to this thesis.
Still Image Photography

As part of the visual–perceptual portion of this study, still images were taken of each larynx after mounting and before phonation trials. Figures 6 and 7 show photographs of an experimental rabbit and a control rabbit, respectively. Photographs were taken with an iPhone XS using both natural light and a commercial light (Genaray Monobright, 2 LED, Genaray LLC., China) held directly superior to the larynx. Photos were standardized with respect to position, crop, and lighting using Adobe Lightroom (version 3.3) photo editing software on a desktop Mac.
Data Segmentation and Analysis

Pressure and airflow data were segmented and processed in Matlab by Megan Hoggan and Amber Prigmore, two research assistants with over one year of experience in data analysis and segmentation (The MathWorks Inc, 2010). Appendix H contains specific instructions for data segmentation and analysis. Data from LabChart were segmented by placing markers for phonation onset, phonation offset, and sustained phonation on the acoustic signal (ADInstruments, 2015). Signals acquired for acoustics, pressure, and airflow were time aligned so that these markers on the acoustic signal were used to determine phonation pressure and
airflow at onset, offset, and during sustained phonation. Researchers used visual and auditory perceptual information from the acoustic signal to determine correct marker placement. In trials with clear phonation onset and offset, the second peak of periodic phonation was marked as phonation onset, and the second to last peak of periodic phonation was marked as phonation offset. In trials with more gradual or breathy phonation onset and offset, the auditory signal was segmented to determine the general location of phonation onset or offset. Then, both auditory and visual information from the acoustic signals were used to make an informed decision about the timing of phonation onset and offset. Sustained phonation was defined as the point mid-way between the onset and offset markers. Researchers randomly re-segmented 10% of phonation trials to determine intra-rater reliability for marker placement. Reliability was greater than or equal to 98% for all marker placements indicating strong consistency of marker placement across phonation trials.

Information collected through LabChart were further analyzed using other data analysis programs. Average F0 of phonation trials was extracted using Praat (Boersma et al., 2019). version 6.0.49. Pressure, airflow, and, acoustic signals were analyzed using a custom Matlab application created by Dr. Christopher Dromey, Ph.D (The MathWorks Inc, 2010). A segment of data from 10 ms before to 10 ms after marker placement was averaged through Matlab to determine PTP and PTF at phonation onset, sustained phonation, and phonation offset. Figure 8 shows the Phonation Aerodynamics window from the custom Matlab application, including 15 phonation trials for one larynx. Figure 9 shows extracted data from one phonation trial, which is further exported into an Excel spreadsheet with information on onset pressure and airflow, sustained pressure and airflow, and offset pressure and airflow.
Figure 8

Matlab Application 15 Phonation Trials
Visual–Perceptual Analysis

Following laryngeal standardization for position, crop, and lighting, all laryngeal images were de-identified and randomly compiled into a slideshow using Microsoft PowerPoint. These slides included instructions for separately rating edema and erythema of both arytenoid and vocal fold tissues, definitions of anatomical locations and physiological presentations of edema and erythema, and external visual anchors on each experimental slide for purposes of consistency in ratings. Approximately 10% of the laryngeal images were randomly repeated in the slides for
purposes of intra-rater reliability. These slides may be referenced in Appendix I.

Six raters were recruited to perform visual–perceptual ratings of severity of vocal fold and arytenoid edema and erythema. Raters included two practicing clinicians with expertise in voice disorders, three graduate students who completed a class on voice disorders at BYU (ComD 657), and one undergraduate research assistant. Ratings were made using an equally appearing interval scale from 0–3, zero indicating no edema or erythema and three indicating the most severe edema or erythema.

**Statistical Analysis**

For purposes of the parent project, summary data for onset and sustained pressure, airflow, F0, and visual–perceptual severity ratings were examined. Data distributions were examined visually using analysis of covariance. For the segmenting process, inter-rater reliability was calculated using intraclass correlation coefficients and intra-rater reliability was calculated using Pearson product-moment correlations. For visual–perceptual ratings, intraclass correlation coefficients were used to calculate inter-rater reliability and percent agreement was used to calculate intra-rater reliability.

Repeated measures one-way analysis of variance was conducted for each of these variables. Post-hoc Student Newman-Keuls analyses were conducted for sustained pressure, sustained airflow, and F0 using an alpha level of .05. Linear regression was used to analyze significance of severity scores from visual–perceptual ratings. Analyses were conducted using SPSS (version 24) and SAS (version 9.4) by Dr. Ray M. Merrill, Ph.D., in Life Sciences at BYU.

**Results**

The following includes a detailed reporting of the results of this thesis, including aerodynamic, acoustic, and visual–perceptual data analyses involving the experimental and
control rabbit larynges. The primary purpose of collecting and reporting these results is to
determine whether experimental rabbit larynges with eight-week exposure to ICs have higher
sustained pressure, airflow, and levels of edema and erythema than control rabbit larynges with
eight-week exposure to an inhaled nebulized isotonic saline solution.

As described in the methods section, data were collected for 15 phonation trials per
excised rabbit larynx. Ambient temperature and humidity were recorded at the beginning and end
of trials for each rabbit larynx. These values are displayed in Table 1.
Table 1

*Ambient Temperature and Humidity During Data Collection*

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Note. Coded rabbit numbers do not represent sequential experimentation or skipped samples.

*Replaced by approximates based on series of rabbits and time-frame comparisons*
**Physical Dimensions**

Tracheal and laryngeal dimensions were measured using an electronic caliper. The trachea width was measured as the inner diameter between the lateral edges of the trachea. The trachea length was measured as the distance from the inferior edge of the anterior thyroid cartilage to the bottom edge of the trachea following resection. The width of the thyroid cartilage was measured at the widest portion as the lateral distance between the outer edges of the thyroid cartilage. The length of thyroid cartilage from prominence to bottom was estimated as the superior portion of the thyroid, including the thyroid prominence, was resected for purposes of vocal fold visualization. Tracheal and laryngeal dimensions are displayed in Table 2. The length of the vocal folds was measured with the vocal folds adducted as the distance from the anterior commissure on the inside of the anterior thyroid cartilage to the vocal process of the arytenoid cartilages. The width of the vocal folds was measured as the width of one vocal fold at its widest point from the medial to the lateral edge. The width from the vocal folds to the thyroid cartilage was measured as the distance from the lateral edge of one vocal fold to the inside edge of the thyroid cartilage at the widest point. Vocal fold measures are shown in Table 3.
Table 2

Tracheal and Laryngeal Dimensions by Rabbit Number

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<td>13.81</td>
<td>2.71</td>
</tr>
<tr>
<td>19-092</td>
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<td>5.96</td>
<td>15.11</td>
<td>4.38</td>
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<td>7.28</td>
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</tr>
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<td>7.04</td>
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<td>15.32</td>
<td>7.28</td>
<td>13.52</td>
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<td>19-098</td>
<td>12.56</td>
<td>7.75</td>
<td>14.22</td>
<td>5.54</td>
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<td>19-099</td>
<td>18.89</td>
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<tr>
<td>Group</td>
<td>Vocal fold Length (mm)</td>
<td>Vocal fold Width (mm)</td>
<td>Width from vocal fold to thyroid cartilage (mm)</td>
<td></td>
</tr>
<tr>
<td>---------</td>
<td>------------------------</td>
<td>-----------------------</td>
<td>------------------------------------------------</td>
<td></td>
</tr>
<tr>
<td><strong>Experimental</strong></td>
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<td>2.78</td>
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<td>2.78</td>
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<td>2.78</td>
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<td><strong>Control</strong></td>
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<tr>
<td>19-088</td>
<td>7.31</td>
<td>1.65</td>
<td>2.85</td>
<td></td>
</tr>
<tr>
<td>19-090</td>
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<tr>
<td>19-100</td>
<td>7.15</td>
<td>1.84</td>
<td>3.53</td>
<td></td>
</tr>
</tbody>
</table>
Aerodynamic Measurements

Aerodynamic data presented in this thesis include sustained pressure and sustained airflow. Two researchers with extensive training segmented raw aerodynamic data by marking phonation onset, mid-point (sustained phonation), and phonation offset. Inter-rater reliability for marker placement at points of sustained phonation was calculated using an intraclass correlation coefficient. Inter-rater reliability was excellent as demonstrated by intraclass correlation coefficients from 0.877–0.995 for sustained pressure and 0.986–0.994 for sustained flow. Intra-rater reliability was calculated using the Pearson product-moment correlation coefficient. Intra-rater reliability was also excellent, with Pearson product-moment correlation coefficients 1.000 for sustained pressure and from 0.999–1.000 for sustained airflow.

The flow and pressure signals were then run through an automated Matlab program for further analysis of aerodynamic data based on the segmentation points (The MathWorks Inc, 2010). The sustained pressure and airflow values of 15 phonation trials were averaged for each larynx individually. Aerodynamic data for rabbit number 19-097 was excluded from data reporting and analysis due to visually damaged vocal folds compromising aerodynamic measurements. Average sustained pressure and airflow for each excised rabbit larynx are presented in Table 4.
Table 4

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

<table>
<thead>
<tr>
<th>Group</th>
<th>Sustained pressure (cm H₂O)</th>
<th>Sustained airflow (L/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Experimental</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>19-023</td>
<td>11.24</td>
<td>0.08</td>
</tr>
<tr>
<td>19-025</td>
<td>8.51</td>
<td>0.09</td>
</tr>
<tr>
<td>19-027</td>
<td>8.75</td>
<td>0.09</td>
</tr>
<tr>
<td>19-032</td>
<td>6.81</td>
<td>0.09</td>
</tr>
<tr>
<td>19-033</td>
<td>10.32</td>
<td>0.09</td>
</tr>
<tr>
<td>19-035</td>
<td>8.23</td>
<td>0.08</td>
</tr>
<tr>
<td>19-036</td>
<td>16.62</td>
<td>0.21</td>
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<tr>
<td>19-039</td>
<td>14.86</td>
<td>0.17</td>
</tr>
<tr>
<td>19-050</td>
<td>13.31</td>
<td>0.17</td>
</tr>
<tr>
<td>19-051</td>
<td>12.57</td>
<td>0.13</td>
</tr>
<tr>
<td>19-052</td>
<td>15.01</td>
<td>0.15</td>
</tr>
<tr>
<td><strong>Control</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>19-088</td>
<td>9.42</td>
<td>0.07</td>
</tr>
<tr>
<td>19-090</td>
<td>9.54</td>
<td>0.05</td>
</tr>
<tr>
<td>19-091</td>
<td>8.31</td>
<td>0.07</td>
</tr>
<tr>
<td>19-092</td>
<td>9.71</td>
<td>0.09</td>
</tr>
<tr>
<td>19-094</td>
<td>9.46</td>
<td>0.10</td>
</tr>
<tr>
<td>19-095</td>
<td>8.14</td>
<td>0.06</td>
</tr>
<tr>
<td>19-096</td>
<td>7.57</td>
<td>0.04</td>
</tr>
<tr>
<td>19-098</td>
<td>7.72</td>
<td>0.07</td>
</tr>
<tr>
<td>19-099</td>
<td>8.42</td>
<td>0.11</td>
</tr>
<tr>
<td>19-100</td>
<td>12.48</td>
<td>0.12</td>
</tr>
</tbody>
</table>
Descriptive statistics for aerodynamic data were calculated using SPSS (version 24) and SAS (version 9.4) by Ray M. Merrill, Ph.D. Mean, median, standard deviation, minimum, and maximum aerodynamic values are presented in Table 5. Repeated measures one-way between-groups analysis of variance was used to analyze the effects of IC use on sustained pressure and sustained airflow. The results indicated significant between-groups effects across phonation trials for both sustained pressure \( F(35, 279) = \text{infinity}, p < .0001 \) and sustained airflow \( F(35, 279) = \text{infinity}, p < .0001 \). Post-hoc Student Newman-Keuls analyses were then performed using an alpha level of .05. Results demonstrate that average sustained pressure was significantly greater in the experimental group than the control group \( p < .05 \). Similarly, average sustained airflow of the experimental group was significantly greater than that of the control groups \( p < .05 \). For a visual comparison between experimental and control group aerodynamic measures, see Figures 10 and 11 for analysis of covariance for mean sustained pressure and airflow, respectively.

**Table 5**

*Aerodynamic Descriptive Statistics*

<table>
<thead>
<tr>
<th>Group</th>
<th>Mean</th>
<th>Median</th>
<th>SD</th>
<th>Minimum</th>
<th>Maximum</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Experimental</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sustained pressure</td>
<td>11.48</td>
<td>11.24</td>
<td>3.24</td>
<td>6.81</td>
<td>16.62</td>
</tr>
<tr>
<td>(cm H$_2$O)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sustained airflow (L/min)</td>
<td>0.12</td>
<td>0.09</td>
<td>0.04</td>
<td>0.08</td>
<td>0.21</td>
</tr>
<tr>
<td><strong>Control</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sustained pressure</td>
<td>9.08</td>
<td>8.92</td>
<td>1.43</td>
<td>7.57</td>
<td>12.48</td>
</tr>
<tr>
<td>(cm H$_2$O)</td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sustained airflow (L/min)</td>
<td>0.08</td>
<td>0.07</td>
<td>0.03</td>
<td>0.04</td>
<td>0.12</td>
</tr>
</tbody>
</table>
Figure 10

Analysis of Covariance for Mean Sustained Pressure in cm H$_2$O

![Graph showing analysis of covariance for mean sustained pressure in cm H$_2$O. The graph plots mean sustained pressure against phonation trial. The data points for experimental and control groups are shown with red and blue circles, respectively, connected by linear regression lines.]
Figure 11

Analysis of Covariance for Mean Sustained Airflow in L/min

Acoustic Data

Rabbit phonation during each trial (n = 15) was recorded acoustically and F₀ data were extracted using autocorrelation algorithms in Praat software (Boersma et al., 2019). Inter-rater reliability was excellent, with intraclass correlation coefficients between 0.978 and 0.986. Intra-rater reliability, calculated using the Pearson product-moment correlation coefficient, was similarly excellent, between 0.925 and 0.955. Experimental larynx F₀ ranged from approximately 403 Hz to 604 Hz with a mean of 519 Hz, while control larynx F₀ ranged from approximately 284 Hz to 673 Hz with a mean of 446 Hz. Significant treatment effects between groups were found using repeated measures one-way between-groups analysis of variance \(F(35, 279) = \)
A visual representation of data between groups, using analysis of covariance, is shown in Figure 12.

**Figure 12**

*Analysis of Covariance for Mean $F_0$ in Hz*

---

**Visual–Perceptual Ratings**

Visual–perceptual ratings of the presence and severity of vocal fold edema and erythema of vocal fold and arytenoid tissues were collected from still-image, color photographs. Study participants rated a total of eight items on a 0–3 scale, including right and left arytenoid edema, right and left arytenoid erythema, right and left vocal fold edema, and right and left vocal fold erythema. Intraclass correlation coefficients, shown in Figure 13, demonstrate generally good inter-rater reliability between five raters for each of the eight items. While all intraclass
correlation coefficients range from an acceptable .792 for left arytenoid edema to an excellent .900 for right arytenoid erythema, notable are the slightly higher inter-rater reliability coefficients for ratings of erythema than those for edema. Intra-rater reliability was calculated using percent agreement for random re-ratings of approximately 15% of laryngeal images. Percent agreement is an incredibly rigorous measure of reliability as it allows for no margin of error. As such, percent agreement does not consider the degree or magnitude of error in the case that any occurred. If data were analyzed on a binary scale (healthy 0–1 versus abnormal 2–3) rather than 0–3 scale, intra-rater reliability measures would likely have increased significantly. Due to structural damage and outlying aerodynamic data, the most consistently rated laryngeal image (rabbit number 19-097) was removed from data analysis and inter- and intra-rater reliability calculations. Average percent agreement for each rater is shown in Table 6.
Figure 13

Intraclass Correlation Coefficients for Inter-Rater Reliability

![Intraclass Correlation Coefficients for Inter-Rater Reliability](image)

Table 6

Percent Agreement for Intra-Rater Reliability

<table>
<thead>
<tr>
<th>Measure</th>
<th>Rater 1</th>
<th>Rater 2</th>
<th>Rater 3</th>
<th>Rater 4</th>
<th>Rater 5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Average</td>
<td>62.6%</td>
<td>42%</td>
<td>79%</td>
<td>83%</td>
<td>33.3%</td>
</tr>
</tbody>
</table>

Severity ratings for all eight items (edema and erythema for the four anatomic structures) were analyzed using a linear regression model. Initially, main effect and interaction effects were observed between treatment and rater for each of the eight items. The interaction term between treatment and rater was not significant for any item. For example, main effects in item one, right arytenoid edema, were observed for treatment \( F(1, 1) = 29.97, p < .0001 \) and rater \( F(4, 4) = 3.42, p = 0.0116 \) with an insignificant interaction effect between treatment and rater \( F(4, 4) = 0.84, p = 0.5040 \). Because there was no significant interaction between treatment and rater for any item, regression analysis was used on all items, controlling for rater. On all items, average
severity ratings were significantly higher for larynges in the experimental versus control group. Average differences in ratings between experimental and control groups for each of the eight items as well as levels of significance are shown in Table 7.

Table 7

<table>
<thead>
<tr>
<th>Item</th>
<th>Difference in Severity Ratings</th>
<th>t</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Right Arytenoid Edema</td>
<td>1.00</td>
<td>5.49</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>Right Arytenoid Erythema</td>
<td>0.45</td>
<td>2.28</td>
<td>.0249</td>
</tr>
<tr>
<td>Right Vocal Fold Edema</td>
<td>0.91</td>
<td>4.99</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>Right Vocal Fold Erythema</td>
<td>0.84</td>
<td>4.33</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>Left Arytenoid Edema</td>
<td>0.84</td>
<td>4.66</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>Left Arytenoid Erythema</td>
<td>0.43</td>
<td>2.20</td>
<td>.0305</td>
</tr>
<tr>
<td>Left Vocal Fold Edema</td>
<td>0.89</td>
<td>4.49</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>Left Vocal Fold Erythema</td>
<td>0.81</td>
<td>4.17</td>
<td>&lt;.0001</td>
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</table>

Discussion

The purpose of this thesis is to describe the differences between experimental larynges exposed to 8 weeks of ICs and control larynges exposed to 8 weeks of an inhaled nebulized isotonic saline solution. These differences are quantified using aerodynamic measures of pressure and airflow and visual–perceptual ratings of edema and erythema. This thesis is part of a 5–year study to determine the effects of ICs, a common drug used to treat asthma, on the voice. As the prevalence of asthma increases, it becomes more important to clearly understand the risks associated with asthma inhaler drugs. Results of this thesis demonstrate a significant treatment effect. When compared to control larynges, experimental larynges required significantly higher sustained pressure and significantly higher sustained airflow to maintain phonation. Similarly,
images of experimental larynges received significantly more severe edema and erythema ratings when compared to images of control larynges.

**Dependent Variables**

Dependent aerodynamic measures in this thesis included sustained subglottal pressure and sustained glottal airflow. These measures have not been traditionally used in ex vivo animal benchtop studies. Typically, PTP and PTF have been used to measure differences between laryngeal models as they are sensitive to presence of vocal fold lesions and changes in glottal width, respectively (Tanner et al., 2016; Zhuang et al., 2013). While changes in PTP and PTF can indirectly indicate changes in vocal fold structure and position, sustained subglottal pressure and airflow are also significant dependent variables. Plant and Hillel (1998) demonstrate the importance of measuring subglottal pressure and airflow at several different points and throughout the phonatory cycle due to the irregularity of pressure and airflow in some clinical populations. For example, PTP and PTF can be measured as well as subglottal pressure and airflow during sustained phonation. Damaged vocal fold tissue demonstrates irregularities in vibration when compared to healthy vocal fold tissue and, therefore, demonstrates variability in associated aerodynamic and acoustic measures (Powell et al., 2020). It follows that measures limited to phonation onset lack important depth and detail, and sustained phonation must be considered to truly describe the irregular phonation of clinical populations. Furthermore, sustained aerodynamic measures are often used in human research to examine correlations between acoustic, aerodynamic, and physical vocal fold characteristics. Thus, the findings of this thesis may be compared to studies of human populations and subsequently translate to clinical work (DeJonckere & Lebacq, 2020; Enflo, 2013; Silva et al., in press).
The acoustic measure $F_0$ was collected due to the importance of $F_0$ data in research regarding voice. $F_0$ is a relatively simple measure to collect and analyze. It changes significantly with changes in vocal fold tissue and with changes in subglottal pressure (Dollinger et al., 2018; Lieberman et al., 1969; Plant & Younger, 2000; Silva et al., in press).

Visual–perceptual judgements are often used clinically and in research literature to describe physical properties of vocal folds (Poburka et al., 2017; Powell et al., 2020). Therefore, to best translate findings from this thesis to clinical applications, visual–perceptual ratings were used to compare the vocal fold and arytenoid tissue between experimental and control larynges.

**Aerodynamic Results**

Results show that sustained pressure and sustained airflow were significantly higher for experimental larynges than for control larynges, $[F(35, 279) = \infty, (p < .0001)]$. Due to the highly controlled environment of specimen acquisition, treatment administration, and collection of aerodynamic measurements, differences in aerodynamic measurements between experimental and control groups can be attributed to differences in treatment (i.e., ICs versus nebulized isotonic saline). Thus, experimental larynges treated with ICs were shown to require higher sustained pressure and higher sustained airflow to maintain phonation than control larynges treated with inhaled nebulized isotonic saline. When viewing the figures on analysis of covariance, it is apparent that aerodynamic measures in both control and experimental larynges seem to follow a similar pattern, with a peak in subglottal pressure occurring approximately every 5 trials. While not completely understood, this pattern is assumed to be related to data collection protocols. This could be due to rehydration of the larynx as well as a brief rest period associated high-speed data collection, which occurred every 5 trials. These effects of these variables on the phonation of ex vivo larynges should be studied more extensively in future
research. The relationship between air pressure and airflow play an important role in interpreting the aerodynamic results of this thesis. In the following paragraphs, physical laws of moving liquids will be referenced, including the Bernoulli effect and Ohm’s law.

Differences between aerodynamic measures obtained from experimental and control larynx phonation can be understood from the basis of the myoelastic aerodynamic theory and the Bernoulli effect. According to the myoelastic aerodynamic theory, vocal fold vibration occurs due to the elastic and mass properties of the vocal folds and the aerodynamic principles of air pressure and airflow (Seikel et al., 2010). Properly preserved tissues maintain mass and elastic properties ex vivo and can, therefore, vibrate when appropriate aerodynamic forces are applied. As air passes from the trachea through the larynx, it passes through a constriction created by the vocal folds in the larynx. According to the Bernoulli effect, given constant airflow through the trachea and larynx, velocity will increase as air passes through the narrowing of the vocal folds (Seikel et al., 2010). This increase in velocity leads to a drop in pressure, which causes the vocal folds to come together (Seikel et al., 2010). As subglottal pressure builds, the vocal folds part again, allowing air to again flow through the constriction and the process to repeat. Thus, vocal fold vibration is a nearly periodic motion created and affected by tissue elasticity, mass, and aerodynamic forces. While consistently following protocols for increasing airflow and pressure between specimen and trials, the need for higher airflow and pressure to maintain phonation in experimental larynges treated with ICs must be related to changes in tissue elasticity or mass. These changes may be further understood on the basis of Ohm’s law.

Ohm’s law states that flow is equal to the product of pressure and resistance (Emanuel & Letowski, 2009). In accordance with Ohm’s law, pressure and airflow are linearly related in phonation and benchtop model research (Dollinger et al., 2016; Master et al., 2015). This
linearity has been replicated in the current thesis as pressure and airflow both increased significantly in experimental versus control larynx phonation. Increases in airflow are related to inherent increases in subglottal pressure that occur following 8 weeks of IC administration. Specifically, increased mass and decreased elasticity related to edema in experimental larynges would increase glottal resistance, thus requiring higher subglottal pressure and higher airflow to initiate and sustain phonation.

Previous research demonstrates that increases in subglottal pressure are related to vocal fold pathologies. Increases in subglottal pressure related to IC administration suggest that ICs might cause damage to vocal fold tissue. Silva et al. (in press) measured PTP, vocal fold contact pressure, and maximum subglottal pressure to demonstrate that each of these measures were significantly increased in pathologic larynges. This study is significant in interpreting current thesis results as not only PTP, but subglottal pressure during sustained phonation was significantly affected by vocal fold pathology. Similarly, Zhuang et al. (2013) found that PTP was significantly lower in healthy individuals than in those with vocal fold mass lesions. PTP and sustained subglottal pressure are both significantly increased due to pathology. Following the examples of past research, increases in subglottal pressure in the current thesis are related to damaged vocal folds, or pathology, caused by eight-week administration of ICs. This relates to the research of Erickson and Sivasankar (2010), who measured PTP in participants following IC treatment administration. They found that, at specific phonation frequencies, PTP was significantly higher for the treatment versus the placebo group. Therefore, in the current thesis, increases in subglottal pressure in experimental larynges are likely related to vocal fold damage due to IC administration. Further, according to Ohm’s law, increases in subglottal pressure are accompanied by increased airflow if resistance does not change. It follows that significant
increases in both sustained pressure and airflow are a result of vocal fold pathology caused by eight-week administration of ICs.

**Acoustic Results**

In addition to increases in subglottal pressure and airflow, pathologic voices with physical vocal fold changes are, typically, associated with decreased F₀ (Silva et al., in press). However, Sahrawat et al. (2014) found that 5 days of IC administration to a group of healthy adults did not significantly affect F₀. With these factors in mind, phonation of experimental larynges treated with ICs was expected to be either similar to that of control larynges if no pathologic changes were noted, or to have significantly lower F₀ in the case that pathologic changes were associated with IC administration. Results of the current thesis are surprising in that F₀ was significantly higher for experimental versus control larynges. When interpreted in conjunction with other data collected, this change in F₀ is likely related to the increased subglottal pressure needed to sustain phonation. F₀ increases as subglottal pressure increases (Lieberman et al., 1969). Higher F₀ and higher subglottal pressures similarly correlate in experimental larynges in the current thesis.

Changes in F₀ may also be due to factors related to the benchtop model that are less-frequently explored in the prevailing literature. Sahrawat et al., (2014) and Silva et al., (in press) reported F₀ changes in relation to in vivo human phonation. The current thesis measures F₀ in ex vivo larynges using the benchtop method. In in vivo phonation, thyroarytenoid, cricothyroid, lateral cricothyroid, and posterior cricothyroid muscle activation control phonation and affect F₀. Using the benchtop model, lateral cricothyroid and thyroarytenoid muscle action is simulated using micropositioners. The vocal folds are vibrated using increased subglottal pressure and
airflow according to the myoelastic aerodynamic theory. It is possible that unexpected changes in
$F_0$ are due to a lack of muscle activation in addition to a treatment effect.

**Visual–Perceptual Results**

In addition to aerodynamic and acoustic data, this thesis utilized visual–perceptual
measures. Results show that visual–perceptual ratings on an equal appearing interval scale of 0–3
demonstrate significantly higher edema and erythema of vocal fold and arytenoid tissues in
experimental versus control larynges. It is surprising to find increases in edema for larynges
treated with ICs, as ICs are used as an anti-inflammatory drug to treat the symptoms of asthma
(Sahrawat et al., 2014; Uhlik et al., 2007). While these drugs have an anti-inflammatory effect on
the tracheal epithelium, their possible inflammatory effect on laryngeal tissue appears to be
contradictory. However, particles of IC drugs settle on and affect laryngeal structures differently
than the trachea. This drug deposition seems to cause a chemical-type injury, resulting in
increased edema of laryngeal structures (Erickson & Sivasankar, 2010; Hassen & Hasseba,
2016). While not initially suspected, the increases in edema for experimental versus control
larynges is likely related to the effects of ICs on vocal fold and arytenoid tissues.

Significant visual–perceptual differences between groups must be interpreted carefully
due to the subjective nature of perceptual studies. Results and data are influenced by the bias of
raters, specifically, their experience with the assigned population, possible time-constraints,
possibly limited sustained attention, or drifting in internal representations due to continued
exposure. This thesis utilized an external reference for purposes of increasing inter- and intra-
rater reliability due to characteristically low reliability in similar studies (Beaver et al., 2003;
Kreiman & Gerratt, 1998). While inter-rater reliability was generally good between five raters
for eight ratings of edema and erythema, intra-rater reliability ranged from poor to acceptable. With low reliability, significant differences between groups must be treated with caution.

**Limitations**

The results of this study may be subject to limitations. While the benchtop method used for aerodynamic and acoustic data collection was highly standardized, the possibility of human error during dissection and trial-and-error laryngeal mounting on the benchtop is one limitation of the current thesis. Researchers received short trainings prior to dissection and data collection and relied on the advice of experienced researchers throughout the data collection process. Despite trainings and expert advice, human mistakes may have affected larynx preparation and, thereby, data collection.

A significant limitation of the visual–perceptual ratings is that intra-rater reliability ranged from 33–83 percent agreement. Although percent agreement is a rigorous measure of reliability, this is not sufficient for data to be considered reliable. Limited resources related to the COVID19 pandemic likely negatively affected the reliability of visual–perceptual results. The importance of rater training is demonstrated by Cammarota et al., (2006), who reported training raters for 2 months prior to collecting visual–perceptual data. These raters had relatively high agreement, with a kappa coefficient of 0.89. In the current thesis, rabbit larynges were limited, and all images were experimentally blinded and rated for severity. Due to restrictions on lab use and physical gatherings resulting from the COVID19 pandemic, raters were not able to be trained in using the 0–3 equal appearing interval scale with an alternate set of comparable laryngeal images. Raters were also required to complete ratings on personal electronic devices rather than in a highly controlled, lab environment. If raters had been able to train and practice reliability prior to participating in ratings, and if they had been able to complete ratings in highly
controlled environments, intra- and inter-rater reliability may have been higher. Limited resources also negatively affected the validity of visual–perceptual measures. Although standard procedures were used in the collection and normalization of images, some images were unclear. This may have made ratings more difficult as well as distracted raters, leading to unforeseen changes in their perception of such images. Factors leading to limitations in reliability and validity of visual–perceptual ratings should be addressed to improve future studies.

While this study may have limitations and results should be interpreted cautiously, all rabbit larynges were subject to the same variables and the experiment was highly controlled. Therefore, significant differences between experimental and control groups noted in this thesis should be considered in the literature base and in decisions regarding future research studies.

**Recommendations for Future Studies**

To overcome possible limitations faced in the current thesis, future studies should consider the following recommendations. First, research assistants and participants should receive proper training. Regarding dissection and preparation of laryngeal tissues for aerodynamic data collection, future studies should ensure sufficient training of research assistants to reduce human error. To increase reliability for future visual–perceptual studies, it is recommended that raters be required to participate in preliminary, intensive training programs. While resources for the current thesis were limited, images collected and normalized for the current thesis may be used as training material for future, related visual–perceptual studies.

If visual–perceptual ratings are used in future benchtop research, the following recommendations should be considered to improve the quality of data. Images should be clear and normalized to ensure valid ratings. To ensure sufficient resources for obtaining adequate images, several images should be obtained for each larynx. These images can be compared, and
the most representative image chosen for use in the study. It is also recommended that external references be used to prevent drifting internal representations. Future studies should consider using increased numbers of subjects. Specifically, repeating a greater number of images for visual–perceptual intra-rater reliability and including a greater number of raters could increase statistical power and significance of study results.

In conjunction with visual–perceptual ratings, this thesis lays the foundation for using sustained subglottal pressure, sustained airflow, and $F_0$ to differentiate between groups of larynges. While PTP and PTF have been typically used in such studies, research demonstrates the importance of including several data points to better understand the physical characteristics of larynges and more easily translate findings to clinical populations (Plant & Hillel, 1998). When interpreted in conjunction, the unique dependent variables in this thesis demonstrated significant differences between experimental larynges treated with ICs and control larynges treated with a nebulized isotonic saline solution. Future studies should include unique and novel as well as traditional dependent variables to better describe the effects of independent variables on vocal fold tissue and phonation.

**Conclusion**

This study found that experimental larynges treated with 8 weeks of inhaled corticosteroid drugs differed significantly from control larynges treated with 8 weeks of an inhaled nebulized isotonic saline solution. Experimental larynges required higher sustained pressure and higher sustained airflow to maintain phonation. Similarly, experimental larynges received overall higher severity ratings for edema and erythema of vocal fold and arytenoid tissue. Rabbit larynges have been shown to have similar histology to human larynges, and previous rabbit benchtop studies have demonstrated that this species is adequate for translational
research (Prigmore, 2020). As all these dependent variables are associated in the literature with pathologic voices and damaged vocal folds, it follows that IC treatment caused significant damage to rabbit larynges when compared to the control treatment, nebulized isotonic saline. These findings should be considered in the planning and fulfillment of research related to the effects of ICs on human populations.
References

ADInstruments (2015). *LabChart data acquisition software* (Version 8) [Software].
https://www.adinstruments.com/products/labchart

https://doi.org/10.1121/1.2908289

https://doi.org/10.1067/mhn.2003.10

https://doi.org/10.1097/00005537-200108000-00001


The MathWorks Inc (2010). *MATLAB* [Computer Program]

https://www.mathworks.com/products/matlab.html


APPENDIX A

Annotated Bibliography

This appendix contains a review of research articles used in the formation of the research questions and experimental design of this thesis, including the use of animal larynges, the benchtop model, acoustic, aerodynamic, and visual-perceptual data. For each article, the purpose, method, results, and conclusion are addressed as well as the article’s relevance to the current work and its reference.


[https://doi.org/10.1067/mhn.2003.10](https://doi.org/10.1067/mhn.2003.10)

**Purpose of this work:** Researchers focused on the use of laryngeal imaging to rate and classify laryngopharyngeal reflux disease both before and after 6 weeks of treatment using a proton pump inhibitor.

**Method:** Participants undergoing videolaryngoscopy were recruited for this study. Still laryngeal images were extracted from endoscopies and then rated by three otolaryngologists. There were 98 experimental images collected from 49 patients with laryngopharyngeal reflux disease, one pre- and another post-treatment for each patient, and there were 10 images collected from the initial examinations of healthy individuals. Using the Laryngopharyngeal Reflux Disease Index, otolaryngologists rated edema and erythema of the supraglottal, glottal, and subglottal regions. Raters were blinded to patient diagnosis and images were presented randomly. Scores were given on a scale of 0 to 3, with 3 indicating the most severity.
**Results:** Scores for patients with laryngopharyngeal reflux disease were significantly elevated when compared to scores of healthy individuals (p < .001). Ratings also indicated significant improvement on all post-treatment scores (p < .001) with a moderate effect size. Intrarater reliability was good, however, interrater reliability was only fair with a low level of agreement between raters.

**Conclusion:** Authors concluded that the Laryngopharyngeal Reflux Disease Index is a reliable and valid assessment of laryngopharyngeal reflux disease and that 6 weeks of proton pump inhibitor treatment is sufficient to make notable improvement in the reduction of edema and erythema of patients with laryngopharyngeal reflux disease.

**Relevance to the current work:** This study differentiates laryngeal images based on ratings of edema and erythema of the supraglottal, glottal, and subglottal regions. The current work differentiates still images of larynges with adducted vocal folds by rating edema and erythema. One major difference between this study and the current thesis is that ratings are made of human subjects in this study and ex vivo rabbit larynges in the current thesis.

https://doi.org/10.1097/00005537-200108000-00001

**Purpose:** Researchers evaluated the use of the Reflux Finding Score in assessing laryngoscopic images of individuals with laryngopharyngeal reflux.

**Method:** Forty subjects with laryngopharyngeal reflux and 40 age-matched control subjects received flexible endoscopy. Laryngeal images were collected before treatment onset and again at 2, 4, and 6 months after the onset of treatment. Images were
scored using the Reflux Finding Score for subglottic edema, ventricular obliteration, erythema/hyperemia, vocal fold edema, diffuse laryngeal edema, posterior commissure hypertrophy, granuloma/granulation, and thick endolaryngeal mucus. Final scores on this scale can range from zero to 26, with zero indicating no pathology, 11 and higher indicating laryngopharyngeal reflux, and 26 indicating severe pathology. Both intra- and inter-rater reliability were determined for the two laryngologists that completed the ratings.

**Results:** Pre-treatment Reflux Finding Scores for subjects with laryngopharyngeal reflux had a mean of 11.5, which improved to 9.3 at two months, 7.3 at four months, and 6.1 at six months post treatment onset. Mean Reflux Finding Scores for control subjects was 5.2. Inter- and intra-rater reliability were determined for both total Reflux Finding Scores and for individual items; all correlation coefficients were greater than 0.90.

**Conclusion:** Longitudinal comparison of Reflux Finding Scores demonstrates good validity and treatment efficacy. Correlation coefficients greater than 0.90 indicate good inter- and intra-rater reliability for both individual items and the total Reflux Finding Score.

**Relevance to the current work:** This study examines different attributes of still images of vocal folds, including edema and erythema, in order to evaluate improvement in subjects with laryngopharyngeal reflux. This relates to the current thesis, which rates still images of excised larynges for edema and erythema to determine the effects of inhaled combination corticosteroid (IC) drugs on vocal fold health.
Purpose: The purpose of this study was to quantify intraglottal pressure during the opening phase of the vibratory cycle during both sustained phonation and voice onset. Researchers explored the relationship between intraglottal pressure and other dynamic vibratory characteristics of the vocal folds during phonation.

Method: This study used previous recordings of phonation from one male participant. Phonation samples within the ranges 95–125 Hz and 60–70 dB were analyzed for glottal area, glottal flow, sound pressure level, and average speaking frequency. Intraglottal pressure was calculated previous to this study. Analysis included measurements during both sustained phonation and phonation onset.

Results: During both sustained phonation and phonation onset, intraglottal pressure was greater during the opening phase than during the closing phase. Because the net force on the vocal folds was sufficiently positive, intraglottal pressure was sufficient to support sustained phonation. Greater intraglottal pressure correlated with higher intensity phonation. Measurements of airflow showed skewing, or a slight lag behind the glottal area curve.

Conclusion: Researchers concluded that sustained phonation is supported by positive intraglottal pressure as the pressure during the opening phase is greater than the pressure during the closing phase. Additionally, the skew of the glottal flow may be due to the compression of the air in the vocal tract and the inertance of the vocal tract. These
characteristics of vocal fold vibration are present, though to a lesser extent, during phonation onset.

**Relevance to the current work:** This study relates to the current work in that it collects measurements during sustained phonation. The current thesis collects aerodynamic measures of subglottal pressure and flow during sustained phonation to describe the effects of ICs on the voice.


**Purpose:** Researchers examined the effects of subglottal pressure, airflow, and glottal adduction on the vibratory patterns of excised human hemilarynges.

**Method:** Three human larynges were harvested within 24 hours postmortem. After dissection, hemilarynges were mounted on an air source alongside a glass plate to replace the mass of the second vocal fold. The vocal fold was adducted to varying degrees of glottal closure via attachment of different sized weights to the arytenoid cartilage. The weights, sizes 10, 50, and 100 g, applied pressure that acted to replace the force of the lateral cricoarytenoid muscle. The medial edge of the vocal fold was tracked via 30 microsutures in the mucosal epithelium that were visible in high–speed video.

Thirty phonation trials with varying levels of glottal adduction and subglottal pressures (between 0.9 and 4.3 kPa) were performed. Data were collected on airflow, sound pressure level, fundamental frequency (F₀), laryngeal airflow, maximum displacement of the vocal folds in lateral and vertical directions, and maximum velocity of vocal fold
vibration. Dynamic movement of the vocal folds was described using empirical eigenfunctions.

**Results:** Statistical analyses were not performed on the data collected due to the small sample size. Subglottal pressure and airflow during sustained phonation varied linearly with the range of subglottal pressures being 0.97–4.30 kPa and the range of airflow being 500–1800 mLs. The three larynges responded to varying levels of adduction differently. With stable subglottal pressure and incrementally increasing adduction, one larynx showed increased airflow while the other two showed decreased levels of airflow. During all experiments, $F_0$ was 97–200 Hz and typically increased linearly with subglottal pressure. Sound pressure level also increased with subglottal pressure and ranged 78.0–98.8 dB. There was no correlation between subglottal pressure and vocal fold displacement. Generally, lateral displacement of vocal folds was greater than vertical displacement.

**Conclusion:** Different larynges responded to levels of sustained pressure and increased glottal adduction differently. As subglottal pressure and airflow were constant, increased adduction led to higher amplitude of vocal fold vibration. The preliminary importance of the balance between lateral and vertical aspects of vocal fold vibration was noted. Future research should use larger sample sizes so that statistical analyses can be performed.

**Relevance to the current work:** This study is relevant to the current work as both measure subglottal pressure and airflow during sustained phonation in excised larynges. While this study measures changes within human hemilarynges, though, the
current work measures differences between rabbit larynges treated with either ICs or a control nebulized isotonic saline solution.

https://doi.org/10.1121/1.5043384

**Purpose:** The purpose of this study was to research the aerodynamic and acoustic parameters of phonation using ex vivo rabbit larynges. Researchers explored the correlation between size of glottal opening and phonation airflow and acoustics.

**Method:** New Zealand White rabbit larynges were harvested and prepared for data collection using a benchtop model. Measurements included subglottal pressure, sound pressure level, and high–speed video. The 11 larynges were each phonated 45 times at the following glottal width configurations: complete closure, partial closure, and no vocal fold contact. The first phonation trial was conducted at the rabbit's phonation threshold pressure (PTP). For each of the subsequent 14 trials, airflow was manually increased 0.5 l/min per trial. Data on glottal area waveform, glottal closure, laryngeal tissue characteristics, opening and closing characteristics, dynamic left–right symmetry, subglottal pressure, harmonics, perturbation, F0, airflow, average subglottal pressure, sound pressure level, and laryngeal flow resistance for each trial were collected and used for statistical analyses. The glottal gap index reflected the glottal width configuration during vibration and was compared to increased vocal fold tension, increased airflow, and other aerodynamic and acoustic measurements. Finally, histological analyses were
performed to ensure that healthy vocal fold tissue was used to collect the data used in this study.

**Results:** There was a significant decrease in glottal gap index both as vocal fold tension and as airflow increased. Significant differences were found in glottal waveform measurements between complete glottal closure and no vocal fold contact configurations, including amplitude-to-length ratio, stiffness, asymmetry quotient, closing quotient, open quotient, maximum area declination rate, speed quotient, and amplitude symmetry index. Between all three glottal configurations, statistically significant differences were found in all aerodynamic measures, including laryngeal flow resistance, average subglottal pressure, and sound pressure level. Measures of harmonics and perturbation, including percent jitter and shimmer, harmonics-to-noise ratio, and cepstral peak prominence were significant in the acoustic signal but not in the subglottic pressure signal.

**Conclusion:** This study confirmed past research claims that airflow, F0, and sound pressure level all increase with increased subglottal pressures. By increasing vocal fold tension and glottal airflow, the glottal gap index was reduced, and aerodynamic measures and acoustic quality are improved. Therefore, treatment for glottal closure insufficiency could include increased vocal fold tension and/or airflow. When comparing findings to past research on ex vivo rabbit larynx phonation, this study found lower PTP, higher average airflow, a wider range of sound pressure levels, and a higher range of F0. The most productive glottal vibration configuration for aerodynamic and acoustic measurements was complete closure.

**Relevance to the current work:** This study relates to the current work in that it measures and controls for subglottal pressure during sustained phonation. Both this study
and the current work phonate ex vivo rabbit larynges on benchtop to collect aerodynamic, acoustic, and visual information about vocal fold vibration.


**Purpose:** This study tested a method for restraining pigs without using anesthetics or chemical sedation so that nebulized isotonic saline could be administered comfortably. Finding a restraining method without anesthesia or chemical sedation is important to solve timing issues and prevent possible confounding side-effects. Isotonic saline was used in these trials because it is comparable to extracellular fluid and considered the gold standard for experimental trials on voice.

**Method:** Pigs voluntarily walked into specially designed sling restraints. Researchers administered nebulized isotonic saline to six adult female pigs three times a day for 20 days. The pigs were then sacrificed so that their upper airways could be examined for any negative effects of the saline solution.

**Results:** Researchers reported that the pigs seemed to enjoy the sling as they were reluctant to leave it after the nebulized isotonic saline was administered. After 60 administrations of the saline solution, the pigs were found to have normal histology nasally, in the lungs, and on the vocal folds.

**Conclusion:** The sling method used in this study is a viable option for administering treatments to large animals without using chemical sedation or anesthesia.

**Relevance to the current work:** The current thesis administered either a treatment (i.e., ICs) or a control (i.e., nebulized isotonic saline) to rabbits in order to
compare the effects that they have on the voice. Isotonic saline is an appropriate control
treatment in the current thesis as it has the same composition as extracellular fluid.
Confounding effects are avoided by not using chemical sedation or anesthesia.

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

**Purpose:** The purpose of this study was to examine the effects of IC treatments on
phonation via measurement of perceived phonatory effort and PTP. The relationship
between perceived phonatory effort and PTP was also examined.

**Method:** Participants included nine women and five men that were taking Advair
diskus® as an IC treatment for asthma. They each participated in two data collection
sessions in random orders, once receiving an IC treatment and once receiving a placebo
treatment. Data collection sessions first included measurements of baseline pitch glides,
PTP, perceived phonatory effort, and forced vital capacity measures. PTP was collected
via pneumotachograph mask, forced vital capacity was measured via spirometer, and
perceived phonatory effort was measured through self-ratings on a visual analogue scale.
These measures were collected immediately, one hour, and again at two hours post-
administration of either the IC or the placebo treatment.

**Results:** Statistical analysis of data revealed a significant raise in PTP at the 80th
percentile pitch for the IC treatment versus the placebo treatment group. There was no
significant increase in PTP for the placebo group, and the increase in PTP for the IC
treatment group was maintained for two hours after treatment. There was no significant
difference in PTP between groups for either the 10th or 20th percentile pitches. No
A significant difference was found between groups for perceived phonatory effort ratings. No significant correlation was found between perceived phonatory effort and PTP.

**Conclusion:** A significant negative effect of ICs on the voice was observed through PTP measurement during high pitch phonation. This concurs with previous research suggesting that vocal fold mucosal changes are easiest to observe during vocally challenging tasks, such as high-pitch phonation. Study limitations include a small sample size, unequal male and female participants, and limitations to participant blinding. Future research may strengthen evidence that ICs have a negative effect on the voice.

**Relevance to the current work:** This study relates to the current work in that it examines the negative effects of ICs on the voice. With a slightly larger sample size, gender control, and no need of participant blinding to IC versus control treatment, the current work overcomes some limitations of this study and contributes a stronger research design.

https://doi.org/10.5606/kbbihtisas.2016.79740

**Purpose:** The purpose of this study was to examine the effects of IC treatment on the voice through acoustic and physical laryngeal measures.

**Method:** Participants for this study included 15 males and 15 females ages 16–27. Each participant received ICs for a minimum of 4 months immediately prior to the current study. For each participant a case history was collected and a speech sample was rated for dysphonia using a modified GRABS scale. Videolaryngoscopy was performed and laryngeal recordings were examined for vocal fold edema and erythema, vocal fold
bowing and atrophy, irregular vocal fold edges, interarytenoid thickening, and supraglottic hyperfunction. A sustained /a/ vowel was acoustically analyzed for F0, percent jitter and shimmer, noise to harmonic ratio, soft phonation index, and phonatory frequency range in semitones.

**Results:** Mild to moderate dysphonia was noted in 53% of participants, however, the correlation between duration of IC use and the severity of dysphonia was not significant. Significant laryngeal findings included interarytenoid thickening and vocal fold erythema in 56.7% of participants, supraglottic hyperfunction and irregular vocal fold edges in 53.3% of participants, and vocal fold edema in 36.7% of participants. Percent shimmer and noise to harmonic ratio were also significantly different for participants taking ICs when compared to normal values.

**Conclusion:** Authors concluded that participants demonstrated dysphonia, physical laryngeal changes, and raised acoustic measures. The physical laryngeal changes were not attributed solely to IC use and could be due to other factors relating to asthma. Individuals taking ICs are at a higher risk for dysphonia.

**Relevance to the current work:** This study relates to the current thesis in that both examine the effects of IC treatments on the voice. The current thesis uses rabbits in a between–groups case–control experimental design in order to isolate the effects of ICs from the effects of asthma or other health concerns on the voice. Both this study and the current work also use physical attributes of the vocal folds, including visual–perceptual ratings of vocal fold edema and erythema, in order to describe the effects of ICs on the voice.

**Purpose:** The research in this article was conducted in order to provide evidence that hydration influences the voice. Specifically, researchers studied the effects of dehydration on perturbation and noise-to-harmonics ratio.

**Method:** Participants for this study included four men and four women, ages 28–43, with no existing voice conditions. They inhaled 10 minutes each of hydrated, standard, and desiccated air in a random order. After each condition, they sustained an /a/ vowel. Recordings were collected and measured for relative perturbation and noise-to-harmonics ratio.

**Results:** Results showed significantly increased vocal perturbation following the desiccated condition and no significant difference between the standard and humidified air. Researchers did not find significant differences in noise-to-harmonics ratio between any of the three conditions.

**Conclusion:** This study concluded that dehydration has a significant effect on vocal perturbation. The vocal folds are very sensitive to conditions of dehydration, as differences in phonation were noted after only 10 minutes of exposure to desiccated air.

**Relevance to the current work:** The current thesis uses excised leporine larynges to collect aerodynamic measures of voice in various conditions. To prevent dehydration from affecting data, the larynges are carefully stored in hydrated conditions, frequently hydrated throughout the desiccation and data collection process, and phonated using hydrated air.

*The Laryngoscope, 103*(8), 872–882.

https://doi.org/10.1288/00005537-199308000-00008

**Purpose:** This study compared the difference in aerodynamic, acoustic, and physiological measurements between excised full larynges and hemilarynges. Researchers aimed to create a reliable, replicable method for excised larynx benchtop studies.

**Method:** Researchers collected nine canine larynges 15 minutes post-mortem. They resected the epiglottis, upper portion of the thyroid cartilage, and false vocal folds prior to benchtop mounting. On the benchtop, they used 2 three-pronged micropositioners to adduct the vocal folds via the arytenoid cartilages. The larynx was attached to a micropositioner positioned anteriorly via a string. The benchtop was equipped with an air source, a humidifier, and a pseudolung. Trials were first run on full larynges, then larynges were cut in half and trials were run on hemilarynges. Data were collected on subglottal pressure, airflow, F0, sound pressure level, and amplitude of vibration. Both PTP and phonation instability pressure were observed. Phonation instability pressure corresponds to the subglottal pressure level at which vocal fold vibration becomes irregular and phonation becomes unstable. Glottal flow, sound pressure level, F0, and vibrational amplitude were all analyzed in relation to subglottal pressure level.

**Results:** Between hemilarynges and full larynges, no statistical differences were found for F0, subglottal pressure, or amplitude of vibration. Airflow in hemilarynges was approximately doubled and sound pressure level was about 6 dB softer than that of full larynges. F0 and vibrational amplitude were both reported to increase as subglottal
pressure increased. Airflow and sound pressure level graphically appeared to increase with increases in subglottal pressure.

**Conclusion:** There was high variability between larynges, making it difficult to draw conclusions based on group averages. Hemilarynges may be a suitable alternative to full larynges in excised benchtop model studies of the voice.

**Relevance to the current work:** This study outlines the dissection and benchtop methods used in the current work. It also highlights the importance of subglottal pressure by using it as the comparison for all other observed aerodynamic, acoustic, and physical vocal fold vibratory measures.


**Purpose:** The purpose of this study was to examine the effect of transglottal air pressure on rate of change of F₀ during phonation.

**Method:** This study collected data from one healthy male participant in two sessions. In the first, subglottal pressure was measured via esophageal balloon during sustained phonation in either a "soft" or a "loud" voice at different pitches. The second session was conducted similarly to the first, however, the participant’s hearing was masked while recording utterances (i.e., the participant could not hear his own voice during phonation and recordings). The rate of change of F₀ was compared to transglottal pressure measurements at both soft and loud intensities to determine whether there were significant correlations.
**Results:** As transglottal pressure decreased, a decrease in signal amplitude (soft versus loud) and F0 were also observed. The rate of change of F0 was 3–18 Hz for each 1 cm H2O change in transglottal pressure. Transglottal pressure had the greatest effect on F0 (i.e., caused the highest rate of change) during both softer and higher pitch phonation. The minimum subglottal pressure recorded to sustain phonation in this study was 2–3 cm H2O. Whether or not the participant could hear his own voice did not significantly impact the rate of change of the F0 in relation to transglottal pressure.

**Conclusion:** Sustained transglottal pressure affects F0, with higher pressure leading to higher F0. It is important to note that vocal fold tension also plays a large role in the change of F0.

**Relevance to the current work:** This study relates to the current thesis in that it explores the effects of different levels of transglottal pressure on the voice during sustained phonation. The current thesis uses subglottal pressure as a measurement of phonation to better understand the effects of ICs on the voice. Any changes in subglottal pressure could also relate to changes in F0 or vocal fold tension, thus it is important to record F0 and avoid vocal fold elongation during vocal fold mounting and data collection.


**Purpose:** Researchers describe the difference between trained and untrained voices using electroglottography and aerodynamic measures.

**Method:** Participants in this study included 40 individuals ages 27–47. They were divided into two groups of 20 with 10 men and 10 women in each group. The first group
included actors with over 5 years of acting experience and at least 3 years of voice training. Individuals in the second group had never used their voices professionally or received voice training. In a single data collection session, individuals completed several phonatory tasks at low, medium, and high acoustic intensities. Measurements were made during repeated /pa/ syllables, sustained /a/ phonation, and a connected speech sample while reading the Grandfather passage. Acoustic measures included sound pressure level (dB) and F0 (Hz). The electroglottograph measured the contact quotient as a percent. During sustained vowel phonation, average phonatory airflow was measured. Aerodynamic measurements during /pa/ repetition included average phonatory airflow, average subglottal pressure, and aerodynamic power, resistance, and efficiency. Aerodynamic measurements during connected speech included inspiratory airflow volume and duration, average phonatory airflow, and average inspiratory airflow. Multivariate linear regression analysis was regarded as the most accurate method of determining statistical significance.

Results: Based on results from multivariate linear regression analysis, individuals from the group with vocal training had higher phonatory airflow, subglottal pressure, and sound pressure levels. They had higher inspiratory volume, mean inspiratory airflow, and inspiratory durations. Those with vocal training also had lower glottal resistance, lower F0, and their F0 was not as dependent on sound pressure level. There was a positive correlation between both increases in sound pressure level and subglottal pressure and increases in sound pressure level and aerodynamic power.
**Conclusion:** The main differences between trained and untrained voices were noted in aerodynamic rather than glottal factors, indicating the importance of respiratory support.

**Relevance to the current work:** This study is relevant to the current work in that it provides a foundation for the use of airflow during sustained phonation and mean subglottal pressure to differentiate between phonation in two different populations.


**Purpose:** The purpose of this study was to report PTP and phonation threshold flow (PTF) in excised human larynges, confirm the presence of hysteresis in human larynges, and determine the effects of posterior glottal width and age on PTP and PTF.

**Method:** Researchers collected nine human larynges and performed all data collection procedures within 24 hours post-mortem. They dissected all tissue above the true vocal folds, including the ventricular folds, to expose the true vocal folds. The larynges were mounted on a benchtop air pipe with a hose clamp and micrometers, and air was passed through them to stimulate phonation. Data were collected via a microphone, pressure manometer, flow meter, electroglottograph electrodes, and a sound level meter. Researchers examined the effects of gender and posterior glottal width on PTP and PTF at onset and offset.

**Results:** Hysteresis, or the change in pressure and flow from onset to offset in the excised human larynges was observed. There was high variability in PTP and PTF between trials and between larynges even when matched for glottal width. Onset PTP and
PTF measures fluctuated more than offset measures. In male larynges, PTF onset and offset values were significantly higher than in female larynges. No significant correlation between glottal area and either PTP or PTF onset and offset was observed.

**Conclusion:** Findings demonstrated high variability between individuals, which should be expected in a clinical setting. Additionally, very different values were seen in male versus female larynges. Gender should always be considered or controlled for in future studies. As offset measures were significantly more stable than onset measures, they might be considered in future research to be more accurate descriptors of voice.

**Relevance to the current work:** Similar to this study, the current work uses a benchtop model to determine subglottal pressure. While this study uses human larynges, rabbit larynges controlled for age and gender are used in the current thesis. Findings from the current thesis will eventually be translated to the possible effects of ICs on the human phonatory system.

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**Purpose:** The purpose of this study was to adapt the ex vivo larynx benchtop model for use with rabbit larynges. Adaptation was necessary because rabbits are much smaller than animals previously used, such as the pig and the canine. Rabbit larynges are a valuable resource for voice studies as they have similar histology to human larynges.

**Method:** Researchers finely dissected five rabbit larynges to reveal the true vocal folds. They were mounted on benchtop through an anterior suture and lateral micropositioners. Data were collected for each phonation trial for each larynx, including
electroglottography, high-speed video, airflow, subglottal pressure at onset and offset, acoustic F₀, jitter, shimmer, signal-to-noise ratio, and amplitude and phase difference. Data were analyzed for variability and were compared to measures in canine larynges.

**Results:** Measurements collected from rabbit larynges had similar coefficients of variation to those obtained from canine larynges, indicating low variability between trials in a single larynx. Discrepancies observed in past research on canine larynges were also observed in rabbit larynges. Mucosal wave was found to have a large standard deviation and there was inconsistency between acoustic F₀ and electroglottography.

**Conclusion:** The rabbit larynx is a viable model for ex vivo studies of the effects of pathologies and environmental factors on the vocal folds. The rabbit is relatively inexpensive to house and care for, is more easily procured than human larynges, and has similar histology to the human larynx.

**Relevance to the current work:** This study contributes to the research base for using ex vivo rabbit larynx models in the current thesis. The rabbit larynx is ideal for the current thesis as it is easy to house, care for, and procure, and it has similar histology to human vocal folds.


**Purpose:** The purpose of this study was to describe in vivo rabbit phonation using humidified airflow through an Isshiki type IV thyroplasty.

**Method:** This study included six male New Zealand White rabbits weighing 3–4 kg. Phonation was elicited in vivo using an Isshiki type IV thyroplasty and humidified
subglottal airflow. Measurements to describe phonation included F0 (Hz), vocal intensity (dB), subglottal pressure (cm H2O), and airflow (mL/s). Rabbits were subsequently sacrificed and larynges were harvested. MRI was conducted in order to validate glottal configuration and laryngeal models used in previous research simulations.

**Results:** Measurements were averaged across specimens. Average vocal intensity was 61.39 dB, average F0 was 590.25 Hz, average airflow rate was 85.91 mL/s, and average subglottal pressure was 9 cm H2O.

**Conclusion:** The phonation elicitation method used in this study was similar to that elicited via neuromuscular stimulation in previous studies. Several benefits to the current model include maintenance of glottal configuration for future imaging and use of constructed models to test other specific aspects of phonation. Stimulated computations can be validated against measurements originally made in the physical model.

**Relevance to the current work:** Though it uses a different elicitation technique, this study relates to the current work in that it uses mean subglottal pressure during sustained phonation as a measure of rabbit phonation.


**Purpose:** Researchers compared the validity and reliability of using videostroboscopy versus high–speed videoendoscopy to make visual–perceptual ratings of individuals with vocal mass lesions both before and after surgical removal.

**Method:** Both videostroboscopy and high–speed videoendoscopy samples were
obtained from 28 patients with vocal fold mass lesions both before and after operational removal. Video samples were also collected for 17 vocally healthy patients to be used as a control group. All samples were rated by two expert raters for mucosal wave, amplitude, phase asymmetry, and vocal fold edge. Ratings were compared within and between groups as well as with measurements of vocal fold lesions to determine the reliability and validity of rating videostroboscopy and high–speed videoendoscopy images in making clinical decisions.

**Results:** For both high–speed videoendoscopy and videostroboscopy samples, ratings of vocal fold edge and amplitude of vibration were significantly related to the total measured area of vocal fold mass lesion. Ratings for mucosal wave changes and left–right phase asymmetry were also significant, though ratings made with high–speed videoendoscopy were more reliable than those based on videostroboscopy. Due to sample limitations (i.e., inability to synchronize with the F0 of pathologic voices in videostroboscopy and/or inability to rate high–speed videoendoscopy due to visual obstructions caused by vocal fold mass lesions), perioperative measures comparing pre- and post-operative ratings were not obtained for 46% of videostroboscopy samples and 11% of high–speed videoendoscopy samples.

**Conclusion:** Due to the difficulty in rating pathologic voices using videostroboscopy, high–speed videoendoscopy may be preferred to measure perioperative differences in vocal fold vibratory characteristics. While ratings of both amplitude and edge were reliable using either technique, ratings of left–right phase asymmetry and mucosal wave were more reliable using high–speed videoendoscopy.


**Relevance to the current work:** The current work uses visual–perceptual measures to contribute to determining the health of vocal fold tissue. This study builds the research basis on the validity of using visual–perceptual ratings as a measure of vocal fold health.


**Purpose:** This study compared different signs of reflux laryngitis to determine the most accurate diagnostic measurements.

**Method:** Researchers examined the larynges of 108 subjects with complaints of some form of gastroesophageal disease and 90 healthy control patients. Images collected via videolaryngoscopy were rated for mucosal lesions, edema, and erythema of the vocal folds, ventricular folds, interarytenoid notch, and the arytenoid cartilages. Ratings were analyzed using logistic regression analysis to determine which laryngeal features could serve as the most accurate diagnostic measures.

**Results:** Rating mucosal lesions and edema of the vocal folds along with mucosal lesions of the interarytenoid notch was the most sensitive and adequately specific diagnostic measure of reflux laryngitis. Diagnostic accuracy increases by 21 times when mucosal lesions on the interarytenoid notch are noted.

**Conclusion:** Signs of edema and erythema of the larynx were significantly greater in those participants with reflux laryngitis diagnoses than in healthy controls (p < 0.001). As laryngoscopy rates mucosal lesions on the vocal folds, edema of the vocal folds, and mucosal lesions on the interarytenoid notch, specificity and sensitivity for diagnosis of reflux laryngitis was high (p < 0.05).
Relevance to the current work: This study relates to the current work as it examines visual–perceptual ratings of physical characteristics in order to diagnose laryngeal pathology. It establishes the validity of examining edema and erythema of different laryngeal structures to determine the health of the phonatory system. The current work uses visual–perceptual ratings of edema and erythema to differentiate between larynges treated with ICs and those treated with a control nebulized isotonic saline solution.


**Purpose:** The purpose of this study was to describe the correlation between phonation threshold power and different variations in excised canine larynges including posterior glottal width, vocal fold lesions, and vocal fold elongation.

**Method:** Researchers collected 30 excised canine larynges and randomly assigned them to one of three groups. They analyzed phonation threshold power with regards to either posterior glottal width, vocal fold length, or presence of vocal fold lesions, depending on the assigned group. After dissection, the larynges were mounted on benchtop to stimulate phonation. Data were collected by a microphone, pressure meter, and flow meter so that PTP and PTF could be determined for each trial and used to calculate phonation threshold power.

**Results:** Phonation threshold power correlated significantly with posterior glottal width and with the presence of vocal fold lesions. It correlated mildly with vocal fold length.
Conclusion: In excised larynges, phonation threshold power is a sensitive measure for various vocal fold pathologies. Experiments on human subjects are necessary to determine whether these findings can translate to human clinical evaluations.

Relevance to the current work: The current work examines PTP and PTF in order to determine the health of vocal fold tissue. This study demonstrates that many variables contribute to changes in these measurements. Effects of ICs on vocal fold tissue are expected to lead to increases in phonation threshold power.


Purpose: The authors examined the short–term effects of ICs on the voice, including a comparison of these effects between genders.

Method: Participants in this study included 30 healthy individuals (15 males and 15 females) ages 18–30 with no history of asthma or voice disorders. Both perceptual and quantitative auditory data were collected during recorded sustained vowels and a reading passage. Following baseline voice measurements on day one of the study, subjects inhaled 500 μg of the corticosteroid fluticasone propionate via metered–dose inhaler and a spacer during both a morning and an evening session. One hour after IC administration, audio samples of sustained vowels and a reading passage were collected. On the second through fifth days of the study, subjects inhaled ICs in the same manner during both morning and evening sessions. No further audio samples were collected until the evening of the fifth day of the study. On the sixth day, audio recordings of the voice were collected without prior IC administration. Audio recordings were analyzed for F0, first
and second formant frequency, first and second formant bandwidth, and long-time spectral analysis, including first spectral peak and spectral tilt. Auditory measurements were statistically analyzed to determine differences due to IC exposure.

**Results:** ICs had no significant effect on F0, the second formant frequency, first and second bandwidth frequency, or long–time spectral analysis. The first formant frequency was found to be significantly lower on the second recording of the vowel /i/ when compared to baseline and the third reading. Spectral tilt was also significantly lower in the second compared to the baseline recording and lower in the fourth when compared to the third recording. There was no significant difference in the effect of ICs between genders.

**Conclusion:** Although results were limited, there is some indication that ICs may have a negative effect on acoustic aspects of the voice. Authors emphasize that this is a preliminary study with several limitations and future studies could further their claim.

**Relevance to the current work:** The bases of both this study and the current work are to examine the effects of ICs on the voice. Acoustic, aerodynamic, and visual–perceptual measures are collected in this study. Aerodynamic and visual–perceptual data are analyzed as part of the current thesis, which introduces greater control by using rabbit subjects and excised larynges for data collection.


https://doi.org/10.1016/j.jvoice.2008.08.005
**Purpose:** Researchers of this study examined the validity of using laryngoelectromyography in diagnosing and measuring treatment outcomes for various laryngeal pathologies.

**Method:** Researchers retrospectively examined medical records for 751 participants. These records were collected from the patients of the principal author and his co-workers. Participants consisted of 492 females and 259 males ages 8 to 85 years with a mean age of 46.6 years. Records were reviewed for results of dynamic voice assessment and strobvideolaryngoscopy in addition to laryngoelectromyography for those patients with observed laryngeal movement disorders. Function of the right and left recurrent laryngeal nerves and right and left superior laryngeal nerves were examined.

**Results:** Stroboscopic examination revealed 689 patients as having paresis and 62 as having normal movement/function. Using laryngoelectromyography, these same patients were classified as 675 having paresis and 76 being normal. With stroboscopy as the "gold standard" diagnostic tool, laryngoelectromyography classified patients with 95.9% sensitivity and 77.4% specificity. For patients with arytenoid dislocation, laryngoelectromyography results were typically normal and movement disorders were typically noted through stroboscopy.

**Conclusion:** Laryngoelectromyography is a valuable tool in diagnosing laryngeal movement disorders, especially when differentiating between nerve damage and structural laryngeal limitations (i.e., cricoarytenoid fixation). Visual–perceptual examination is not sufficient to accurately determine the nature of laryngeal pathologies.

**Relevance to the current work:** This study demonstrates the need for different forms of assessment to understand the complete nature of laryngeal pathologies. The
current thesis uses both visual–perceptual ratings and aerodynamic measures to contribute to understanding the nature of vocal fold health of excised leporine larynges.


**Purpose:** The purpose of this study was to measure the contact pressure of the vocal folds in individuals with Reinke's edema and compare it to the contact pressure measured in individuals with healthy vocal folds.

**Method:** Researchers harvested two human larynges 24–48 hours post-mortem. Both were from female subjects; one was healthy and the other had grade I Reinke's edema. During benchtop phonation trials, subglottal pressure, airflow, sound pressure level, electroencephalography signals, and contact pressure between the vocal folds were measured. Subglottal airflow was increased slowly until sustained phonation was achieved; it was subsequently reduced until phonation ceased. Subglottal pressure at onset and offset were estimated at the threshold of 65 dB SPL due to high levels of ambient noise, though subglottal pressure was also measured and recorded throughout sustained phonation. Absolute contact pressure was calculated from the assumed baseline of zero contact during the open phase of the glottis.

**Results:** In the healthy larynx, PTP had a mean of 2.78 hectopascals (hPa) and a standard deviation (SD) of 0.35 hPa; corresponding contact pressure had a mean of 18.5 kilopascals (kPa) with a SD of 1.0 kPa. Maximum subglottal pressure in the healthy larynx had a mean of 8.58 hPa and a SD of 0.67 hPa. The maximum contact pressure had a mean of 34.0 kPa and a SD of 5.0 kPa. In the pathologic larynx, PTP was 4.46 hPa with
a SD of 0.09 hPa; contact pressure at phonation threshold had an average of 100 kPa with a SD of 70 kPa. Maximum subglottal pressure in the pathologic larynx reached an average of 14.00 with a SD of 1.10 hPa and corresponding maximal contact pressure reached a mean of 296 kPa with a SD of 24 kPa. Other measurements showed that in the pathologic larynx, $F_0$ was lower, harmonic noise energy level was lower, replaced with higher noise energy level.

**Conclusion:** Researchers concluded that there is increased contact pressure between the vocal folds in conjunction with Reinke's edema. This may lead to recurring vocal fold damage and prevent the lesion from healing.

**Relevance to the current work:** This study relates to the current work in that both measure subglottal pressure during sustained phonation in an excised larynx, benchtop phonation study.


[https://doi.org/10.1016/j.jvoice.2013.03.006](https://doi.org/10.1016/j.jvoice.2013.03.006)

**Purpose:** The purpose of this article was to report subglottal aspects of vocal fold vibration. Researchers examined subglottal resonant frequencies, subglottal pressure in relation to supraglottal pressure and flow, the extent to which supraglottal formants affect subglottal acoustics, and relations between subglottal resonance frequencies and pitch breaks between vocal registers.

**Method:** The subject for this study was one male vocalist with experience in phonation studies. Materials examined included a story recitation at comfortable pitch and loudness, sustained vowel phonation at a comfortable pitch and loudness, both
ascending and descending pitch glides, and the sustained vowel /æ/ with normal, breathy, and pressed phonation. Oral airflow was measured through a pneumotachograph, subglottal pressure was measured directly through cricotracheal puncture, and the acoustic signal was measured with a microphone.

**Results:** Researchers reported differences between normal, breathy, and pressed phonation types. Airflow and acoustic intensity were highest in normal phonation and lowest in breathy phonation. Subglottal pressure was lowest in breathy phonation and highest in pressed phonation. Average subglottal pressures were reported for breathy phonation as 9.5 cm H₂O, for normal phonation as 13.7 cm H₂O, and for pressed phonation as 20.1 cm H₂O. Subglottal pressure varied slightly between different vowels; peak-to-peak amplitude was smallest for the vowel /u/ and greatest for the vowels /i/ and /æ/. Maximal subglottal pressure correlated with the point of the maximal flow declination rate.

**Conclusion:** Due to differences in subglottal pressures for different vowels, researchers concluded that vocal tract resonance must influence subglottal pressure. This could be due to reflected sound energy during the open phase of vocal fold oscillation. Variations in subglottal pressures were found to be greatest for normal versus breathy and pressed phonations.

**Relevance to the current work:** This study reported on measures of airflow, subglottal pressure, and resonant frequencies during sustained phonation. This relates to the current work, which measures sustained phonation of ex vivo larynges to differentiate between those larynges treated with ICs and those that received control treatment.


https://doi.org/10.1080/01913120701425951

**Purpose:** Because of its frequent use as a treatment for asthma, researchers examined the histological effects of inhaled glucocorticosteroid (GSC) beclomethasone diapropionate (BDP) on the epithelium of the trachea and lungs.

**Methods:** New Zealand White rabbits were used as participants for this study as they have similar airway epithelium to humans. Researchers separated 15 rabbits into three groups. All rabbits were initially administered an anesthesia. The treatment group contained three rabbits, which received two puffs each (a single dose) of BDP treatment via metered–dose inhaler. The treatment control group consisted of six rabbits that received a single dose of a similarly administered inhaler containing a control treatment. The third group, containing six rabbits, was an untreated control group that only received the anesthesia. Rabbits in all three groups were sacrificed thirty minutes after treatment administration and airway epithelial tissues were examined through an electron microscope. Measurements included the number of goblet cells stimulated, effects to ciliated, Clara, and kinocilia cells, changes in secretions, and changes in the ability of the epithelial tissue to self-clean.

**Results:** The BDP inhaler significantly increased the quantity and speed of secretions of the goblet cells, which were subsequently exhausted, degenerated, and lost. The numbers of Clara cells remained largely unimpacted in all three groups, however, in both the treatment and treatment control groups, pathological changes were noted in these cells. The number of kinocilia cells was mildly decreased in both treatment and treatment control groups, though there was no significant difference between these two groups.
**Conclusion:** Relatively minimal injury was noted in response to placebo treatment and moderate injury was noted in response to BDP treatment. Ultimately, airway epithelium was impacted by administration of both the BDP treatment control and the treatment control. Researchers concluded that when compared to the untreated control, ICs have a detrimental effect on the health of airway epithelial cells.

**Relevance to the current work:** Similar to this study, the current work examines the negative effects of IC treatments. This study examines histological pathologies, which would be related to the aerodynamic and visual–perceptual changes of the vocal folds that are examined in the current work.


**Purpose:** The purpose of this study was to demonstrate the effects of dehydration on PTF in excised canine larynges.

**Method:** Researchers harvested 11 canine larynges for use in this study. Larynges were separated into three groups. The dehydration (i.e., experimental) group contained eight larynges, the hydration (i.e., control) group contained two larynges, and one larynx was phonated initially as a hydrated larynx and later under dehydration conditions. Phonation trials on dissected larynges were performed on benchtop. Each larynx was mounted, and subglottal airflow was increased until phonation occurred. Airflow was maintained for 10 seconds of sustained phonation and was removed for a three second rest period. This process was repeated 23 times for each larynx. Trials continued in two
larynges in the dehydration group until phonation ceased. For the dehydration group, subglottal air was not humidified, and the larynx was not hydrated with a saline solution between trials. For the control group, subglottal air was humidified and larynges were sprayed with a hydrating saline solution during each three second rest period.

**Results:** Average initial PTF in the dehydration groups ranged 133.9–661.8 mL/s compared to an average final PTF ranging 196.5–1219.2 mL/s. The difference in PTF between initial and final trials was significant in the dehydration group but not in the control group. For the larynx that was run first as a control and then as dehydrated, the difference in PTF between initial and final trials was significant only for the dehydrated condition.

**Conclusion:** Dehydration of the vocal folds leads to increased difficulty in phonation as measured through increased PTF. The greater the dehydration, the greater the PTF. Researchers hypothesized that increased PTF related to dehydration was likely specifically due to dehydration of the lamina propria of the vocal folds. As increasing dehydration eventually led to cessation of phonation, this study also supports the use of hydration therapy in treating dysphonia.

**Relevance to the current work:** This study relates to the current work by demonstrating the importance of airflow measurements in evaluating vocal fold vibration. Increases in airflow indicate increasing difficulty in phonation and may be due to dehydration or vocal fold pathology. While this study specifically calculated significance based on PTF, the current work examines airflow during sustained phonation.

Purpose: While several studies have examined phonation threshold power as a measure of vocal health in excised animal larynges, none had examined it in humans. This study compares phonation threshold power to PTP and PTF to determine whether it is a viable quantitative clinical measure to distinguish between a healthy control population, a population with vocal fold movement disorders, and a population with mass lesions on the vocal folds.

Method: This was a large study, including 100 control participants with no voice complaints or pathology, 94 individuals with vocal fold mass lesions (including cysts and polyps), and 19 individuals with vocal fold immobility (including paralysis and arytenoid dislocation). Of the participants with mass lesions of the vocal folds, 41 had polyps and were examined both before and after surgical polyp removal. Subjects were instructed to repeat the /pi/ syllable with an orally placed pressure transducer for collection of PTP at onset. They were also instructed to sustain /a/ through a flow meter with decreasing intensity for collection of PTF at offset. These values were multiplied to calculate phonation threshold power.

Results: Phonation threshold power significantly distinguished between the control group and the group with vocal fold movement disorders and vocal fold mass lesions and proved to be a more accurate measure than PTP and PTF. It did not significantly distinguish between the group with vocal fold movement disorders and the group with vocal fold mass lesions. PTF best distinguished between the control group and
the group with vocal fold movement disorders. PTP best distinguished between the control group and the group with mass lesions on the vocal folds.

**Conclusion:** Researchers concluded that phonation threshold power may be a viable quantitative measure to identify individuals with possible vocal fold pathology, including either a mass lesion or a movement disorder. Additionally, this study was consistent with previous research in concluding that PTP is more sensitive to vocal fold tissue pathologies while PTF is more sensitive to factors relating to adduction or abduction of the vocal folds.

**Relevance to the current work:** This study relates to the current thesis in that it - animal larynges, this study demonstrates that these findings can relate to humans. The current thesis analyzes the health of the vocal fold tissue in excised rabbit larynges by measuring subglottal pressure and airflow during sustained phonation. Because differences between groups in the current thesis are expected to be related to changes in vocal fold tissue, measures of subglottal pressure are expected to be the most accurate quantitative measure.
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-ST™)

Materials for data acquisition
- Dell computer
- Dell computer monitor
- PowerLab™ data acquisition hardware (ADInstruments)
- LabChart data acquisition software (ADInstruments, 2015)
- Microphone (Model SM-48, Shure, Niles, IL)
- High-speed camera (KayPentax, Montvale, NJ)
- Medical-grade air tank (2) containing compressed, low-humidity air (30 psi, <1% relative humidity)
- Physiological pressure transducer (Model MLT844, AD Instruments)
- Sphygmomanometer (AD Instruments)
- Syringe (25 cc/ml)
- Pressure calibration block
• Gauze (to decrease reverberation under pressure transducer)
• Velcro™ for securing transducers during calibration and data collection
• Pneumotach Calibration Unit (MCU-4, Glottal Enterprises)
• Audio Output Extension
• Bose™ Amplifier
• Pulse transducer (AD Instruments)
• AcuRite™ Hygrometer (Model 01083M)

Materials for benchtop and phonation trials
• Anterior (one) and lateral (two) Micropositioners (Model 1460, Kopf Industries)
• Micropositioner single prong attachments (Kopf Industries)
• Plastic syringe tip (25 cc/ml)
• Tubing
  o Vinyl: 1 ½” ID outer diameter (OD), 1” inner diameter (ID)
  o Clear Vinyl: 1 1/8” OD, 7/8” ID; 1”OD, ¼”ID; ¾” OD, ½” ID; 7/8” OD, 5/8” ID;
    5/8” OD, ½” ID; ½” OD, ¾” 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
• Screw driver
APPENDIX C

LabChart Protocol, Computer Set-up

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

Pressure Calibration, LabChart Protocol

1. Zero the pressure transducer before collecting data
   a. Attach the pressure transducer to the clear piece with the white cap
      i. Pinch the clear prongs together and fit circle around the rim of the golden piece
   b. Attach the pressure transducer to a small wooden block for stability.
   c. Fasten the transducer wire between the Velcro pieces on the benchtop.
   d. Attach the manometer (sphygmomanometer dial piece) using 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 to 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 the “Mains filter” box
         6. Press the “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
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

Airflow 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 airflow 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 “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 airflow (left). Do not remove the clear tube attachments between the Lab Chart box and the flow head meter.
APPENDIX F

Rabbit Tissue Dissection and Preparation Protocol

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

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

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

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

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

Data Acquisition Protocol

These procedures occur immediately following pressure and airflow calibration and specimen fine dissection. To collect data on pressure and airflow 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 airflow 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.
Data Segmentation and Analysis Protocol

1. Selecting Signals for Segmenting
   a. Open Lab Chart™ version 8 and the file from Desktop folder “LabChart Data”
   b. Select the pre-collected animal signals that you want to segment
   c. Select “File” -> “Save Selection”
      i. Rename file and save in designated folder
      ii. Do not save changes to main LabChart Data File
   d. 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 and upload to custom Matlab program for further analysis

5. Open Matlab application
   a. Click “Open File” -> select segmented txt file
   b. Drag the yellow boxes on the screen out of the way
   c. Count trials to verify that all 15 trials have been included in txt file

6. Selecting Results
   a. Move red markers on microphone signal data to surround one trial of phonation
      i. Note the placement of the vertical lines between pressure signal peaks.
      The red markers should be placed as close to these lines as possible but must be within the vertical markers.
   b. Select “play” for application to register line placement

7. Select “save”
   a. Save as “rabbit#_trial#”. It will save as a CSV file (both sound and excel file)

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

9. Repeat steps with each trial
APPENDIX I

Visual-Perceptual Slides

Figure II

Introductory Slide for Visual-Perceptual Ratings

Preliminary Visual Perceptual Ratings

Thank you for your participation in these preliminary visual perceptual ratings. They should take no longer than 60-90 minutes for you to complete. Rate all images in one sitting and in the order in which they appear. Before starting, please ensure that there will be consistent lighting and computer settings for the entirety of the experiment. Instructions for rating will be on the following pages. You may scroll through these introductory pages and reference them throughout the experiment as you feel necessary.

You are not required to participate in this experiment and may discontinue participation at any time for any reason. Should you discontinue prematurely, none of your ratings will be considered in the results of this study.
Figure 12

Instruction Slide for Visual-Perceptual Ratings

Instructions

- You will be presented with 26 images of excised rabbit larynges, mounted on benchtop with vocal folds adducted by micropositioners. Each image has been normalized for lighting and crop.
- Rate each image for
  1) edema, defined as swelling
  2) erythema, defined as redness
- Rate edema and erythema on a scale of 0-3 as you deem appropriate. Please feel free to use the entire scale, but you are not required to do so.
  - 0 being no redness or swelling
  - 1 being slight redness or swelling
  - 2 being moderate redness or swelling
  - 3 being the most severe redness or swelling
- Rate both left and right true vocal folds and left and right arytenoid mucosa, as shown on the next slide.
- Enter a total of 8 ratings for each larynx into a table that will be provided on each slide.

Figure 13

Anatomical Markers Slide for Visual-Perceptual Ratings

Example Ratings

Locations that you will rate for edema and erythema are shown in the images below. Circled and labeled are left and right true vocal folds and left and right arytenoid mucosa.
Instructions, continued

- Some images will be repeated for purposes of reliability measurements.
- Please rate each picture as it appears in order that it appears.
- Do not go back and change previous ratings.
- Two images will be provided for your reference throughout the experiment. These images will serve as an external anchor for you to reference when making visual perceptual judgements. The images will appear on the next slide and on each experimental slide following.

Figure 15

Example Ratings Slide for Visual-Perceptual Ratings

Example of best/healthiest larynx in terms of no edema or erythema on true vocal folds or arytenoid mucosa. This image will be referenced as "normal larynx reference".

Example of worst/least healthy larynx in terms of severe edema and erythema on true vocal folds and arytenoid mucosa. This image will be referenced as "severe larynx reference".
Figure 16

*Image 5 to be Rated for Visual-Perceptual Ratings*

Image 5. Provide Ratings (0-3) in the Tables Provided.

<table>
<thead>
<tr>
<th>Image 5, right side</th>
<th>Edema Rating</th>
<th>Erythema Rating</th>
</tr>
</thead>
<tbody>
<tr>
<td>Right Arytenoid Mucosa</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Right True Vocal Fold</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Image 5, left side</th>
<th>Edema Rating</th>
<th>Erythema Rating</th>
</tr>
</thead>
<tbody>
<tr>
<td>Left Arytenoid Mucosa</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Left True Vocal Fold</td>
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<td></td>
</tr>
</tbody>
</table>

Figure 17

*Image 9 to be Rated for Visual-Perceptual Ratings*

Image 9. Provide Ratings (0-3) in the Tables Provided.

<table>
<thead>
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<td></td>
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</tbody>
</table>
**Figure 18**

*Image 17 to be Rated for Visual-Perceptual Ratings*

![Image 17](image17.jpg)

Image 17. Provide Ratings (0-3) in the Tables Provided.

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<thead>
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<tr>
<td>Left True Vocal Fold</td>
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</tbody>
</table>

**Figure 19**

*Image 25 to be Rated for Visual-Perceptual Ratings*

![Image 25](image25.jpg)

Image 25. Provide Ratings (0-3) in the Tables Provided.

<table>
<thead>
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</table>
APPENDIX J

Thesis Timeline

9/19
- Training for lab use, including orientation to instruction manuals and videos in cloud storage, hard drive data storage, lab computer and program usage, and pressure and airflow calibration
- Training in fine dissection of rabbit larynges and benchtop setup. Training in collecting acoustic, aerodynamic, and visual data

10/19
- Fine dissection and collection of acoustic, aerodynamic, and visual data for experimental larynges

11/19
- Training for data segmentation of raw data on LabChart to prepare for upload to Matlab program for analysis

12/19
- Preparation for control rabbit acquisition for further data collection

1/20
- Fine dissection and collection of acoustic, aerodynamic, and visual data for all control larynges

2/20
- Completion of BIOMED CITI training, affiliated with University of Utah
- Initial draft of IRB X18007 edited to adapt current IRB2020-503 to meet new electronic requirements

3/20
- Maintain lab
  - Back-up collected data on hard drive
  - Computer maintenance via crash-plan download
  - Medical grade compressed air USP gas cylinder replacement
  - Reset precautionary ThermoScientific™ battery

4/20
- Complete data analysis of phonation pressure and flow using Matlab and Audacity programs performed by Amber Prigmore and Meg Hoggan
6/20
- Analyze data for significant differences between experimental and control groups in phonation pressure and airflow completed by Dr. Ray M. Merrill, Ph.D., using SPSS, (version 24) and SA (version 9.4)

9/20
- Complete Prospectus meeting with thesis committee, discussing specific thesis questions, importance of current study, and alterations to visual-perceptual study for increased accuracy and reliability

10/20
- Edit Prospectus documents to align with feedback received from thesis committee members

12/20
- Standardize images of mounted rabbit larynges for position, crop, and lighting using Adobe Lightroom (version 3.3)
- Prepare visual-perceptual study through blinding and randomization in Microsoft PowerPoint

1/21
- Complete second draft of IRB2020-503 with recommended edits from IRB review board at Brigham Young University
- Visual-perceptual study distributed to potential participants
- Collect and organize visual-perceptual ratings
- Analysis of visual-perceptual data for inter- and intra-rater reliability and differences between groups of larynges completed by Dr. Ray M. Merrill, Ph.D., using SPSS (version 24) and SAS (version 9.4)

2/21
- Prepare for thesis defense by completing first written draft of thesis
- Schedule oral thesis defense

3/21
- Complete oral thesis defense