Modeling Subglottic Stenosis Effects on Phonation Threshold Pressure in the Porcine Larynx

Jessica Maryn Murphey
Brigham Young University

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Modeling Subglottic Stenosis Effects on Phonation Threshold Pressure

in the Porcine Larynx

Jessica Maryn Murphey

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

Master of Science

Kristine Tanner, Chair
Christopher Dromey
Scott L. Thomson

Department of Communication Disorders
Brigham Young University

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ABSTRACT

Modeling Subglottic Stenosis Effects on Phonation Threshold Pressure in the Porcine Larynx

Jessica Maryn Murphey
Department of Communication Disorders, BYU
Master of Science

Subglottic stenosis (SGS) is a narrowing of the airway below the vocal folds and above the trachea. This narrowing may be idiopathic or caused by scarring in the airway due to prolonged endotracheal intubation, radiation therapy, trauma, or gastroesophageal reflux disease. People who present with SGS often experience respiratory difficulty both at rest and during exertion. Breathing difficulty increases with stenosis severity. SGS is also associated with voice problems. Research has identified relationships among stenosis severity, voice function and certain types of surgical management; however, many aspects of these relationships are not fully understood due to the complexities of studying human phonation in this population. The purpose of the present study was to examine the effects of SGS on aerodynamic features of voice function using an excised larynx benchtop mechanical model. Specifically, this research involved the comparison of excised porcine vocal fold vibration at baseline and under experimental conditions of 50% and 75% stenosed. The dependent variable was phonation threshold pressure (PTP), the minimum pressure needed to initiate and maintain vocal fold vibration. PTP was analyzed for nine excised porcine larynges, sampled three times each, at baseline and the two stenosis conditions. The results of this study revealed no differences in PTP based on within-subjects comparisons. Because airflow changes with airway narrowing, this finding might indicate that other factors are responsible for the voice problems associated with SGS that were not accounted for in the current mechanical model. Vocal fold tone is not easily simulated in a benchtop setup and might be an important consideration for future studies. The quantification and manipulation of vocal fold adduction, as well as the study of high-speed imaging, could be useful in future work involving excised larynx mechanical models for the study of SGS. The results from this pilot work represent an important step toward optimizing the experimental setup for studying aerodynamic features of SGS.

Keywords: subglottic stenosis, larynx, voice disorders, benchtop model, phonation threshold pressure, phonation threshold flow
ACKNOWLEDGMENTS

First and foremost, I acknowledge God’s hand in this research and within every aspect of my own life. For I know that “I can do all things through Christ who strengthens me” (Philippians 4:13). Next, I want to thank Dr. Kristine Tanner, my thesis chair, who has tirelessly guided and mentored me throughout this entire process. I am also extremely grateful for my thesis committee members, Dr. Dromey and Dr. Thomson, and their enormous contribution and involvement in this project. I would like to express my gratitude to Robin Pratt, my data collection partner, as well as Amber Prigmore and Meg Hoggan, who were indispensable to this project and became true friends. I would also like to acknowledge Ben Hilton of the BYU mechanical engineering school, for his massive contribution to this project in creating the SGS device. Mark Berardi was also extremely influential in the analysis portion of this project as he helped analyze the raw data using a specialized Matlab software program. Most importantly, I thank my incredible husband Tate, for his endless love and support to me and my dreams. His never-ending encouragement has truly made this all possible. Lastly, I am grateful for my son Cayson and his constant patience and love for me throughout this process. This work was supported by grants 2R56DC009616-06 and 2R01DC009616-06A1 from the National Institute on Deafness and Other Communication Disorders, National Institutes of Health.
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DESCRIPTION OF THESIS STRUCTURE

The format of this thesis, *Modeling Subglottic Stenosis Effects on Phonation Threshold Pressure in the Porcine Larynx* is written in a hybrid format; it includes classic thesis requirements combined with standard journal publication format. This thesis is based upon a larger five-year project which will be used for developing larynx-specific MRI coils and protocols for subglottic stenosis imaging and determining the impact of glottic and subglottic morphology on voice function in upper airway stenosis patients. This work is also a companion study to the 2019 thesis by Robin Smith, *Modeling Subglottal Stenosis Effects on Phonation Threshold Flow in the Porcine Larynx*. This work is supported by grants 2R56DC009616-06 and 2R01DC009616-06A1 from the National Institute on Deafness and Other Communication Disorders, National Institutes of Health. The annotated bibliography of a comprehensive literature review is included in Appendix A. A Food Handler’s Permit, which was acquired in compliance with Risk Management requirements at Brigham Young University, is included in Appendix B. Lastly, the study’s experimental checklist is found in Appendix C.
Introduction

Stenosis is an atypical narrowing of a passage in the body. It can occur in many structures, such as the neck, heart, spine, and vocal tract. The most common forms of stenosis include pulmonary, cervical, lumbar, foraminal, and laryngotracheal (Monnier, Lang, & Savary, 1999). Laryngotracheal stenosis is a congenital or acquired narrowing of the airway that may involve one or more associated structures, including the supraglottis, glottis, subglottis, and trachea (Damrose, 2008). Supraglottic stenosis is an unusual manifestation of laryngotracheal stenosis that may result from acute swelling above the vocal folds secondary to bacteria, trauma, irradiation, scarring from prolonged intubation, autoimmune disorders, or gastro-esophageal reflux disease. Glottic stenosis is a narrowing of the larynx at the level of the vocal folds. It is characterized by webbing, fibrosis, or scarring and most often involves the posterior glottis. The most common cause of this particular stenosis is prolonged endotracheal intubation. Subglottic stenosis (SGS) is a narrowing of the airway below the vocal folds and above the trachea, typically in the region of the cricoid cartilage. This form of stenosis is often caused by scarring in the larynx just below the vocal folds. Tracheal stenosis is a narrowing of the trachea that can occur after radiation therapy, prolonged intubation or tracheostomy, or other procedures affecting the trachea (Gelbard, Francis, Sandulache, Simmons, Donovan, & Ongkasuwan, 2015). Several of these stenoses are associated with adverse effects on voice function, particularly laryngotracheal stenosis and SGS (Ettema, Tolejano, Thielke, Toohill, & Merati, 2006).

The present investigation will focus on SGS, which specifically affects the diameter and shape of the upper airway between the vocal folds and first tracheal ring (Conley, 1953). Clinically, SGS is characterized by a visually-identified narrowing of the airway lumen at
the level of the cricoid cartilage, causing dyspnea, hoarseness, and increased mucus production (Hseu, Benninger, Haffey, & Lorenz, 2014). The common etiology of SGS is idiopathic, however, other risks for SGS include intubation, airway irritation, and trauma-related factors (Damrose, 2008).

Recent research has established the adverse effects of certain types of stenosis and associated treatments on voice function (Ettema et al., 2006; Hseu et al., 2014). These effects on voice function can negatively impact an individual’s quality of life through impaired communication, job performance, psychosocial function, and self-esteem. Clinical and scientific studies have identified important relationships between voice function, stenosis severity, and the type and frequency of treatment. Historically, surgery has been found to improve airway patency in SGS, but at the expense of voice function (Mattei, 2016). Other procedures improve patency, but often require repeated interventions. Due to the substantial variability that exists in the presentation and surgical management of SGS, pretreatment and posttreatment research is needed to guide surgical interventions and optimize outcomes (Tanner, Roy, Merrill, Kendall, Miller, Clegg, & Elstad, 2013).

Treatment for SGS differs depending on the level of severity, exact location and shape of the stenosis, and the manifestation of other health-related variables. Myer, O’Connor, and Cotton (1994) proposed a four-point grading scale for SGS severity, where grade one included up to 50% obstruction, grade two was 51% to 70%, grade three exceeded 70% obstruction, but included a detectable lumen, and grade four indicating no detectable lumen. The range of treatment options is fairly broad for SGS, with the most severe requiring invasive surgical procedures. Cricotracheal reconstruction has emerged as a favorable surgical tool for managing SGS and without relying on a modified airway (Ettema et al., 2006). Generally, this form of
surgical management involves the removal of a large portion of the anterior cricoid cartilage, disturbing the cricothyroid musculature bilaterally. Although recent revisions to this surgical procedure have identified a variant that spares or attenuates these adverse voice outcomes, it is important to understand the effects of SGS before and after surgery (Tanner et al., 2013). For example, in a study performed in 2006, 31 patients with SGS from a three-year period at a single academic center were retrospectively reviewed regarding their vocal quality after surgery. The grade, roughness, breathiness, asthenia, and strain scale results indicated that collectively patients were perceived to have a mild to moderate dysphonia. Patients who had previous stenosis surgery had worse voice quality than those who had not undergone any previous surgery; the nonsurgical history group were also found to have more normal vocal fold motion (Ettema et al., 2006). To better understand voice disorders in this population, more research needs to be done to clarify the physiologic underpinnings of how stenosis affects vocal fold vibration. Understanding these effects will facilitate treatment planning and the optimization of voice-sparing surgical procedures (Mattei, 2016).

Most of what professionals understand about stenosis is based on imaging: laryngoscopy, intraoperative observations, and sometimes CT scans. However, many of these sources are very limited, especially the CT scans due to the risks of radiation (U.S. Food and Drug Administration, 2010). The MRI imaging would be a potentially favorable choice; however, MRI imaging is not the standard of care for preoperative and postoperative assessment in this population (Mattei, 2016). Due to the lack of instrumental assessments employed to quantify stenosis effects on voice function, it has been difficult to establish precisely why people with SGS have voice problems. Additionally, the types of voice symptoms associated with SGS are varied and have not been examined systematically. Furthermore, the complexity of respiratory
and phonatory factors that influence voice function in SGS makes alternative research methodologies appealing, such as mechanical modeling and simulation (Mattei, 2016).

The specific aim of the proposed research is to assess the aerodynamic consequences of upper airway stenosis at the vertical level of the subglottis. Excised porcine larynx experiments with adjustable stenoses will be used to explore fundamental physical relationships between stenosis geometry and changes in air pressure and flow patterns at the onset of vocal fold vibration. The anticipated outcomes of this study include deeper insight into the sources of dysphonia in stenosis patients and into the aerodynamic characteristics associated with SGS. The ultimate aim is to develop tools and understanding that will lead to improved voice outcomes for patients with upper airway stenosis.

**Excised Larynx Models**

Excised animal larynges have for many years made valuable contributions to voice research in ex vivo studies (Pipkin-Litster, 2018). Ex vivo refers to research or experimentation that uses tissue from an organism in an environment that is outside of its natural conditions with marginal modifications. Excised larynges have in the past been examined for vocal structure and function in a variety of voice studies. Many of these research groups completed studies that established which animal larynges had the most physical similarities to human larynges. In 2008, Alipour and Jaiswal examined the characteristics of phonation in excised cow, sheep, and pig larynges and studied how close they were to the human larynx. The study’s results suggested that although the larynges of each animal had qualities that made them suitable models for studying the human larynx, the porcine larynges were the most similar in respect to physical characteristics as well as having a more extensive frequency range, comparable to human phonation. Porcine larynges also have a vocal ligament, unlike other animal larynges that are
also widely used in voice research but do not have a vocal ligament. Furthermore, the study
discovered that the porcine larynges’ average fundamental frequency was approximately 220 +/-
57 Hz and the onset pressure was approximately 7.4 +/-2 cm H₂O (Alipour, Jaiswal, & Finnegan,
2007); these values are similar to the aerodynamic features of human females.

A later study (Alipour, Finnegan, & Jaiswal, 2013) used the measurements of an excised
human larynx to compare with their earlier study’s results. The study showed that pig larynges
have similar oscillation patterns to the human larynx. A later study (Howard, Mendelsohn, &
Berke, 2015) confirmed that phonation of the porcine larynx is comparable to the phonation of a
human larynx in both fundamental frequency and onset pressure. The findings of these studies
reveal that using porcine larynges for an ex vivo study is an appropriate selection for comparison
with human phonation due to their general size, features, laryngeal structure, and characteristics
of phonation (Alipour & Jaiswal, 2008; Jiang & Titze, 1993). These findings all support the
current method in using porcine larynges to study the effects of SGS on vocal fold vibration,
specifically with regard to PTP.

**Phonation Threshold Pressure**

The minimum amount of pressure required to initiate and maintain vocal fold vibration is
operationally defined as PTP (Titze, 1994). In a more recent study, investigators documented the
relationships among vibratory features of the vocal folds and PTP using an excised human larynx
mechanical model (Mau, Muhlestein, Callahan, Weinheimer, & Chan, 2011). Disorders of the
vocal folds often cause disruption to these properties, resulting in an increased PTP (Pipkin-
Litster, 2018). To better understand the vibratory features of SGS including the distinction
between a normal and disordered voice, PTP was chosen as the outcome variable for the current
investigation.
Statement of Purpose

The purpose of the present study was to examine the effects of airway narrowing at the vertical level of the subglottis on PTP using a within-subjects experimental design. Specifically, this study documented vocal fold patterns between stenotic and normally patent airways in the same excised porcine larynges. The principal goal of this study was to establish preliminary aerodynamic data for the air pressure and flow required to initiate and sustain phonation across several grades of stenosis as well as a standard subglottis. The outcome of the current work will lay an important foundation for further research in voice disorder treatments for SGS. Another anticipated outcome of this study includes a deeper insight into the source of vocal deficits in stenosis patients and into the aerodynamic changes associated with upper airway stenosis. The ultimate aim is to develop tools and understanding that will lead to improved voice outcomes for patients with upper airway stenosis.

Method

This study was undertaken in accordance with regulations from Brigham Young University Risk Management and the Institutional Animal Care and Use Committee. Excised porcine larynges were used in this study and were donated from Circle V Meats in Spanish Fork, Utah. All of the operational procedures in this study involving the excised porcine larynges were performed in rooms 105 and 106 of the John Taylor Building, 105 of the John Taylor Building Annex, and room 126 of the Nicholes Building at Brigham Young University.

Research Design

This study employed a within-subjects design, in which all participants were exposed to every condition. 13 excised porcine larynges were included in this study, each one experiencing three different conditions of normal or stenosis. The three conditions used in this study were: (a)
0% stenosis, (b) 50% stenosis, and (c) 75% stenosis measured by radius. Each larynx underwent a randomization procedure such that 0% was either first or last; this was related to the amount of time required to mount the larynx on the stenosis apparatus. The other two stenosis conditions, 50% and 75%, were assigned randomly. For each stenosis condition, three phonation trials were completed. For the stenosis conditions, each larynx was placed on a silicone artificial stenosis mechanism that was made from a 3D printed mold created by researchers in the mechanical engineering department at Brigham Young University. Larynges that began the process without any grade of stenosis were mounted on the bench model and phonated without any modifications or artificial narrowing. Larynges would then go through three phonatory trials of 0% stenosis. Subsequently, the larynges were removed from the bench mount, the stenosis mechanism put in place, and the larynx remounted; larynges were not removed between stenosis conditions. It is essential to note that the placement and removal of each larynx on the benchtop was operationalized extensively such that any potential effects secondary to the mounting process were minimized. In counterbalanced fashion, half of the larynges underwent the stenosis conditions first, again randomly assigned, followed by the 0% stenosis condition. For all three groups, isotonic saline was sprayed throughout the data collection to prevent desiccation of the vocal folds. Pressure and flow were recorded during each phonatory trial. The independent variable was percent stenosis of the subglottis and the dependent variable for this study was PTP (cmH2O).

Larynges

Many researchers have used excised larynx models to examine the structural and physiological properties of the larynx. These models usually involve a larynx that has been excised from an animal within 24 hours postmortem (Alipour et al., 2013; Jiang & Tao, 2007);
(Zhang, Jiang, Tao, Bieging, & MacCallum, 2007); (Jiang & Titze, 1993). In this study, 13 excised porcine larynges, from pigs sacrificed for nonresearch purposes, were obtained from a local slaughterhouse (Circle V Meats, Spanish Fork, UT). All the larynges were collected from adult food-grade pigs, each at least two years in age. After collecting the larynges from the Circle V Meats, the larynges were immediately brought back to room 105 of the John Taylor Building at Brigham Young University to be inspected and roughly dissected. During this time, the larynges were methodically inspected for any structural abnormalities or punctures and were discarded if exhibiting any malformations. Following inspection, the larynges were each dissected to remove excess fat, tissue lining, the esophagus, and unnecessary muscles attached to the larynx. The trachea was then trimmed to approximately 6 cm in length while taking precautions to ensure it was not punctured. Following dissection, the larynges were rinsed off with water and placed in gallon sized zip-locked bags, filled with 0.9% sodium chloride irrigation USP fluid (i.e., isotonic saline, 0.9% Na^+Cl^-). These were then transported to the Chemistry Central Stockroom located in room 126 of the Nicholes Building at Brigham Young University to be flash frozen for preservability using liquid nitrogen. While submerged in isotonic saline within the gallon sized zip-lock bag, each larynx was fully immersed in a cooler of liquid nitrogen for approximately seven minutes. After being completely frozen through using liquid nitrogen, the larynges were transported back to the John Taylor Building and placed in an industrial freezer with a temperature of approximately -18 degrees. The entire process of picking up the larynges from the slaughterhouse and preparing them for experimentation took approximately two hours. Seventy-two hours before the initiation of each experiment, the larynges were removed from the industrial freezer and placed in a refrigerator to be allowed to thaw.
When the larynges were completely thawed through, excess fat, cartilage, supraglottic tissues, and false vocal folds as well as the surrounding portion of the thyroid cartilage were dissected while making sure to not puncture the trachea. The epiglottis was also carefully removed, followed by the trimming of the trachea to ensure that it was approximately 6 cm in length. The arytenoid cartilages were kept intact for additional support to assist in the adduction of the vocal folds during experimentation. The thyroid cartilage was cut at a level approximately 0.5 cm above the true vocal folds and the cartilage trimmed at an upward angle from anterior to posterior to provide additional support during arytenoid adduction. Two different-sized scalpels were used to dissect each larynx. After dissection, the larynges were each submerged in isotonic saline within the gallon sized zip-locked freezer storage bag and labeled by number. The larynges were then mounted on an artificial stenosis simulation device used to create the different levels stenosis below the glottis.

**Procedures**

Benchtop model. The benchtop model that was used during the procedure of phonatory trials of excised larynges was based on a study conducted by Jiang and Titze (1993). This study used a similar mechanical model, including how the porcine larynges were placed vertically on plastic tubing, and using a foam-insulated custom pseudolung surrounding the plastic tubing placed below the breadboard benchtop (Thorlabs, Ann Arbor, MI). The base of each trachea was positioned on the vertical tubing and locked in place with ¾ inch Teflon tape and an adjustable metal hose clamp (Pipkin-Litster, 2018). Three micropositioners (Model 1460, Kopf Industries, Tujunga, CA) were also secured to the tabletop by ¼-20 headless screws with custom bases (Pipkin-Litster, 2018). The two micropositioners that were positioned laterally, had three prongs; these gently penetrated the lateral surface of each arytenoid cartilage to create the effect of
adduction in the vocal folds. To standardize the procedure, the lateral micropositioners were
simultaneously pushed 10 notches into each porcine larynx to stabilize the mechanism. The third
micropositioner was situated near the anterior commissure so that the suture thread could be tied
to it. This allowed the micropositioner to be used to adduct and lengthen the vocal folds until
phonation was achieved. To standardize the setup, the third micropositioner was pulled four
notches for each larynx to provide adequate tension in the string to lengthen the vocal folds and
facilitate phonation.

**SGS device.** The device simulating subglottic stenosis (i.e., SGS device) consisted of a
silicone sleeve (Smooth On Dragon Skin 10, E = 22 psi) that was constricted by a series of
threads which can be tightened by cranks shown in Figures 1 through 5. The gears were
calibrated such that the desired diameter or grade of stenosis, could be achieved by turning the
gears to the desired marking. Each crank constricted the sleeve in an elliptical shape along
perpendicular axes; if both cranks were tightened, then the constriction was circular. Each
gear was marked from 1 to 12 mm around the edge. To set the desired diameter, the crank
was turned until the designated mark lined up with a mark on the base. This created the effect
of stenosis within the larynges during the procedure. To create the 50% stenosis, both cranks
were set to the five marker. To create the 75% stenosis, both cranks were set to the 10 marker
as shown in Figure 4.
Figure 1. Manual subglottic stenosis (SGS) device on the optic table.
Figure 2. Superior view of the subglottic stenosis (SGS) silicone sleeve.
Figure 3. Dimensions of the subglottic stenosis (SGS) silicone sleeve.
Figure 4. Manual subglottic stenosis (SGS) device presenting a 75% artificial narrowing.
Figure 5. Porcine larynx mounted on top of the manual subglottic stenosis (SGS) device.
**Measurement of pressure and flow.** An adjustable flow regulator at 50 psi and compressed air tank (<1% relative humidity) were attached to an in-line thermal flow meter. This was then attached to a tube connected to a Theraheat temperature-controlled humidifier (Model RC70000, Smiths Medical, Dublin, OH), which was attached to clear plastic tubing that passed through a 20-cm aluminum pseudolung insulated with foam. This tube passed through a hole in the table top and had a subtracheal outlet where a pressure transducer (Model PT-25-S, Glottal Enterprises, Syracuse, NY) was connected perpendicular to the direction of the flow.

**Phonatory trials.** Once properly placed on the benchtop and SGS device, each larynx underwent a succession of phonatory trials testing the three conditions of stenosis (0%, 50%, and 75%). Compressed air was circulated through the humidifier and pseudolung to reach the vocal folds subglottally. Using the anteriorly placed micropositioner, the length of the vocal folds was adjusted until phonation was achieved. This length remained stable for the rest of the trial. The vocal folds for each trial were only vibrated until phonation was achieved and then the subglottal air was turned off.

**Signal acquisition.** The acoustic signal, air pressure, and airflow signals were obtained at baseline and following each phonation trial. A DATAQ A/D (DI-720 Series) converter and Windaq software (Windaq Pro+, Akron, OH) were used to acquire signals at 10 kHz per channel. A dynamic microphone (Model SM-48, Shure, Niles, IL) was positioned about 6 inches above the true vocal folds and an audio mixer (Samsung MIXPAD 4, New York, NY) preamplified the signal. Prior to data collection, a pressure calibrator (PC-1H, Glottal Enterprises, Syracuse, NY) was used to calibrate the pressure transducer to 0 and 10 cmH2O and the flow meter was calibrated at 0 and 15 L/min. Prior to the study, a HygroSet II Digital Hygrometer (model DHYG-Round; HygroSet, Weston, FL) was calibrated using the Humidipak calibration kit and
was then used to monitor environmental humidity during the experiment. Each Windaq file was
coded and saved for subsequent pressure and flow analysis.

**Data analysis.** Windaq files were segmented and then imported into Matlab
(MathWorks, Natick, MA). Phonation onset was identified using the acoustic signal via a custom
Matlab program. The PTP values were obtained by identifying the subglottal pressure and flow
of 10 ms prior to and after the onset of phonation and calculating the average.

**Statistical analysis.** The data gathered from the within-subjects design comparing the
three conditions within the same larynx, were evaluated for central tendency and variability at
baseline. All evaluations were accomplished using SPSS, version 24 (IBM Corp., Armonk, NY).
Analysis of the differences between the stenosis conditions were performed using a repeated
measures analysis of variance (alpha = .05).

**Results**

Among the excised larynges obtained for data collection, four were not viable due to
tears in the tissue or other anatomical anomalies that precluded benchtop mounting. The
remaining 13 larynges completed the experimental protocol; however, the final data collection
day resulted in equipment failure during the signal acquisition phase. Therefore, data from a total
of 9 larynges were available for data analysis. For each larynx, vocal fold anatomical dimensions
are provided in Table 1. The width and height of each thyroid cartilage are reported in Table 2.
Tracheal anatomical dimensions such as width and height are included in Table 3. The weight of
each larynx both pre and post trachea cut without thyroid cartilage are recorded in Table 4. Prior
to the initiation of each phonatory trial, all measurements were made in mm or oz.
Table 1

*Vocal Folds Anatomical Size and Dimension*

<table>
<thead>
<tr>
<th>Group</th>
<th>Session Date</th>
<th>Length of Vocal Folds (mm)</th>
<th>Width of Vocal Folds (mm)</th>
<th>Width from Vocal Folds to Thyroid Cartilage (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pig 1</td>
<td>7/6/2018</td>
<td>16.47</td>
<td>2.53</td>
<td>11.02</td>
</tr>
<tr>
<td>Pig 2</td>
<td>7/6/2018</td>
<td>16.05</td>
<td>2.09</td>
<td>11.03</td>
</tr>
<tr>
<td>Pig 4</td>
<td>7/12/2018</td>
<td>18.00</td>
<td>4.00</td>
<td>11.00</td>
</tr>
<tr>
<td>Pig 5</td>
<td>7/12/2018</td>
<td>17.00</td>
<td>3.00</td>
<td>12.00</td>
</tr>
<tr>
<td>Pig 6</td>
<td>7/16/2018</td>
<td>19.37</td>
<td>2.37</td>
<td>10.64</td>
</tr>
<tr>
<td>Pig 7</td>
<td>7/16/2018</td>
<td>17.03</td>
<td>1.87</td>
<td>10.29</td>
</tr>
<tr>
<td>Pig 8</td>
<td>7/16/2018</td>
<td>20.51</td>
<td>2.12</td>
<td>13.28</td>
</tr>
<tr>
<td>Pig 10</td>
<td>8/9/2018</td>
<td>17.79</td>
<td>1.36</td>
<td>12.04</td>
</tr>
<tr>
<td>Pig 11</td>
<td>8/9/2018</td>
<td>19.46</td>
<td>1.62</td>
<td>10.33</td>
</tr>
<tr>
<td>Pig 13*</td>
<td>9/21/18</td>
<td>16.63</td>
<td>1.44</td>
<td>10.02</td>
</tr>
<tr>
<td>Pig 14*</td>
<td>9/21/18</td>
<td>19.42</td>
<td>1.87</td>
<td>10.28</td>
</tr>
<tr>
<td>Pig 15*</td>
<td>9/21/18</td>
<td>18.99</td>
<td>1.55</td>
<td>9.58</td>
</tr>
<tr>
<td>Pig 16*</td>
<td>9/21/18</td>
<td>15.78</td>
<td>1.86</td>
<td>8.90</td>
</tr>
</tbody>
</table>

*Note.* * = Pigs not included in the data analysis due to equipment malfunction
Table 2

*Thyroid Cartilage Anatomical Dimensions*

<table>
<thead>
<tr>
<th>Group</th>
<th>Session Date</th>
<th>Height (protuberance to top)</th>
<th>Height (protuberance to bottom)</th>
<th>Width of Thyroid Cartilage (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pig 1</td>
<td>7/6/2018</td>
<td>51.24</td>
<td>35.56</td>
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<tr>
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<tr>
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<tr>
<td>Pig 14*</td>
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<td>45.85</td>
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<tr>
<td>Pig 15*</td>
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<td>27.86</td>
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<tr>
<td>Pig 16*</td>
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<td>49.71</td>
<td>28.38</td>
<td>46.75</td>
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</table>

*Note.* * = Pigs not included in the data analysis due to equipment malfunction
Table 3

*Trachea Anatomical Dimensions*

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<th>Group</th>
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<th>Trachea Length (mm)</th>
<th>Trachea Diameter (mm)</th>
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<td>Pig 16*</td>
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</table>

*Note.* * = Pigs not included in the data analysis due to equipment malfunction
Table 4

*Larynx Weight*

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<th>Group</th>
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<td>3.10</td>
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<td>Pig 6</td>
<td>7/16/2018</td>
<td>3.70</td>
<td>2.89</td>
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<td>Pig 7</td>
<td>7/16/2018</td>
<td>2.36</td>
<td>2.15</td>
</tr>
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<td>Pig 8</td>
<td>7/16/2018</td>
<td>3.49</td>
<td>2.61</td>
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<td>Pig 10</td>
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<td>Pig 15*</td>
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<td>2.86</td>
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<td>Pig 16*</td>
<td>9/21/18</td>
<td>3.49</td>
<td>2.61</td>
</tr>
</tbody>
</table>

*Note.* * = Pigs not included in the data analysis due to equipment malfunction
Descriptive Statistics

Summary data for PTP were analyzed for central tendency and variability. For each observation (i.e., each specimen at each percent stenosis), larynges were phonated three times and the data averaged, resulting in one data point for each observation. The mean of PTP values for 0% stenosis was 5.29. The means for the two stenosis conditions were 5.74 for 50% stenosed and 5.81 for 75% stenosed. The standard deviation for PTP values were .88 for 0% stenosed, 1.38 for 50% stenosed, and 2.52 for 75% stenosed. The range of PTP values for 0% stenosis was 4.16 to 6.67 cmH2O (median = 5.22 cmH2O). Ranges for the two stenosis conditions were 3.67 to 7.80 cmH2O (median = 5.23 cmH2O) for 50% stenosed and 3.80 to 12.31 cmH2O (median = 5.06) for 75% stenosed.

Inferential Statistics

Examination of the distribution for PTP indicated that the data were not normally distributed. Additionally, the data violated Mauchly’s test of Sphericity, Mauchly's W = .380, p = .034. Therefore, the Friedman Analysis of Variance by Ranks was performed. The results from the Friedman test were not significant, p = .459. Data for PTP mean and standard deviation for each percent stenosis are displayed in Figure 6.
Discussion

The present investigation aimed to determine if different grades of subglottic stenosis produced changes in PTP using an excised porcine larynx benchtop methodology and a within-subjects experimental design. The primary purpose of this study was to collect and examine preliminary aerodynamic data from a mechanical model to lay the groundwork for further research in SGS. Specifically, this pilot investigation developed an operational methodology that can be incorporated and improved in future modeling studies of SGS toward clinical translation and treatment optimization. Another anticipated outcome of this study was to gain deeper insight into the sources of dysphonia in stenosis patients by examining aerodynamic changes associated
exclusively with upper airway stenosis (i.e., independent of other in vivo covariates). Analysis of the results indicated no differences in PTP between the normal, nonstenosed condition and the two stenosis conditions. These results have important research-related methodological and clinical implications and are discussed below.

Theoretical Framework

In this investigation it was hypothesized that PTP would change predictably with narrowing of the subglottis. This hypothesis was based on several theoretical constructs that served as a framework for this study. First, Ohm’s Law indicates that resistance and flow are inversely related when pressure is constant (Definition of 'Ohm's Law', 2019). Second, the Bernoulli Effect describes the drop in the pressure of liquids or gasses when the velocity of flow increases at constant temperature (Bernoulli’s Principle, 2018). Third, Poiseuille’s Law states that flow through a tube is affected by the pressure of the current, the radius and length of the tube, and the viscosity of liquid (i.e., given a liquid flow model); as applied to this study, Poiseuille’s Law implies that decreases in the diameter of the tube creates resistance that reduces flow (Flow and Poiseuille's Law in Operation, 2019). Collectively, these principles may be applied to SGS wherein air pressure and flow interact to affect voice function.

Air Pressure and Voice Function

Respiratory pressure is the driving force during phonation. More specifically, subglottal pressure and PTP are related to laryngeal geometry as well as the integrity of the vocal folds (Titze, 1994). PTP is described as the minimum phonation onset pressure and has a modest correlation with vocal effort. During vocal fold vibration, the vocal fold mucosal rapidly closes and opens using the lamina propria and TA muscle, building up and then releasing air pressure. The PTP is generally used to quantify vocal fold vibration as it is highly influenced by the vocal
fold cover, the mucosal wave, and pre-phonatory glottal width (Mau et al., 2011). Disorders of the vocal folds often cause a disturbance within these properties, causing PTP to increase.

Despite PTP being a widely used and accepted measure of voice function, it has its limitations. Onset pressure relates to the phenomenon of overcoming momentarily constant vocal fold closure via the buildup and release of subglottal air. While healthy vocal folds have fairly regular vocal fold vibratory patterns, the onset of phonation is always characterized by this initial perturbation, or interruption, of pressure and flow. Because of this, PTP can be a variable measure. Similarly, differences in how PTP is measured can influence raw PTP values and how data are interpreted (Plexico, Sandage, & Faver, 2011). Conversely, it is also possible that some of the variability observed in PTP values relates directly to its level of sensitivity to overall voice function and not simply variability of the measure itself; that is, PTP is sensitive to subtle voice function changes. It is possible that due to this sensitivity, the researchers in the present study did not see significant changes in PTP across the stenosis conditions.

In Vivo Versus Ex Vivo Models

The current study examined the effect of three different levels of subglottic stenosis (0%, 50%, and 75%) on PTP. Although the expected outcome for this study was that PTP would increase due to the disruption of the stenosis in the larynx, the effects from the stenosis were not significant. It is important to consider, however, the nature of this experimental setup. The current investigation involved an ex vivo benchtop mechanical model. The advantage of this design was the ability to manipulate stenosis parameters independent of any other covariates that occur in vivo. Vocal fold adduction and tension were standardized using precise micropositioners for arytenoid adduction and vocal fold elongation. Although this process has been used routinely in excised larynx studies (Jiang & Titze, 1993), it does not permit some of
the dynamic responsiveness that occurs during voice production in vivo. In human voice production, other speech subsystems, neurological control mechanisms, and behavioral modifications interact to produce speech and voice. Even though the current experiment permitted the examination of aerodynamic factors only, it did not permit other relevant modifications such as contraction of the lateral cricoarytenoid and thyroarytenoid musculature. There is precedent for externally stimulating the vocal fold adduction musculature (Alipour & Jaiswal, 2008); however, adding this variable also requires accounting for any covariate effects. In future studies, the manipulation of vocal fold adduction might induce vocal fold vibration that is more similar to in vivo phonation in SGS. Several studies have included this methodology to better represent in vivo phonation during ex vivo modeling (e.g., Hottinger, Tao, & Jiang, 2007). Perhaps as stenosis-related airflow decreases in the benchtop model, a more abducted position might better represent human phonation. This would correspond with the reduced vocal loudness observed in people with SGS (Tanner et al., 2013).

Larynx Preservation

The preservation techniques used in this investigation such as the method used for freezing and thawing the tissue should also be considered as these can both contribute to histological changes. Pipkin-Litster (2018) conducted a study which investigated features of three preservation methods: flash freezing in liquid nitrogen, storage in Ringer’s solution, and storage in isotonic saline. The results of this study indicated that freezing the larynx resulted in the least variability and suggested that quick freezing each larynx may result in a model that more closely resembles human phonation (Pipkin-Litster, 2018). As such, the current study used liquid nitrogen to flash freeze the tissue and then a refrigerator to thaw the larynges. During the first test run for the study, a single larynx was frozen for six minutes. However, this was found to
not be enough time in the liquid nitrogen so the new time allowed for freezing was changed to seven minutes. The first test run for thawing a larynx was done over a 24-hour period, which also proved to not be long enough, as the tissue was still substantially frozen through. For the remainder of the study, liquid nitrogen was used to flash freeze the tissue for seven minutes and then a refrigerator was used to thaw the larynges for a 72-hour period. However, research has shown that a gradual rate of thawing can lead to bigger ice crystals forming on the tissue (Young, Armitage, Bowerman, Cook, & Easty, 1994). This extended process of thawing before trialing could have led to bigger ice crystals developing and compromised the compliance of the vocal folds as well as PTP. The transformation that occurs in the tissue from this procedure may be unique from subject to subject which could result in higher inconsistency within the data collected from these subjects.

**Sample Size**

One factor that may have influenced the results of the study is having a small data set. Over the course of the study, the researchers obtained 21 porcine larynges to be used for the study. However, through the course of the study, only 13 larynges completed the experimental protocol. This was due to a number of reasons such as anatomical anomalies or damage in the tissue that precluded benchtop mounting, having larynges thawed and dissected but unusable due to equipment malfunctions, the industrial freezer breaking overnight while specimens were inside, and other contributing factors. The final data collection day was affected by equipment failure during the signal acquisition phase which left only nine larynges available for data analysis. Having a smaller sample size means that outliers could significantly have influenced the statistical analysis and thereby the entire study outcome. Larger numbers are needed to fully interpret the effects of stenosis on PTP.
Increasing Trials

Another possibility that may have altered the outcomes of the study is that the specimens did not go through enough trials to reveal the anticipated effect. Since this study used live tissue, the researchers did not want to leave the larynges out too long, so as to minimize other factors that could raise PTP, such as dehydration; however, this pilot study showed that PTP did not increase the longer the tissue was mounted within a 30-minute time frame. This indicates it would be possible and beneficial in the future to perform many trials for each condition such as vibrating the vocal folds 10 times for each observation instead of just three. This would also expand the data and reduce the influence of outliers in order to better represent how PTP is affected by stenosis in an ex vivo model.

Variations in Manual SGS Device

An additional issue of concern for this study is that while analyzing the results, the flow transducer was early in the airflow line; however, the pressure transducer varied with respect to its distance from the vocal folds, the size of the larynx, and the addition of the stenosis mechanism. These three variables created inconsistencies in the data regarding pressure, which affected the overall outcomes of the study especially with regard to PTP. This is a consequence of having to move and remount the larynx as well as rearrange the micropositioners each time the researchers changed larynges or as they alternated trials specifically before or after the 0% grade of stenosis. This was due to the addition and removal of the manual SGS device to the benchtop model, as it is slightly raised and changed the vertical distance of the larynx. However, if a modified manual SGS device were allowed to stay on the bench top for the entirety of the procedure, the variability of the remounting of the larynx would be reduced and optimize the
benchtop setup to be more standardized for vertical distance of the pressure transducer and the larynx.

**General Limitations**

During this study, a few limitations occurred that may have affected the study’s outcomes. One source of variation in this study is the wide range of physical dimensions, age groups, and genders of the pigs used in the study. Although the dimensions and weight of the pig larynges were documented after each dissection, the age and gender of each pig were unknown to the researchers. These factors may have contributed to variability within the data set. As mentioned previously, porcine larynges possess many structural resemblances to human larynges. Nevertheless, human vocal folds do vary from the porcine vocal folds in that human vocal folds lie at a 0° angle within the larynx while porcine vocal folds lie at a 45° angle. Although these differences are not greatly significant, they may affect the results since the dissimilar angles could have altered outcomes with measures of PTP over time.

Another limitation of this study concerns the gradual learning process for each of the researchers during the procedural processes of dissection and phonatory trials. The skills and abilities of the researchers during dissection, using equipment, and the mounting of the larynges all increased with experience by the end of the study. Ex vivo experimentation, such as this, involves dissection steps that vary depending on the individual dissecting. An incision that is slightly larger or in a marginally different location could theoretically influence the data, therefore altering the final outcome. However, this issue was mitigated by having regular trainings to keep the procedures for dissection, calibration, and mounting consistent during each trial, as well as having one person be in charge of the same task whether it be the dissection, calibration, or mounting over the course of the experiment to increase reliability. Another
possible source of variability in the data could have resulted from variation in the flash freezing procedure. Although each of the porcine larynges was submersed in liquid nitrogen for seven minutes, each larynx differed in weight and size, thus changing the amount of time needed for it to be completely frozen. The visibility for the center of the larynx was very limited when trying to determine whether or not the larynx was completely frozen through. However, this concern was addressed early on in the study and the proper precautions were taken to ensure each larynx was fully inspected to determine if additional freezing time was needed, but variability could still occur in this process.

Another concern was the overall complexity of the dissection process. It included several detailed steps which, if not followed completely, could have led to inconsistencies in the data. An example of this is that in order for the larynges to properly phonate, the trachea must be carefully trimmed without being damaged or punctured to ensure adequate pressure and flow.

Some equipment malfunctions limited the scope of the study. The industrial freezer failed after the first round of trials, leaving five dissected and frozen larynges unusable. This resulted in a smaller sample size, meaning that outliers could significantly have influenced the statistical analysis and thereby the entire study outcome. Additionally, this experiment was not conducted in a sound booth, potentially allowing unwanted noise to affect the recognition of the onset of phonation. Another limitation with the equipment was a malfunction of the WinDaq system, which delayed the study. Equipment failure on the final data collection day also resulted in the study’s sample size becoming smaller. Having a small data set meant that outliers could have significantly influenced the statistical analysis. A larger sample size is needed to fully interpret the effects of stenosis on PTP.
Implications for Future Research

Future research could involve larger sample sizes to reduce the effects of outliers as well as consider the age and gender of the specimens to minimize variability within grades of stenosis and trials. The preferable gender would be the male larynx as it is larger and more similar to the human larynx (Pipkin-Litster, 2018). Future studies should also consider performing multiple trials for each condition such as vibrating the vocal folds 10 times for each observation and averaging instead of just three as was done in this study. As this pilot study demonstrated that PTP did not increase the longer the tissue is mounted within a 30-minute time frame, many trials could be run to increase the statistical power.

Future studies might also involve making adduction adjustments along with the stenosis (e.g., 80% adducted, 90% adducted, etc.), since the vocal folds in this study were not adjustable for their level of adduction. In 2008, Alipour and Jaiswal conducted studies on phonation while successfully manipulating vocal fold adduction using ex vivo models. In their study, the lateral cricoarytenoid muscle was stimulated to manipulate adduction of the vocal folds to create a more realistic representation of how the vocal mechanism engages in phonation. The levels of adduction in the study were low, medium and high. Including these elements would be beneficial in investigating how PTP is affected by stenosis while properly manipulating vocal fold adduction. As this is a viable option, another pilot study manipulating adduction may be beneficial to observe how different adduction levels in the vocal folds might interact with a narrowed airway.

Future studies would also benefit from modifying the stenosis device to allow it stay on the bench top for the entirety of the procedure. This would remove the variability that was introduced as a consequence of having to remount the larynx as well as rearrange the
micropositioners each time the researchers changed larynges or alternated trials before or after the 0% grade stenosis. This would optimize the benchtop setup to standardize the vertical distance of the pressure transducer and the larynx as well as eliminate the dismounting of the larynx altogether.

**Conclusion**

Although we hypothesized that PTP would increase due to the disruption of the stenosis in the larynx, the results from the current study indicated that the effect of two different levels of SGS (i.e., 50% and 75%) on PTP were not statistically significant. Although the findings were inconclusive, this pilot study revealed that PTP does not increase the longer the tissue is mounted; this suggests that completing multiple trials at one time would be efficient and beneficial in future studies. This would also increase the sample size and statistical power to provide a clearer understanding of how PTP is affected by stenosis in an ex vivo model.
References


APPENDIX A

Annotated Bibliography


**Purpose of the study.** The purpose of this study was to compare the similarities and differences between human larynges and cow, sheep, and pig larynges. Another aim of this study was to find other possible substitutes as models of the human larynx during phonation.

**Method.** This study used eight porcine larynges, six cow larynges, and eight sheep larynges. The researchers purchased these larynges from a butcher shop and then immediately cleaned and slow froze them until they needed to be used for experimentation. Twenty-four hours before the experiment began, the larynges began to be thawed in 0.9% saline. Each larynx was also dissected, removing the epiglottis prior to the experiments. During the procedure, the larynges were each mounted onto a tube that delivered humidified air that was pressurized and heated to simulate the air coming from the lungs. Subglottal pressure, mean flow rate, audio signal, electroglottograph, and the sound pressure level were each measured as variables in the study. The electroglottograph was used to determine the fundamental frequency during the phonatory trials. The researchers also stimulated the lateral cricoarytenoid muscle during the procedure to manipulate adduction of the vocal folds. The stages of adduction were low, medium and high.

**Results.** The researchers found that the porcine larynges had the largest range of fundamental frequency ($F_0$) compared to the sheep and cow larynges. Additionally, the porcine larynges phonated the loudest. The researchers did find differences between human and porcine larynges which are listed hereafter: the false vocal folds were found to participate in the production of sound in porcine larynges. This may have added to the loud phonation in the porcine larynges. The highest number of the maximum frequency range was also produced by the porcine larynges, with the lowest number being produced by the cow larynges. It was thought that this was due to the larger size of the cow larynges. Anatomically, the porcine larynges were also identified to be the most similar to the human larynges partially because these were the only ones with ventricles.

**Conclusions.** The results from this experiment identified the porcine larynges to be most similar to the human larynx in comparison to the larynges of the cow and sheep larynges.

**Relevance to current work.** The study’s method for obtaining and preserving the larynges is the same for the current investigation. The information gathered from this study also gave support for similarities between porcine larynges and human larynges. This study shows that the use of porcine larynges may give a more accurate representation of human larynges than a different study involving a different animal excised model.


**Review Article.** The purpose of this article was to identify what subglottic stenosis (SGS) is as well as determining possible treatments. Six different techniques for treatment were described
and illustrated in great detail. Six therapeutic techniques were used on six different case studies. The six different techniques included direct approximation, dilation, free graft and stent, free graft only, reconstruction of the trachea, and utilization of mucoperichondrial flap. The purpose of each of these techniques as well as the instructions on how to perform each were discussed and then compared to the outcomes of each technique. Some important discussions within this article that relate to the current study are the surgical outcomes of reconstruction in SGS, the symptoms of SGS, and descriptions of patient vocal quality posttreatment.


**Purpose of the study.** The classification of perceptual voice irregularities and documentation of potential risk factors for perceptual voice disorders associated with patients exhibiting SGS were the intentions of this study.

**Method.** Medical records 22 females and 9 males (31 patients total) who present with SGS were retrospectively reviewed from a three-year period in a single academic center. The Grade, Roughness, Breathiness, Asthenia, and Strain (GRBAS) scale, which is used as a four-point auditory-perceptual evaluation instrument for examining overall vocal quality. The GRBAS was administered to the patients individually during their first visit to the clinic in order to determine relationships between patient characteristics. Audio recordings were statistically analyzed from each patient with SGS as well as other patients with benign vocal disorders for assessment and reliability.

**Results.** Average baseline data for all patients was $G_{1.4} R_{1.2} B_{0.5} A_{0.5} S_{1.1}$. Average female Grade scores were better than males. Patients with single-stenosis scored better than patients with multi-stenosis in Grade, Breathiness, and Asthenia. As a whole, patients who had not had airway surgery had substantially better vocal quality across the scale. A greater probability of moderate or severe dysphonia in the categories of GBS were linked with patients who had vocal fold motion impairment.

**Conclusions.** The GRBAS scale outcomes suggested that as a whole, patients were perceived to have a mild to moderate dysphonia. Substantial vocal quality distinctions were noted between patients with single-site and multiple-site stenosis. Those patients who had stenosis surgery previously were observed to have poorer voice quality than patient who did not have any previous surgery and experienced typical vocal fold motion.

**Relevance to current work.** This study is relevant because it shows that individuals with SGS presented with mild to moderate dysphonia at baseline. This suggests that both SGS does have an effect on the voice.


**Purpose of the study.** The purpose of this study was to establish whether or not laryngotracheal stenosis (LTS) is heterogeneous when looking at biological history, etiology, and clinical outcome.
Methods. The objective of this study was completed using a retrospective cohort study design with patients over the age of 18 with LTS, between the years 1998 and 2013. The patient’s individual characteristic information was documented, including age, gender, comorbidities, race, and follow-up duration. The information that was asked for comprised of treatment approach, etiology of surgical dates, stenosis, and if a tracheostomy was present at the previous follow-up.

Results. Etiologies were grouped into four categories; traumatic (8%), idiopathic (18.5%), autoimmune (18.5%), and iatrogenic (54.7%). Autoimmune and iatrogenic etiologies made considerably more patients continue to be tracheostomy-dependent in comparison with idiopathic and traumatic.

Conclusions. LTS is generally diverse in nature, coming from multiple etiologies with a variety of rates for long-term tracheostomy dependence.

Relevance to current work. LTS is very similar to subglottic stenosis in that it distresses the same anatomical mechanisms, but continues to affect further down into the trachea as well as further up the larynx.


Purpose of the study. This study observed the phonation instability flow, or the amount of airflow necessary for chaotic phonation, in order to identify a range at which typical vocal fold vibration begins.

Method. Seven excised canine larynges were used in this study. Airflow and pressure were recorded at chaos and phonation onset. Three experimental conditions were used: 0 and 20% elongation without a glottal gap, and 20% elongation with a 3-mm posterior glottal gap. Effects of elongation and posterior glottal gap were assessed using paired t-tests.

Results. Phonation instability flow and phonation flow range indicated dependency regarding abduction, but not for elongation of the vocal folds. Phonation instability pressure did not indicate dependency on either elongation or abduction. Phonation instability flow and phonation flow range indicated more significant differences for vocal fold abduction compared to phonation threshold pressure (PTP) and phonation threshold flow (PTF).

Conclusions. This study explored other aerodynamic measures and found that these may be more reliable measures to determine physiological differences regarding elongating or abducting the vocal folds. The results may be useful to detect pathologies such as bowing, vocal nodules, or posterior glottal chinks.

Relevance to current work. This study provided additional information about PTP and PTF being useful measures for observing the biological mechanism of the vocal folds. This confirms that PTP and PTF were appropriately chosen in the present study to examine changes in pressure and flow within excised porcine larynges exhibiting ranging levels of stenosis.

**Purpose of the study.** This study manipulated prephonatory glottal width and varied it in order to compare PTF and PTP and see how sensitive there were to these mechanical changes.

**Method.** This investigation used 10 excised canine larynges. These were then inspected for abnormalities and then tested in a benchtop model measuring subglottal pressure and airflow at phonation onset. The posterior glottal width of each larynx was abducted at five different glottal widths using a metal shim of widths ranging from 0 mm to 4 mm. The onset airflow and onset pressures were each measured.

**Results.** According to the results evaluated by a one-way analysis of variance; Significant changes in the PTF were observed when the glottal width was larger than 1.0 mm. PTF increased along with posterior glottal width. However, the results also showed that PTP was insignificantly impacted by glottal width at all values.

**Conclusions.** The data collected indicated PTF is more sensitive to changes in the posterior glottal width than PTP.

**Relevance to current work.** The PTP and PTF were both evaluated in the current study. If PTP was less sensitive to a posterior gap than PTF, then this may occur in the present study. Knowing this may be useful in understanding the results of the current study, as PTP will be measured while excised larynges exhibit ranging levels of stenosis.


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**Purpose of the study.** The intention of this research was to examine the endoscopic surgical management and treatment outcomes of adults presenting with SGS.

**Method.** The following study was a retrospective review of 92 adult patients (69 female, 23 male) presenting with SGS. These patients underwent 247 endoscopic dilations between 2001 and 2010.

**Results.** The results showed that each patient reported at least one comorbid condition. Out of the 92 patients, 25% had a history of prolonged intubation, 33% were idiopathic, and 45% had their SGS connected to a previous history of granulomatosis with polyangiitis. The mean age for first time of surgery was 48 years. The researchers discovered that 41 patients underwent a single procedure and 51 patients required multiple surgeries. It was also found that 13.7 months was the average time period for patients needing a succeeding procedure. The overall results of this investigation showed that aberrations in surgical technique did not display differences in time to the next procedure.

**Conclusions.** The information gathered from this 10-year review showed that SGS continues to be a treatment challenge. Although patients with SGS are often symptomatically improved after receiving an endoscopic dilation, the reappearance rates continue to be high.

**Relevance to current work.** The results collected regarding the surgical management and outcomes of patients presenting with SGS show that this continues to be a problem and further research regarding aerodynamic patterns such as PTP and PTF need to be conducted to improve surgical outcomes.

**Purpose of the study.** The purpose of this study was to observe the efficiency of rehydrating dehydrated larynges and examine the differences seen in phonation.

**Method.** The vocal folds of 13 excised canine larynges were dehydrated using warm dry air until phonation ceased. After the desiccation challenge took place, each larynx was placed in saline solution for 30 min. These same larynges were then mounted onto a bench apparatus which provided subglottal humidified airflow. Measurements were then recorded in order to compare PTP, amplitude and glottal airflow.

**Results.** Rehydrating the vocal folds significantly decreased the PTP and also increased efficiency.

**Conclusions.** The results of this study reflected the importance of vocal fold hydration in the physiology of normal hydration. Additionally, vocal fold hydration contributed to the decrease in PTP as well as perceived vocal effort.

**Relevance to current work.** This is relevant to the current study as the researchers used a similar benchtop model using excised larynges in an ex vivo model.


**Purpose of the study.** The purpose of this investigation was to identify the minimal airflow required to begin phonation. This study also compared PTF to PTP.

**Method.** A one mass model was used in this investigation to study the minimal glottal airflow necessary to initiate vocal fold vibration.

**Results.** The results of the study indicated that glottal shape significantly influenced PTF measurements. Additionally, PTF also changed depending on the tissue properties or viscosity of the vocal folds. Moreover, PTF could have been reduced by decreasing vocal tract resistance.

**Conclusions.** This study found PTF may be used more reliably than PTP in order to evaluate clinical information of the vocal folds or laryngeal function. Additionally, measurements of PTF could detect more efficiently laryngeal dysfunction caused by a vocal pathology.

**Relevance to current work.** Although this study did not look at excised larynges, it included the evaluation of PTP and PTF which are the factors which will be analyzed in the present investigation.


**Purpose of the study.** This study compared the differences between phonation characteristics of a hemilarynx to data collected from phonation of a full larynx.

**Method.** Nine excised canine larynges were included in this study. Each larynx was dissected and refrigerated in 0.67% saline before being thawed and mounted on a benchtop model. Data was then collected from the whole larynx as each was phonated. Afterwards, the left vocal fold was removed from each specimen and a vertical plexiglass plate was put in its place. Each plexiglass plate contained pressure transducers. After dissection, each larynx was phonated once more. This process included recording sound pressure level, F0, PTP, and the average glottal flow.

**Results.** This study found similarities in full larynges and hemilarynges such as PTP, perceptual...
sound quality, amplitude, and frequency of vibration. Some of the main differences found were observing that airflow in the hemilarynx was almost half of the airflow found in the full larynx. Researchers also discovered that the sound pressure level in the hemilarynx was 25% less than the full larynx.

**Conclusions.** The results showed that the hemilarynx has many similarities and characteristics of a full larynx. This knowledge could be useful to those patients who have surgery and receive a partial vertical laryngectomy. The similar frequencies ranges seen in the hemilarynges and the full larynges give researchers useful information about phonation to patients who may be in need of a partial laryngectomy. This is also valuable information for SLPs concerning improving therapy techniques meant for patients who have had a partial laryngectomy.

**Relevance to current work.** This study is relevant to the current study as they both collect similar data. The PTP and frequency range were two essential measurements taken, showing PTP is a valuable measure in regards to vocal quality and efficiency. This experiment also used canine larynges, which confirms the idea that animal larynges may be beneficial in accurately represent the human larynx.


**Purpose of the study.** The purpose of this study was to quantify the form and structure of collagen in the vocal fold lamina propria. A secondary objective was to identify the effects of pepsin on the form and structure of collagen in the lamina propria of the vocal folds.

**Method.** Twenty-six vocal folds from pigs were collected for this experiment. The following data was taken for each larynx: the d-periodicity (characteristic axial pattern), diameter, and roughness of the collagen fibers. As a part of the study, the lamina propria of 13 larynges was dissected and these were then imaged with atomic force microscopy. The secondary objective was tested by exposing the lamina propria of another set of pig larynges to pepsin and sham prior to the dissection of the lamina propria and atomic force microscopy. An additional group was also tested by directly exposing pepsin to the epithelium of the vocal folds. Each group exposed the epithelium of the vocal folds directly to pepsin or sham every 15 min for a total of two hours.

**Results.** The data collected from the d-periodicity, diameter and roughness of the collagen fibers supported previous literature reports about collagen fibers. The results gathered from the atomic force microscopy contribute to the knowledge about other tissues. Additionally, the results of exposing the vocal folds to pepsin did not prove to alter the structure and form of the collagen fibers of the lamina propria. However, a slight difference in the thickness of the collagen fibers was detected from the results of this study.

**Conclusions.** The knowledge gained from the results of this experiment may contribute to the improvement of biomaterials used in place of healthy lamina propria. This may especially be helpful for clients who have deteriorated vocal folds due to aging or scarring.

**Relevance to current work.** This study included pig larynges because they are the most similar in structure and form to human larynges. Additionally, the knowledge gathered about the morphological properties of collagen fibers of vocal folds of the pig larynges may be useful in conducting other studies. Knowing the structure of the collagen fibers may aid in better understanding the physiology of the vocal folds during phonation.

**Purpose of the study.** An x-ray stroboscopic system was used to analyze the movement of vocal tract fluid in relationship with vocal fold vibration. The flexibility of the mucous membrane of the vocal folds was also observed.

**Method.** Two types of excised canine larynges were included in this study: one group of larynges had normal vocal folds and the other group had a unilateral stiff vocal fold lesion. An x-ray stroboscope system was used to observe and record vibratory patterns on the frontal plane. The vocal folds were sutured at the posterior portion of the vocal folds in order to increase adduction. A nebulizer was also used to stimulate the movement of air tract fluid from the subglottis to the supraglottis. The frontal and superior views were captured simultaneously by two cameras.

**Results.** This study found the upper surface of the medial edges of the normal vocal folds to correspond very closely to the location where the mucous membrane wave motion disappeared. However, the larynges with the unilateral stiff lesion had a much smaller amount of accumulated fluid on the surface of the stiff vocal fold. Unlike the normal vocal folds, which accumulated fluid in a column, the fluid accumulated in a flat layer for the lesioned vocal folds. However, the vocal fold with a lesion also vibrated up and down.

**Conclusions.** The results of this experiment indicated that traveling waves of the vocal folds’ mucous membrane of the vocal folds may have contributed to the role of relocating air tract fluid from the subglottis to the supraglottis. The fluid column formed with the driving force of typical wave motion of the mucous membrane. However, when a stiff lesion was present on the vocal folds, the fluid column could not form because the membrane lacked flexibility.

**Relevance to current work.** The design of this study was very similar to the current study because it involved an animal model and also included a nebulizer to observe the accumulation of fluids on the vocal folds. This may be pertinent to the current study because the lesions some of the pigs had may not have been detected and these may have affected the manner in which the vocal folds accumulated the aerosolized saline, causing them to dry out faster or slower.


**Purpose of the study.** The purpose of this study was to quantify PTP and PTF of excised human larynges and determine how these measures are affected by posterior glottal width, glottal area, and gender. An additional objective of this study was to determine the presence of hysteresis in human vocal fold oscillation.

**Method.** This study included nine Excised human larynges were obtained and preserved in a sealed beaker filled with phosphate-buffered saline. Prior to testing, the exterior laryngeal muscles, except for the strap muscles, and the ventricular folds were dissected away to expose the true vocal folds. The larynges were mounted on a bench apparatus where the PTP and PTF
were measured at phonation onset and offset. Additionally, screws secured some structures of the larynges. This was done when mounted on the apparatus in order to maintain consistency throughout the study. The larynges were tested by having compressed, desiccated airflow provided subglottally. The air passed through an inline flow meter that monitored the mean flow. Electrode plates were also attached to the strap muscles to measure the electroglottograph signal. Additionally, a sound level meter was attached for the later experiments.

**Results.** This study included 197 trials with a PTP mean range of 0.783 ± 0.093. The PTF mean range was 0.880 ± 0.087. The onset PTP and PTF measurements were more variable than the offset PTP and PTF. The results of testing the effect of the posterior glottal width on PTP and PTF supported that these did not have a significant aerodynamic difference. Moreover, when studying the effect of prephonatory glottal area, the study concluded a positive relationship between PTF onset and offset with the glottal area, however there was not a correlation noted for PTP onset or offset. This study also found PTP and PTF onset and offset to be much greater for males than for females.

**Conclusions.** This study supported the presence of hysteresis, wherein the onset differed from the offset of the vocal fold oscillation. According to the smaller variability noted in PTP and PTF offset, it was inferred that the offset parameters may be more reliable than onset parameters. This study also supported the use of canine larynges instead of human larynges for testing the PTF.

**Relevance to current work.** This study provided relevant information about setting up an excised larynx for experimentation. Moreover, it provided data from human larynges and described the benefits of using animal larynges to gather data pertinent to human laryngeal pathologies.


**Purpose of the study.** The main objective of this study was to develop an animal model similar to humans, with consistent severe subglottic stenosis (SGS).

**Method.** An artificial stenosis was created in the subglottal region of the larynx using either a nylon or polypropylene brush. This was done in 16 New Zealand White rabbits. Using a right-angled probe, the subglottic cross-sectional area of the rabbits was measured endoscopically and analyzed using an open source image analysis software. The native airways and cross-sectional areas of the stenotic airways were compared to find the Myer-Cotton grade classification and SGS percentage.

**Results.** The data gathered during this study showed that the mean SGS percentage was discovered to be 73% for all subjects. During the experiment, three of the rabbit larynges that were damaged with the nylon brush had a 30%, 52%, and 76% stenosis. Nine of the 12 surviving rabbits that were damaged with the polypropylene brush had a >86% SGS. Lastly, four of the rabbits in the polypropylene brush cohort died from procedural complications.

**Conclusions.** This study gives a dependable model for using a polypropylene brush to create severe acute SGS. Researchers in the study found that cartilage exposure after creating the injury was linked with more extreme stenosis and determined that the mortality rate could have been reduced if endoscopic balloon dilation was performed seven days after injury, rather than emergently nine days after the damage.
Relevance to current work. This study supports the use of ex vivo animal models of the larynges similar to humans. This study also used an artificial stenosis device similar to the present study to simulate severe subglottic stenosis in the larynx region. However, the present study will a silicone sleeve consistent with the current research to simulate 50% and 75% stenosis.


Review Article. The goal of this article is to display cricotracheal resection (CTR) as an equivalent or even better surgical technique instead of laryngotracheal resection (LTR). This was done through explaining the preoperative and operative techniques in detail which are needed to perform a successful CTR for patients with extreme SGS. Data sets from a variety of other research studies were also used to provide supporting evidence for the airway patency, positive decannulation rates, and vocal quality after a CTR. The article suggested that CTR should be considered for being the primary surgical intervention for improving severe SGS for both adults and pediatric patients. As this surgical technique continues to develop it is important to examine the secondary effects of this procedure such as the negative effect it has on the patient’s voice quality. The current study is examining the change of PTP in an ex vivo model displaying different grades of SGS to gain a better understanding of its impact on the voice.


Purpose of the study. The intention of this study was to empirically examine the consequences of cricotracheal resection (CTR) on pediatric patients presenting with severe, Cotton grade III or IV, stenosis.

Methods. In this study, thirty-eight infants and children presenting with severe, Cotton grade III or IV, SGS between the years 1978 and 1998 went through a partial cricoid resection procedure with primary thyrotracheal anastomosis. For most of the patients, parents reported an etiology of prolonged intubation while congenital SGS was only documented for seven patients.

Results. The outcomes of the investigation reported no mortalities, one complete restenosis, two patients reached a grade 1 stenosis, and 35 patients had a normal lumen. Twenty-four patients exhibited a normal voice postoperatively while thirteen patients showed symptoms of moderate dysphonia, mostly pertaining to pitch of voice.

Conclusions. The outcomes of this research suggest that the CTR is a better surgical approach in comparison to the currently preferred method of laryngotracheoplasty, in managing severe subglottic stenosis in children.

Relevance to current work. The current study is examining the change of PTP in an ex vivo model displaying different grades of SGS to gain a better understanding of its impact on the voice and how surgical outcome could improve in the future as to not compromise vocal quality.

**Purpose of the study.** The purpose of this study was to determine how viscosity changes of the laryngeal mucous influenced vocal fold vibration.

**Method.** The study used 8 excised larynges divided into two groups. Each group was assigned a different solution with varying viscosities and applied to the larynges. The high viscosity fluid was made up of dissolved chondroitin sulfate sodium salt and the low viscosity fluid was made up physiologic saline. Before piping the fluid onto the vocal folds, the vocal fold’s surface mucous was taken off with swabs. The 0.1 ml of the fluid was then covered over the superior surface of the vocal folds in a randomized order. A laryngostroboscope and an X-ray stroboscope were used to measure the vibration of the vocal folds.

**Results.** The study found that the high viscosity solution required a greater number of frames to capture the opening and closing phases. Additionally, the open quotient was increased and the normalized glottal peak area was lessened significantly for the high viscosity fluid. Also, the horizontal and vertical components’ amplitudes decreased with the high viscosity fluid when the image was seen from the frontal plane.

**Conclusions.** Due to this study, researchers may conclude high viscosity vocal fold surface fluid affects mucosal wave. However, further research is needed to determine the preferred level of viscosity in the mucosal layer.

**Relevance to current work.** The tissue storage and dehydration methods being used in this study are similar to the preservation and hydration procedures in the present study, all of which may impact the observed vibratory patterns of the vocal folds.


**Purpose of the study.** This objective of this study was to identify the effectiveness of a multimodality approach used to treat idiopathic subglottic stenosis (ISGS), as well as determine predicting factors of treatment success, and improve definitions of the limitations and roles of endoscopic and open surgery.

**Method.** Fifty-four patients exhibiting idiopathic subglottic stenosis were treated with either endoscopic or open surgical procedures between the years 2004-2012. Patients were all female and had a mean age of 47.8 +/- 12.1 years at the time of diagnosis. 39% of patients received prerereferal treatments prior to the initiation of this study.

**Results.** Minimal invasive surgery was used for managing 78% of endoscopically treated patients. The five-year actuarial success rate for treatment was determined to be greater for patients with SGS only stenosis than it was for associated glottic and subglottic stenosis. According to the airway-dyspnea-voice-swallowing system, each of the patients, but one, who had a laryngectomy were able to maintain prosthesis-and stoma-free airways by the last follow-up. Twenty-six patients had D1 and D2 dyspnea grades, 41 patients ha a V1 voice, 9 had V2 voices, four received V3 or V4 voices, and 51 patients presented with a normal swallow (S1). The only substantial feature associated with dyspnea and vocal outcomes on a multivariable regression analysis was whether or not the stenosis affected the glottis.
Conclusions. This study confirmed that idiopathic SGS is a treatable, subtle, but progressive fibromatosis disease that currently predominantly affects females more than males and presents between the ages 40 to 60. If the stenosis is less severe, fewer endoscopic interventions are needed. For individuals with SGS who cannot benefit from endoscopic treatment, the use of airway framework expansion and imbedding of biological inhibitors of fibrosis, as described in the article, may accomplish long-term disease remission without affecting vocal gender aberrations. The only identified factor that was independently associated with dyspnea and diminished voice outcomes was when the glottis was affected at the time of presentation.

Relevance to current work. This article quantified voice outcomes of patients with idiopathic SGS after endoscopic and open surgical treatments. The current study is studying the effects of different levels of SGS on PTP as well as vocal quality.


Review Article. This journal article provided a review of an evidence-based summary of SGS including its history, clinical appearance, anatomy, etiology, presentation, outcomes, and possible treatments. Within the study, the main symptoms stated were stridor, a sharp cough, hoarseness, dyspnea, and aphonia. Anatomical abnormalities of the larynx and trachea and previous prolonged intubation were the common etiologies that were presented and discussed. A variety of treatments were reviewed from the least invasive endoscopic procedure to the most invasive laryngotracheal reconstruction. With each treatment, there were both positive and negative outcomes associated with each. The most significant drawback discussed was permanent voice disorders following grafting procedures. This article offered a good overview of SGS as a whole and suggested supporting evidence for the unfortunate side effects of SGS and the surgical management that is associated with it to alleviate the patient from symptoms.


Purpose of the study. This report collected multiple experiences of surgeons in a single institution concerning the surgical treatment of SGS. The investigators compared various SGS treatments and results of individuals presenting with SGS.

Methods. In this report, thirty patients in a tertiary care academic institution were treated for acquired SGS between the years of 2004 to 2009. Seventeen of these patients’ information was used retrospectively while the other 13 patients’ information was collected at the time of the study. Both endoscopic and external surgical interventions used were, including CTR, depending on the patient’s report.

Results. Patients within this research study were categorized into one of two etiological groups: endotracheal intubation injury or external neck injury. Specific acquired SGS was discovered in 3 out of 30 patients. However, stenosis of the subglottis as well as the trachea was found to be the most common etiology. Surgical intervention was dependent on multiple factors, such as the amount of time that has passed since injury, the severity and length of stenosis, the placement of stenosis, and the type of stenosis such as mucosal edema only or cartilaginous framework involvement as well. After surgery, 29 of the 30 patients had their lumen restored. However,
luminal restoration was achieved with a single procedure in 11 patients and multiple procedures were required for the other 19 patients. External factors proved to be big contributors to surgical success in 27 of the 30 patients. When endotracheal procedures were used primarily they were only found to be successful in 2 out of the 8 patients.

**Conclusions.** SGS that is caused primarily by intubation injury has a better profile than stenosis caused by external injury. Choosing a surgical procedure can be successfully directed by the particular anatomical categorization of stenosis. Endoscopic procedures are also beneficial as adjunctive procedures rather than primary procedures.

**Relevance to current work.** This investigation adequately showed that SGS is rarely limited to just the subglottis, but more commonly includes both the trachea and glottis. This information can bring better understanding to the vocal changes that accompany SGS before surgical management.


**Purpose of the study.** This study examined the amount of airflow required for phonation onset is greater than the airflow required during sustained phonation.

**Method.** This study included 10 excised canine larynges which were inspected for pathologies and trauma, and later stored in 0.9% saline solution and frozen for later use. For experimentation, the larynges were mounted on a benchtop after having been stored in 0.9% saline. The larynges were attached to a pseudolung to provide airflow. A subglottal flow of humidified air was introduced to the larynges being tested. This airflow was increased until phonation occurred. The airflow was then decreased until phonation ceased. Some specimens had elongated vocal folds to determine effects on flow threshold; this methodology was used to simulate the increased tension that can occur with vocal fold pathology.

**Results.** The study found that the offset airflow was less than the onset in all cases. The measurements taken during the study illustrated a higher onset PTF than offset PTF. Additionally, the ratios of onset to offset PTF were between 0.515 and 0.972. Moreover, the ratio included a larger range than expected.

**Conclusions.** The knowledge gained from this study may be beneficial in treating and identifying vocal fold dysfunction. PTF could be useful when diagnosing voice disorders particularly with respect to laryngeal resistance. These results added to the knowledge base involving the physics of phonation.

**Relevance to current work.** This relates to the current study because it involved excised animal larynges as well as a bench setup. The setup and procedures of this study may be helpful in delivering the new study as well as providing airflow data that could serve as a comparison during the pilot phase of the current study.

Purpose of the study. This investigation sought to examine the clinical differences between the four post-tracheostomy types (PTTS) according to stenosis mechanism and site: subglottic, stoma, cuff, and tip granuloma.

Method. This study included 99 PTTS participants who were evaluated retrospectively after an interventional bronchoscopy between the years 2004 and 2014. These participants were separated into two groups in regards to their pathophysiological similarities such as subglottic or stoma type and cuff or tip type.

Results. The data gathered from this study showed that there were no differences in baseline characteristics between groups. However, investigators found that silicone stents were needed to preserve airway patency more often in patients that had subglottic or stoma type stenosis (76%) than those that had cuff or tip type stenosis (55%). Investigators also found that permanent tracheostomies were performed more often in patients with cuff or tip type stenosis (50%) than those who had subglottic or stoma type stenosis (19%). Lastly, the investigators in this study found that effective removal of the tracheostomy tube without surgery and procedure- or disease-related mortality was accomplished more often in patients with subglottic or stoma type stenosis (71%) than those with cuff or tip type stenosis (45%).

Conclusions. The results of this study showed that although there were no significant differences in baseline characteristics between PTTS types, the patients that had subglottic or stoma type stenosis had better outcomes than those with cuff or tip type stenosis. This means that it could be critical to differentiate between types of PTTS when considering prognosis.

Relevance to current work. This investigation is similar to the present study since it also involved excised animal larynges. The format of this study could be helpful in carrying out the new study. A pseudolung was also used, but unlike the present study, the larynges were not previously frozen.


Purpose of the study. A laryngeal desiccation challenge and two nebulized hydration treatments were used in this study to determine the differences between the PTP, vocal effort and throat dryness. Patients who experience chronic airway dryness were used as participants in this study.

Method. The following study examined 11 patients (10 females and 1 male) with Primary Sjögren’s Syndrome. Each participant underwent a laryngeal desiccation challenge for 15 minutes. This experiment was conducted once a week for two weeks. After dehydration, each participant received either a nebulized isotonic water treatment or a nebulized isotonic saline (0.9%). These were given transorally at a rate of 8 L/min.

Results. The information gathered from this study suggested a significant increase in PTP, vocal effort, and oral dryness after the dehydration challenge. The outcomes also suggested the nebulized isotonic saline treatment was more effective for hydration than the nebulized isotonic water treatment.

Conclusions. This experiment caused the participants with chronic dry throats to have phonatory changes after the dehydration challenge. The nebulized isotonic saline was thought to have contributed to vocal hydration causing PTP to decrease as well as vocal effort and dryness of throat or mouth.
Relevance to current work. In comparison to the present study, the information gained from this article that PTP decreases and vocal effort due to the nebulized isotonic saline treatment was helpful in keeping the vocal folds hydrated during the present study’s phonatory trials.


Purpose of the study. This investigation examined the movement of the superficial water layer of the vocal folds through the process of vocal fold oscillation.

Method. The researchers in this study investigated multiple studies that were conducted previously that did not identify the movement of liquid during vocal fold oscillation. Hyaluronic acid (HA) and its properties were also explored in this article.

Results. Vocal folds with more gelatinous liquid on the surface, were observed to require a greater lung pressure to initiate phonation. If the vocal folds were phonated for extended amounts of time, then the PTP increased. This may have occurred due to the time the tissues needed to evenly allocate the water after phonation. Additionally, a portion of the study looked at hyaluronic acid and its effects on dry absorption paper. The results showed a mean evaporation rate of 3.08 mg/min for pure water, 2.7 mg/min for 0.5% HA and 2.5 mg/min for 1% HA.

Conclusions. This study’s outcomes suggested that much research is still needed to see how fluid travels through the extracellular matrix as well as how hyaluronic acid affects hydration of the vocal mechanism.

Relevance to current work. This article supported the preservation methods chosen for the current study. Determining whether PTP was affected by overturning the dehydration process of the vocal folds may have given ideas about the movement of the water and how dehydration takes place in the vocal folds.


Purpose of the study. This study sought to examine the variations of the phonation instability pressure and the phonation pressure range of a normal larynx.

Method. Twelve canine larynges were excised postmortem and submerged in a saline solution 48 hours before being used in the experiment. The larynges were then mounted onto plastic tubing that was connected to a pseudolung and then put onto a base using two laterally positioned micropositioners with three prongs. In order to stimulate phonation, warmed humidified air was passed through the pseudolung to simulate air coming from the lungs. Phonation was then stimulated at 0% elongation and then 5%, 10% and 15% elongation of vocal folds.

Results. The characteristics of the spatiotemporal analyses demonstrated an irregular vibratory pattern for the irregular vibration. In comparing the results of 0% elongation and 15% elongation of the vocal fold length, results suggested that phonation instability pressure had no significant change and pressure range showed a substantial decrease.

Conclusions. The results from this study indicated that elongation of the larynx resulted in more stiffness in the vocal folds. Greater stiffness in the folds needed a higher PTP to initiate
phonation, which was seen to create phonation instability. Various vocal fold pathologies, such as scarring, can cause stiffness in the vocal folds, which may change the phonation instability pressure of the larynx.

**Relevance to current work.** On top of providing benefits for using an excised larynx instead of an in vivo model, this study examined vocal fold pathologies, such as scarring, which may change the phonation instability pressure of the larynx. SGS can be caused by scarring which is examined in the current study by degrees of stenosis. The current study also used high speed imaging to help analyze vocal fold vibration.


**Purpose of the study.** This study sought to quantify the regular and irregular phonation of excised larynges.

**Method.** This experiment included 10 excised canine larynges and took place within 48 hours after collecting the larynges. The larynges were mounted onto a base with a tube connected to the trachea and then attached to a pseudolung and secured with metal prongs. Regulated air was blown through below the glottis and several measurements were then taken, such as the phonation instability pressure and PTP. After the data was collected, the vocal folds were elongated at 5%, 10% and 15% with a precise micrometer system. The reason for this was to determine what impact vocal fold elongation has on PTP. To confirm the data was accurate, the measurements were each taken three times at each elongation percentage.

**Results.** The data collected from the excised larynges found that PTP, pressure range, and instability pressure can be successfully found using bifurcation analysis. Additionally, the researchers discovered that when the vocal folds were elongated, there was significant increase in the PTP, and a substantial decrease in the phonation pressure range.

**Conclusions.** The information gained from this study provided a knowledge that instability pressure and pressure range can be influential measurements when assessing phonation. Knowing that these are measures that can be used to better understand the physiological properties of normal and disordered larynges can also help future studies in the diagnosis and treatment of the larynx.

**Relevance to current work.** This study used a similar setup, measures, and methods as the present study. Although, this article noted using canine larynges instead of porcine larynges, this still supports the use of ex vivo animal models when testing phonation compared to human larynges.
APPENDIX B

Food Handler’s Permit

Utah Food Handler Permit

Name: Jessica Murphey
ID No: SFK00044441
Expires: 8/24/2020
Issued by Utah County Health Dept
This permit is NOT a legal form of identification
APPENDIX C

Experimental Checklist

Materials for Dissection and Preservation:
1. Scalpels (No. 11 and No. 21 stainless steel scalpels)
2. Apron
3. Gloves
4. Green dissection paper (to be laid on the dissection table)
5. Saline spray bottle
6. Ziploc bags
7. Hemostats
8. Sutures (1 for each larynx)
9. Dissection table
10. Red hazard box (rinse scalpels and then place them in this box)
11. Clorox wipes (for clean-up)
12. Paper towels (to hold your larynx steady)
13. Mini fridge
14. Industrial freezer
15. 0.9% sodium chloride irrigation USP fluid (isotonic saline)
16. Styrofoam cooler (to place liquid nitrogen in)
17. Liquid nitrogen (from the Chemistry Central Stockroom located in room 126 of the Nicholes Building at Brigham Young University)

Additional Notes for Dissection:
- Remove all surrounding tissues of the larynx such as the esophagus, thyroid gland, fat, excess tendons, innervation, vascularization. Make sure the trachea and thyroid cartilage are intact and without any abnormal openings or damage.
- Use the largest tracheas—these are best for phonation and mounting onto custom tubing
- Tracheas should be cut superiorly of the true vocal folds
- The true vocal folds should not be punctured (this will prevent air leakage)
- The shape should be a smile formed from the anterior commissure to the lateral posterior ends of the thyroid cartilage
- The arytenoid cartilages should be left intact (this will aid in adduction)
- The epiglottis should be removed by cutting a triangle posterior and in between the arytenoid cartilages
- Remove false folds completely (may use a hemostat for better precision)
- Remove any leftover tissue superficial and superior to the vocal folds (this prevents flopping of tissue during vibration of true vocal folds
- Trim the trachea leaving the trachea about 8-10 cm in length. (verify the inferior end of the trachea fits around the custom tubing connecting to the pseudolung
- Suturing: should be placed above the anterior commissure on the thyroid cartilage. First tie the end of the string attached to the suture in a knot (make several knots in the same location in order to prevent the string from going
through the cartilage). Hold the sharp end of the suture using a hemostat to provide support to puncture the anterior end of the thyroid cartilage (located just above and in front of the anterior commissure) (repeat this 4 times) make sure suture is tight and tug at it to observe its strength

**Materials for Experiment:**
1. 4 LED lights (make sure fresh batteries are in place)
2. Macropositioners
3. Micropositioners
4. Teflon tape (used to seal edges of trachea onto the custom tubing which is attached to the pseudolung)
5. Flow meter (Aalborg mass flow meter GFM-47)—flow should be calibrated at 0, 10 and 15 cmH2O
6. Medical Flow Meter- attached directly to the air tank and to the Aalborg mass flow meter GFM
7. 2 Air tanks (one will attach to the flow meter and the humidifiers; the other will be for desiccated air)
8. Pressure transducer (should be plugged in from computer to inferior lateral portion of larynx or the custom tubing)
9. Pressure calibrator box (should be used only to calibrate pressure transducer) calibration occurs at 0, 10 and 15 PSI
10. Check all plugs
11. WinDaq should be turned on and 4 different waves should be showing (wave 1 measures: microphone signal; Wave 2: pressure; Wave 3: Flow; Wave 4: High Speed Trigger)
12. Humidifier (make sure tubing is plugged in to pseudolung and air tank)
13. High Speed video camera: Trigger should be on and plugged into the sound board
14. Microphone (SHURE SM-48) should be on and plugged in (before starting experiment make sure the wave shows up on WinDaq by tapping the mic lightly) (position microphone about 4 inches away from the larynx. )
15. High Speed - make sure trigger is plugged in
16. 4 Metal clamp (secure trachea onto the custom tubing which attaches to the pseudolung)
17. Metal clamps (hold flashlights & Microphone)
18. Clorox Wipes
19. Paper towels

**Measuring Flow**
1. Make sure flow meter (Aalborg mass flow meter GFM) is plugged into outlet
2. Verify computer is turned on and the WinDaq window is opened
3. Verify flow signal is not peaking (max should be 100 liters/min)
4. Should be directly attached to WinDaq box which is attached to the computer
5. Record when flow is at 0 (mark exact number ~ -. 6)
   a. Shift space-to make a comment
6. Record when flow is at 15 (mark exact number)
7. System is ready to record
   a. Hit F4 to record
b. Hit shift F4 to standby

c. Hit shift space to apply comment (comment does not appear until you hit enter)

**Measuring Pressure**

1. Make sure pressure transducer is plugged into the WinDaq box which is connected to the computer
2. PSI or cm H2O
3. Insert pressure transducer directly into PC-IH box
4. Verify WinDaq is picking up pressure signal by observing wave 2
5. Calibrate pressure at 0 and 10 PSI
   a. Record F4 at 0 PSI
   b. Hit shift space to apply the comment (insert press_cal_0)
   c. Do the same for 10 PSI
6. Remove pressure transducer from PC-IH box
   a. Press button before releasing syringe
   b. There should not be any tension when releasing the syringe
7. Insert pressure transducer into opening inferior to the mounted trachea
8. Ready to record
   a. Record F4
   b. Hit shift space to apply the comment (e. g., D3P01) (trial type and pig number along with trial number)
   c. Do the same for all trials

**Recording High Speed**

- Unit should be plugged in and on
- Verify all components are turned on in order (high-speed, computer, monitor)
- Login to computer, open Kay Pentax software
- Verify camera and waveform signals are on, and ensure settings are to record “END”
- Click record, wait for camera to lock
- Click trigger when ready to record (records 4 seconds prior to trigger)

**Microphone signal**

- SHURE SM-48
- Make sure the microphone is plugged into an outlet.
- The microphone should be about 4 inches away from the glottis

**Humidifier**

- (Thera-Heat Heated Humidifier-Portex) by Smiths Medical
- Make sure this is plugged into an outlet
- Use standard settings
- Should be plugged in directly to the flow meter (clear tube) and into the custom tubing of the pseudo lung. (blue tube should be attached to the pseudolung.)

**Flashlights:**

- UltraFire XML-T6
- Verify these have fresh batteries and are working prior to beginning the experiment.
- Should be equidistant from the glottis.
- Position one directly anterior to the glottis.
- 2 will be positioned laterally equidistant from the glottis.
- 1 should be positioned posteriorly.
- Use as many as are necessary (check prior to beginning experiment).