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Examining the Reversal of Vocal Fold Dehydration Using Aerosolized Saline in an Excised Larynx Model

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ABSTRACT

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Previous studies have found vocal fold hydration to be crucial for healthy function of the vocal mechanism. Surface tissue hydration facilitates efficient vocal fold oscillation. The composition of vocal fold surface fluid includes protective water and mucus layers, similar to the fluid that covers the mucosa and epithelia of the upper airway. Laryngeal dehydration has been linked to several factors such as mouth breathing, obstructive sleep apnea, dry air exposure, upper airway hypersensitivity, and certain diseases or behavioral voice use factors. Laryngeal dehydration affects phonation threshold pressure (PTP) and phonation threshold flow (PTF), defined as the pressure and flow observed at the onset of phonation, respectively. The application of topical nebulized isotonic saline (0.9% Na^+Cl^-) has been shown in previous work to decrease PTP. However, there are no studies examining the effects of aerosolized saline, administered supraglottally, on dehydrated excised porcine larynges. Examining the effects of aerosolized saline in an excised model is essential to determine any independent effects of this treatment in the absence of other physiologic mechanisms such as mucus secretion. This study sought to investigate the effects of aerosolized saline on dehydrated animal vocal folds to determine if the administration of supraglottic aerosolized saline, via a nebulizer, could reverse the adverse effects of laryngeal dehydration. The study included a prospective, mixed experimental design with two groups, one desiccation/aerosolization (A/B) group and a control (A) group, each comprised of five bench-mounted porcine larynges. Larynges in both groups received desiccated air (<1% relative humidity) supraglottally via custom tubing for 1-min doses until the vocal folds ceased audible phonation. Following the desiccation challenge, the A/B group received 2-min doses of aerosolized isotonic saline until phonation began again. The PTP and PTF were measured during phonation trials following each dose of the desiccation or aerosolization treatment. Significant changes in PTP and PTF were observed following both the dehydration and aerosolization treatment. The PTP increased significantly following the dehydration challenge and returned near baseline following the aerosolization treatment. The results of this investigation supported the hypothesis that the administration of aerosolized saline may reverse the adverse effects of vocal fold dehydration. Moreover, in a more physiologically realistic excised model, applying the mechanics of respiration, this study advanced the development of innovative theories related to the reversal of the adverse effects of dehydration, which may prevent the development of voice disorders.

Keywords: aerosolized saline, vocal fold hydration, larynx, bench model, phonation threshold pressure, phonation threshold flow

ACKNOWLEDGMENTS

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DESCRIPTION OF THESIS STRUCTURE

The following thesis, *Examining the Reversal of Vocal Fold Dehydration Using Aerosolized Saline in an Excised Larynx Model,* is written in a hybrid format. That is, the format of this thesis blends journal publication formats and classic thesis requirements. A portion of this research was presented at the Voice Foundation Annual Symposium and an article was submitted for publication to the *Journal of Voice*. An annotated bibliography of a comprehensive literature review is included in Appendix A. Appendix B contains the study's experimental check list and Appendix C contains the Food Handler's Permit, which indicates compliance with Risk Management requirements at Brigham Young University.

Introduction

Vocal fold hydration has been found to be essential for optimal voice function (Hanson, Zhang, & Jiang, 2010; Hemler, Wieneke, & Dejonkere, 1997; Sivasankar & Fisher, 2002; Verdolini-Marston, Titze, & Druker, 1990). The reduction of hydration in the upper airway may have adverse consequences on vocal health. However, recommendations regarding vocal health and the prevention of vocal fold injury caused by dehydration necessitate an understanding of how hydration affects voice production. Vocal fold hydration is believed to be largely dependent on two biological mechanisms—systemic and surface tissue hydration—which will be discussed below.

Systemic Hydration

Systemic hydration may be defined as the body's current state of hydration (Hartley & Thibeault, 2014). Systemic dehydration may result from insufficient liquid consumption, excessive fluid loss, and lack of fluid replenishment. These factors may lead to vocal fold dehydration and adverse voice changes (Fisher, Ligon, Sobecks, & Roxe, 2011). Upper airway dehydration affects more than 100 million individuals in the United States alone, as well as many more throughout the world (Erickson & Sivasankar, 2010; Hamdan, Sabra, Rifai, Tabri, & Hussari, 2008; King & Blumin, 2009; Sivasankar & Erickson, 2009; Trangsrud, Whitaker, & Small, 2002). Some of these vulnerable individuals include those who suffer from asthma, obstructive sleep apnea, Sjögren's Syndrome or those who have hypersensitive upper airways (Tanner et al., 2013). Occupational voice users such as teachers and female singers also tend to be at a higher risk for developing upper airway dehydration symptoms (Roy, Merrill, Thibeault, Gray, & Smith, 2004). Many of the individuals from at-risk populations are advised to drink plenty of water and to stay well hydrated (DiRenzo, Tanner, & Thibeault, 2016). However, in an

extensive multidisciplinary literature review, Hartley and Thibeault explained some of the flaws and unanswered questions that exist between systemic hydration and the vocal mechanism. Water travels through several main routes in the body, such as the respiratory tract, the digestive system, the skin, and the kidneys. Through these systems, a sedentary human loses about 2 to 3 L of water per day (Jequier & Constant, 2010). Our body automatically responds to this loss of water with small hormonal changes; however, the hypothalamus does not trigger the thirst response until approximately 1% of the total body's water has been depleted (Hartley & Thibeault, 2014). Additionally, studies have shown that the thirst response decreases with age (Bossingham, Carnell, & Campbell, 2005). Moreover, DiRenzo, Tanner, and Thibeault disputed the axiom that drinking water directly improves voice quality; that is, poor systemic hydration can contribute to the development of an illness but may not be directly related to vocal function. Ultimately, the vocal folds may not be targeted directly through the ingestion of additional liquids beyond what is necessary to maintain general health.

Surface Tissue Hydration

Surface tissue hydration may be defined as the level of hydration found on the surface of the vocal fold and airway epithelia, which is made up of a mucous blanket and deeper water layer governed by transepithelial water fluxes from ionic transport (Sivasankar & Fisher, 2007). The presence and replenishment of this hydrating mucous blanket has been found to significantly decrease phonation threshold pressure (PTP), the pressure observed at the onset of phonation, and self-perceived vocal effort (Roy, Tanner, Gray, Blomgren, & Fisher, 2003; Tanner, Roy, Merrill, & Elstad, 2007; Titze, 1994). The water layer of this vocal fold surface fluid may be referred to as the sol layer and its main functions are to protect, hydrate, and lubricate the vocal folds during oscillation (Witt et al., 2009).

Additionally, the level of viscosity of the fluid located on the surface of the vocal folds is a key factor involved in vocal fold hydration. An increase in viscosity causes an increase in PTP, possibly due to greater lung pressure required to initiate phonation (Finkelhor, Titze, & Durham, 1988; Titze, Klemuk, & Lu, 2014; Witt, Taylor, Regner, & Jiang, 2011). A study completed by Nakagawa, Fukuda, Kawaida, Shiotani, and Kanzaki (1998) observed the effects of surface fluid viscosity on the amplitude of vibration during vocal fold oscillation. Traditional isotonic saline (0.9% Na^+Cl^-) and dissolved chondroitin sulfate sodium salt, two physiologic saline solutions of different viscosities, were topically applied to the vocal folds of an excised animal model. The results indicated that isotonic saline did not decrease the amplitude of oscillation as much as dissolving chondroitin sulfate sodium. This was perhaps due to dissolving chondroitin sulfate sodium having a higher level of viscosity. Previous literature has reported a decrease in the efficieny in the vocal fold oscillation as the viscosity of the vocal fold surface fluid increases (Verdolini et al., 1990). Moreover, previous literature has provided evidence regarding the negative effects of thick secretions on the surface of the vocal folds during voice production (Roy et al., 2003). Negative effects include less efficient and more effortful vocal fold oscillation. Nevertheless, many questions remain unanswered about the mechanical influence of vocal fold surface fluid on vibration. Previous studies have found liquid on the surface of the vocal folds tends to gravitate towards the midline during oscillation (Tao, Jiang, & Czerwonka, 2010). Some researchers have examined hyaluronic acid and its influence on vocal fold oscillation due to its ability to attract large amounts of water (Titze et al., 2014). However, little is known about the direction in which liquid travels on the surface of the vocal folds or if any molecules actually penetrate the epithelial cells and the extracellular matrix.

An additional factor that may relate to voice function and surface hydration is mucociliary clearance. From a respiratory perspective, mucociliary clearance is a protective process that transports mucus from the lungs in a vertical direction towards the vocal tract via cilia (Antunes & Cohen, 2007; Wanner, Salathe, & O'Riordan, 1996). This process aids in the lubrication and maintenance of surface tissue hydration of the vocal folds (Kawaida, Fukuda, Kano, Shiotani, & Kohno, 1990). Mucociliary clearance may be affected by several factors, such as the health of epithelial cells, ciliary function, and the viscosity and depth of the sol layer (Tanner et al., 2007). Due to the wavelike motion created by the cilia during mucociliary clearance, it is suspected that the movement may be related to the level of flexibility of the vocal fold epithelia (Kawaida et al., 1990). However, it is also possible that mucociliary clearance does not fully explain the presence of mucus on the vocal folds. For this reason, excised larynx models do not completely replicate the manner in which vocal fold surface fluid may reach the airway. Additional work is needed to examine the effects of vocal fold topical hydration independent of the mucociliary clearance.

Quantification of Vocal Fold Hydration

A traditional aerodynamic measure of voice function is PTP. The operational definition for PTP used in this study was the minimum amount of pressure at the onset of phonation (Titze, 1994; Hottinger, Tao, & Jiang, 2007). This measure has been considered useful in aerodynamic assessment of vocal fold properties and laryngeal function to discriminate between typical and pathologic phonation (Hottinger et al., 2007). In a study conducted by Hoffman et al. (2012), PTP decreased as glottal abduction increased; a relationship was not found between vocal fold elongation and PTP. The authors of this study recommended using PTP for the initial assessment of vocal pathology due to its sensitivity to the self-perceived vocal effort required to

initiate oscillation (Hoffman et al., 2012). On the other hand, PTP significantly increased with vocal fold elongation in a study involving the elongation of excised canine vocal folds at 0%, 5%, 10% and 15% of the vocal fold length at rest (Zhang, Reynders, Jiang, & Tateya, 2007). Further studies should be completed to determine if vocal fold elongation has a significant effect on PTP. Several studies have arrived at a similar consensus involving hydration and PTP. As hydration increases, PTP decreases, vocal effort decreases, and phonation becomes more efficient (Jiang, Ng, & Hanson, 1999; Roy et al., 2003; Tanner et al., 2007).

Phonation threshold flow (PTF) is a more recent aerodynamic measure that was first described in a study by Jiang and Tao (2007). The operational definition for PTF is the flow level at the onset of phonation. In 2009, a study by Witt and colleagues used excised canine larynges mounted on a traditional bench apparatus to observe the effects on PTF as the excised vocal folds were dried; the results indicated that PTF increased as hydration of the vocal folds decreased. A study by Jiang and Tao observed PTF values in a single mass model and discovered PTF may decrease by the following influences: decreasing the glottal area, decreasing tissue viscosity or mucosal wave velocity, and vertically increasing the glottal duct (Hottinger et al., 2007). Furthermore, Mau and colleagues conducted the first study observing the effects of hydration on PTP, PTF and hysteresis, the phenomena associated with nonlinearities in vibration, on excised human larynges (Mau, Muhlestein, Callahan, Weinheimer, & Chan, 2011). These researchers found offset PTP and PTF values to be lower than onset values in human larynges, which is similar to the results of previous canine studies that also observed hysteresis (e.g., Regner, Tao, Zhuang, & Jiang, 2008). Mau and colleagues inferred that bowing of the excised human vocal folds, caused by presbylaryngis, contributed to the absence of relationships among posterior glottal width, PTP, and PTF. An additional benefit of measuring PTF is that it may be

acquired noninvasively using an external flow transducer or a circumferentially-vented pneumotachograph mask, whereas PTP is only estimated intraorally and requires tracheal puncture for direct measurement (Hoffman et al., 2012; Regner et. al., 2008). For this reason, PTF may be a potentially useful and practical measure of voice function (Jiang & Tao, 2007).

Excised Larynx Models

Several authors have reported the advantages of using porcine excised larynx mechanical models to examine the main effects of independent variables on voice function (Alipour & Jaiswal, 2008; Jiang, Raviv, & Hanson, 2001; Johanes, Mihelc, Sivasankar, & Ivanisevic, 2011). Alipour and Jaiswal (2008) conducted a study involving excised pig, cow, and sheep larynges with the purpose of comparing phonatory characteristics to determine the most similar excised larynx animal model to human phonation. The findings of this study indicated that pigs had the most similarities to human phonation because of the extensive range of frequencies compared to the other two models. Excised porcine larynges had an average fundamental frequency (F_0) of 220 ± 57 Hz and PTP of 7.4 ± 2 cm H_2O, which are similar values to those found in humans (Alipour & Jaiswal, 2008). Porcine models are particularly useful for informing human studies due to similarities in larynx size, structure of vocal fold layers, presence of similar intrinsic muscles, a vocal ligament, and vibratory characteristics (Alipour & Jaiswal, 2008; Jiang & Titze, 1993; Johanes et al., 2011). One study was undertaken to examine differences between human, porcine, white-tailed deer, and canine larynges. These results also indicated that the porcine larynx was most similar to humans in the following respects: similar cricothyroid muscle, similar rotational ability of the cricothyroid joint, similar cartilaginous framework, and similar structure and thickness of the vocal fold cover (Jiang et al., 2001). Lastly, this study found the F_0 produced by the porcine larynx to be most similar to the human F_0 range. These studies provided

the rationale for the use of pig larynges to model the effects of laryngeal dehydration and rehydration in humans.

Although much research has been completed in the area of vocal fold hydration in excised larynx animal models, extensive research has not been done employing a more physiologically realistic respiratory model; additionally, no studies exist examining the use of aerosolized saline to reverse vocal fold dehydration. The present study included an isotonic nebulizer to return severely dehydrated vocal folds to their optimal state of hydration (Kumazawa, Asako, Yamashita, & Ha-Kawa, 1997). Kumazawa and colleagues found that using a nebulizer accompanied by intermittent vocalizations was one of the most successful methods of improving the distribution of aerosol particles into the upper airway. Isotonic saline (0.9% Na^+Cl^-) has been widely used in hydration studies due to its ability to lubricate the surface of the upper airway epithelia and presumably not influence the deeper water layers found in the tissue of the vocal folds. Tanner and colleagues (2007) found that nebulized isotonic saline could reverse the effects of vocal fold dehydration in healthy women with typical voices. Additionally, a more recent study found nebulized isotonic saline to be useful in treating and preventing voice problems in individuals with Sjögren's Syndrome (Tanner et al., 2015). Therefore, an excised larynx mechanical model offers an ideal experimental paradigm for investigating the potential reversibility of vocal fold dehydration using aerosolized saline.

Rehydration

Previous studies have attempted to examine the rehydration capacities of the vocal folds after dehydration (Jiang & Hanson, 1999). Jiang and Hanson completed a study in which 10 excised canine larynges were dehydrated until phonation ceased and then rehydrated for 30 min through complete immersion in isotonic saline. The results indicated that subglottic pressure and

flow decreased following the immersion in saline compared to the pressure and flow data collected from the freshly harvested larynges (Jiang & Hanson, 1999). Hanson, Zhang and Jiang (2011) found that the level of dehydration may impact the ability of the tissue to return to its original hydrated state after attempted rehydration. Furthermore, a study observing the rehydration abilities of muscle, fat, lung, tendon, skin, and cartilage also found that the original dehydrated state may influence the final mass restored upon rehydration and the amount of time required to rehydrate (Meyer, McAvoy, & Jiang, 2013).

Statement of the Problem

Collectively, previous research has explored causative factors associated with vocal fold dehydration, but few have reported potentially viable treatments to reverse surface vocal fold dehydration. Quantifying changes in surface tissue hydration using *in vivo* models is difficult due to numerous physiologic intricacies, which are essential processes in living humans and animals. This issue of *in vivo* covariate complexities supports the rationale for using excised larynx models, where variables can be isolated and manipulated with the purpose of determining their influence on voice production. Furthermore, existing literature includes methodologies such as the administration of subglottic dehumidified air via a pseudolung during phonation as well as the supraglottic application of saline drip, which may not be translational due to essential physiologic components in humans. Therefore, a more physiologically realistic model is needed to quantify variables related to vocal fold dehydration.

Statement of Purpose

This investigation was undertaken to determine if the supraglottic application of aerosolized isotonic saline, delivered topically via an ultrasonic nebulizer, could reverse the negative effects of vocal fold dehydration on voice function. The study will contribute to the

theoretical framework for vocal fold hydration perturbation. This translational research will also inform clinical practice patterns related to hydration recommendations.

Research Questions

The following research questions were addressed:

1. What are the effects of supraglottic laryngeal desiccation on PTP and PTF in an excised porcine larynx mechanical model?

2. Can the adverse effects of laryngeal desiccation be reversed with the topical application of aerosolized saline?

Method

All operational procedures involved in this study were performed in rooms 105 and 106 of the John Taylor Building at Brigham Young University. The present study was performed in accordance with regulations from Brigham Young University Risk Management and the Institutional Animal Care and Use Committee. Excised porcine larynges were donated from a local butcher shop and were used for the purpose of this study. For this reason, a copy of the author's Utah food handler's permit for purposes of working with food grade porcine larynges is included in Appendix C.

Larynges

Ten excised porcine larynges were collected from a local slaughterhouse (Circle V Meats, Spanish Fork, UT). All larynges were collected from adult food-grade pigs, each at least two years in age. Following collection from the slaughterhouse, the larynges were kept in their own secretions and refrigerated until dissected. Experimental procedures were accomplished within 24 hours postmortem. Prior to being included in the study, larynges were meticulously

inspected for any structural abnormalities or punctures and were discarded if any abnormalities were present. Following inspection, the larynges were each dissected to reveal the true vocal folds. Dissection took place the day of the experiment and involved removing the cartilage and supraglottic tissues, specifically the false vocal folds and surrounding portion of the thyroid cartilage. Two different-sized scalpels were used to dissect each larynx. A larger scalpel was used to dissect excess tissue, muscle, and cartilage. A smaller scalpel was used to carefully dissect away the false vocal folds without puncturing the true vocal folds. Metal hemostats were also used to abduct the false vocal folds and aid in precise dissection. The arytenoid cartilage remained intact for additional support to adduct the vocal folds during experimentation. The thyroid cartilage was transected at a level approximately 0.5 cm above the true vocal folds and the cartilage trimmed at an upward angle from anterior to posterior. This angle was found to provide additional support during arytenoid adduction during the pilot phase of this investigation. The epiglottis was carefully removed. The tracheas of the larynges were then cut to approximately 6 cm in length. For elongation purposes, a suture was added to the thyroid cartilage about 0.25 cm above the anterior commissure, superior to the true vocal folds. During and following dissection, the larynges were sprayed liberally with isotonic saline, sealed in zipped plastic bags, and temporarily refrigerated until experimentation. This was done to reduce premature dehydration of the laryngeal tissues. Immediately prior to initiation of each experiment, larynges were again sprayed liberally with isotonic saline, including the subglottis, trachea, supraglottis, and vocal folds.

Research Design

The present study used a prospective mixed experimental design with a control group, including between and within group comparisons. All larynges were randomly placed into either

an experimental or control group; the experimental A/B group received desiccated air (<1% relative humidity) followed by aerosolized saline whereas the control A group received only desiccated air. For both groups, 1-min doses of desiccated air were administered until phonation ceased. Observed PTP (cmH$_2$0) and PTF (L/min) were recorded after each 1-min dose. For the experimental group, aerosolized saline was subsequently administered in 2-min doses until phonation resumed or until at least six doses had been administered. Both groups (A/B and A) were collected alongside another experimental group which examined potential prophylactic effects of 4-min doses of aerosolized saline prior to 1-min doses of dehydration trials; this will be referred to as the B/A to permit comparison between the current study and the prophylactic study (Hansen, 2016). By including an isolated variable (A), the effects of dehydration without rehydration were observed and the average number of trials required to dehydrate the porcine vocal folds to the point of ceased phonation was quantified. The independent variables are considered to be the group and time. The dependent variables included PTP, PTF, and the number of doses administered prior to terminating audible phonation.

Procedures

    Benchtop setup. Jiang and Titze originally reported a benchtop mechanical model for excised larynx experimentation (1993). The present study adopted a similar experimental configuration, which included an excised larynx mounted onto a vertical plastic tubing protruding from a foam insulated custom engineered pseudolung. This tubing was passed through a circular hole in a standard stainless steel breadboard tabletop (Thorlabs, Ann Arbor, MI). The larynx was held in place by micropositioners (Model 1460, Kopf Industries, Tujunga, CA), which were secured to the tabletop by ¼-20 headless screws via custom bases. Two micropositioners with three custom prongs were used to adduct the vocal folds by piercing the

arytenoids bilaterally. The third micropositioner was positioned in front of the larynx. The vocal folds were elongated by tying the suture thread from the anterior commissure to the third micropositioner located in front of the larynx. The micropositioners were used to adduct and lengthen the vocal folds until phonation was achieved. The trachea was affixed to the vertical plastic tubing. To provide a tighter seal from the custom tubing of the pseudolung to the porcine larynx, an adjustable metal hose clamp as well as ¾ in Teflon tape were wrapped around the junction when necessary, depending on the tracheal width.

The following briefly describes the mechanical bench model setup following the direction of flow. A compressed air tank (<1% relative humidity) and adjustable flow regulator at 50 psi led to an in-line thermal flow meter which was attached to tubing which led to Thera-heat temperature controlled humidifier (Model RC70000, Smiths Medical, Dublin, OH). The tubing was attached to clear plastic tubing which passed through a 20-cm aluminum pseudolung, insulated with foam. This clear tubing then passed through the circular hole in the table top which had a subtracheal outlet that allowed for a pressure transducer (Model PT-25-S, Glottal Enterprises, Syracuse, NY) to be attached perpendicular to the direction of the flow.

Signal acquisition. An acoustic signal, indicating phonation onset, pressure and airflow were obtained simultaneously following each 1-min or 2-min dose, depending on the type of challenge or treatment (e.g., dehydration or aerosolization), using the DATAQ A/D (DI-720 Series) converter and WinDaq software (Series Di-720, Akron, OH) programmed at 10 kHz per channel. The acoustic signals were obtained through a dynamic microphone (Model SM-48, Shure, Niles, IL) positioned about 6 in above the true vocal folds. Additionally, an audio mixer (Samsung MIXPAD 4, New York, NY) was used to preamplify the signal. The pressure transducer was calibrated to 0 and 10 cmH_2O with a pressure calibrator (PC-1H, Glottal

Enterprises, Syracuse, NY) and the flow meter was calibrated at 0 and 15 L/min prior to data collection. Two examiners verified vocal fold vibration using high speed imaging (Pentax Medical, Montvale, NJ). All files were marked and saved prior to PTP and PTF analysis. An acoustic signal was used to verify the onset of phonation. A HygroSet II Digital Hygrometer (model DHYG-Round; HygroSet, Weston, FL), which was calibrated using the Humidipak calibration kit, was used to monitor environmental humidity during each data collection session.

 Desiccation. Each 1-min desiccation dose was administered supraglottally via custom tubing directly attached to a different compressed air (<1% relative humidity) tank. A 5-mm shim (Allen wrench) was placed in between the arytenoids, at the posterior 2/3 point. This was done to ensure the air reached the infrasurface and medial edges of the true vocal folds while keeping vocal fold length and adduction constant for following phonation trials. Desiccation trials took place until audible phonation ceased.

 Aerosolization. All 2-min doses of aerosolized isotonic saline (0.9% Na+Cl-) were administered supraglottally through an Omron ultrasonic nebulizer (Model NE-U22V, Omron Healthcare Inc., Lake Forest, IL) and custom tubing. A 5-mm shim was also placed in between the arytenoids and true vocal folds during each administration. The aerosolization treatment involved rehydrating the vocal folds for 2-min doses until phonation restarted.

 Data analysis. Following data acquisition, pressure, flow, and acoustic waveforms recorded in the WinDaq software during experimentation were extracted and imported into MatLab (MathWorks, Natick, MA). The acoustic recordings taken intermittently during each phonation trial were used for audible discrimination of phonation onset. Both PTP and PTF were obtained by averaging the subglottal pressure and flow of 10 ms prior to and after the onset of phonation. PTP and PTF values were then exported to Excel for more in-depth analysis.

Statistical analysis. Data from the experimental and control groups were evaluated for central tendency and variability at baseline. For within group analysis, PTP and PTF were both normalized to baseline values, meaning, that baseline values were subtracted from all subsequent values. Afterwards, the normalized values were averaged by each trial number, and all porcine larynges in the study were included. Changes in the dependent variables were analyzed through linear and polynomial trend analyses with associated formulas and R^2 values. Furthermore, a criterion of greater than .80 was set to a polynomial model, which provided the best fit. All statistical analyses were completed using SPSS, version 23 (IBM Corp., Armonk, NY).

Results

Table 1 includes baseline PTP, PTF, and F_0 values for the experimental A/B and control A groups for this study. For purposes of comparison, PTP, PTF, and F_0 values from another study that included a B/A experimental group are also included (Hansen, 2016). Additionally, environmental humidity was recorded at the beginning and end of each data collection session; these data are reported in Table 2.

Laryngeal Desiccation

Figures 1 and 2 illustrate normalized linear trend models for PTP and PTF during laryngeal desiccation. Both the experimental and control groups demonstrated increases in PTP and PTF based on linear trend analysis. Additionally, as compared with other study data from Hansen, PTP and PTF values increased at a greater slope compared with those larynges prophylactically treated with aerosolized saline (Hansen, 2016). Specifically, PTP values increased by 12 cmH_2O and PTF by 25 L/min for this experimental group; these increases are greater than those reported by Hansen, who observed increases of 7 cmH_2O and 15 L/min during laryngeal desiccation.

Aerosolized Saline

Figures 3 and 4 illustrate normalized polynomial trend models for PTP and PTF during aerosolized saline for the experimental group from this study versus the data reported in the comparative study (Hansen, 2016). Both experimental groups demonstrated decreases in PTP of approximately 5 cmH$_2$O, with no obvious pattern of change for PTF, during aerosolization (Hansen, 2016). Of note, the present study aerosolization data were normalized to the last obtained PTP value during laryngeal desiccation. That is, the first normalized aerosolized saline value was calculated as the difference between that PTP trial and the previous PTP trial during which phonation was obtained; the process for PTF normalization was identical.

Duration of Effects

The number of desiccation trials required to cease vocal fold vibration in the current study's experimental and control groups were compared with that observed in the comparative study (Hansen, 2016). As hypothesized, a one-way ANOVA identified no differences between this study's experimental and control groups, but significant differences as compared to other study data examining prophylactically treated larynges $F(2, 12) = 10.562, p = .002$ (Hansen, 2016). Figure 5 presents these findings. The mean number of trials required to desiccate the larynges in the A and AB groups were 10.8 (range = 5 to 15 trials) and 9.6 (range = 7 to 15 trials), respectively. The mean number of trials required to desiccate the BA group (Hansen, 2016) was 22.6 (range = 17 to 35 trials).

Discussion

The present study sought to determine if the adverse effects of dehydration following a desiccation challenge, consisting of 1-min dehydration doses to the point of ceased phonation, could be reversed by topically hydrating the larynx with aerosolized saline via a nebulizer.

Researchers in the present study found that all excised larynges phonated once again after *about five 2-min doses* of topically applied aerosolized saline. This study was also designed to observe changes in PTP and PTF during the dehydration challenge and aerosolization treatment. As hypothesized, the results of this study found topical application of aerosolized saline to be successful in decreasing PTP and PTF values following a desiccation challenge. Moreover, results showed PTP values increased as the larynx became more dehydrated through topical exposure to dry air at a flow of 8 L/min. This observation was noted for both the A/B group and the control group. PTF demonstrated similar trends to PTP, or an increase in PTF during each desiccation trial, but was not statistically significant.

Physiologically Realistic Model

The present study included a more physiologically realistic model by duplicating the manner in which vocal fold dehydration typically occurs in humans. Previous studies have desiccated the vocal folds subglottally by attaching a humidifier to the pseudolung which carries pressurized heated air to the glottis (Jiang, Verdolini, Aquino, Ng, & Hanson, 2000; Witt et al., 2009). However, several issues exist with this model since vocal fold dehydration typically occurs during inhalation when dry air enter the oral cavity and flows over the surface of the vocal folds. The present model used aerosolized saline to supraglottally hydrate the vocal folds. In this manner, the aerosolized saline acted as a second lubricant in addition to the heated and humidified air humans breathe out. More specifically, this study dehydrated the vocal folds supraglottally which simulated the manner in which vocal folds are typically dehydrated during speaking or breathing. Moreover, the present study used a 5-mm shim to abduct the vocal folds, which is the position the vocal folds are in during inhalation in a human. The inclusion of components observed in a typical breathing human such as vocal fold abduction as well as

supraglottic dehydration, enhance the model making it a physiologically realistic model that is ecologically valid and reflects what occurs *in vivo*. This is crucial for the application of the findings of this model into clinical practice.

An *in vivo* study conducted by Kumazawa and colleagues (1997) observed the effects of inhaled aerosolized particles via a nebulizer on six male subjects. Their findings corresponded with the present study and found intermittent vocalizations following the aerosolization treatment enhanced the positive effects of hydration through nebulized particles. Additionally, their findings reflected that faster inhalation of the particles with a high respiratory rate also enhanced the effects of rehydration (Kumazawa et al., 1997). Although some studies have found evidence supporting the benefits of using a nebulizer to topically hydrate the surface of the vocal folds, the appropriate dosage is yet to be discovered. To further advance the research being done regarding dosage, the amount of nebulized saline delivered to the surface of the porcine larynges was quantified by the amount of time the aerosolized saline was delivered per 2-min trial.

It is noteworthy that, despite model modifications, a traditional bench setup was used to perform this experiment due to its efficacy and reliability in other dehydration studies involving excised animal models. The traditional bench is sturdy, provides enough adduction support for the excised animal model, and has proven to be useful in conducting aerodynamic measures. Isotonic saline was used in this study due to its similar level of tonicity to the human mucosa found on the surface of the vocal folds. A similar level of tonicity between the isotonic saline and the human mucosa prevents water and ions from diffusing into or out of the tissue from the deeper epithelium of the vocal folds to the more superficial saline applied to the surface of the vocal folds and vice versa.

Phonation Threshold Pressure

Previous studies conducted on human subjects and excised animal models have reported that the administration of topical nebulized saline may contribute to hydration of the sol layer found on the surface of the vocal folds and upper airway (Finkelhor et al., 1988; Sivasankar & Fisher, 2002; Tanner et al., 2007). The present study found the topical application of aerosolized saline on the surface of the vocal folds decreased PTP values. Since this finding is similar to previous literature, it is assumed that PTP decreased due to the hydrating effect the aerosolized saline had on the surface of the vocal folds. However, the present study also identified an increase in PTP during the dehydration challenge using dry air at a rate of 8 L/min. Previous authors observed the effects of three nebulized osmotic agents and discovered that PTP significantly increased as subjects were exposed to low humidity air (Tanner et al., 2007). Another excised canine model study conducted by Jiang et al. (2000) subglottally dehydrated 17 vocal folds during continuous phonation and found a significant increase in PTP as dehydration levels increased. Likewise, Sivasankar and Fisher compared the effects of nasal breathing and oral breathing of 20 human females on dehydration of the sol layer, which is found on the surface of the upper airway. Their findings also reflected an increase in PTP as dehydration increased. Moreover, Finkelhor et al. (1988) used excised canine larynges to study the effects of dehydration on PTP by causing the liquid to travel in and out of the laryngeal tissue through exposure to different osmotic agents, and found an increase in PTP as dehydration levels increased and an opposite effect when hydration levels increased. A study completed by Titze (1988) led researchers to believe that this change in PTP values due to varying hydration challenges may occur due to the physical changes occurring in the vocal folds. Titze hypothesized that PTP values would increase when hydration levels decreased and the vocal

folds became more thin and stiff. As dehydration increases, the tissue which makes up the vocal folds becomes stiffer and more pulmonary pressure is required to initiate vocal fold oscillation (Finkelhor et al., 1988). In a study conducted using porcine larynges, PTP was detected to be about 9 cmH$_2$0 (Alipour & Jaiswal, 2008). During the present study, dehydration and elongation of the vocal folds made them less pliable and more stiff, increasing PTF as observed in this study. For this reason, PTP values may have been elevated compared to previous studies using excised porcine larynges (Alipour & Jaiswal, 2008). Additionally, the results of this study proved the effects of vocal fold dehydration may be reversed and corresponding PTP values may be decreased by the topical administration of aerosolized particles via a nebulizer.

Phonation Threshold Flow

Phonation threshold flow is a newer aerodynamic measure which has previously been used to evaluate vocal function, and is a measure which is dependent on pressure and glottal resistance. In a study involving 10 excised canine larynges, Hottinger et al. (2007) observed the effects on phonation threshold flow when changing the posterior glottal width from 1 - 4 mm. Phonation threshold flow was found to gradually increase as posterior glottal width increased. For this reason, PTF has previously been found to be a more sensitive measure than PTP to physiologic changes affecting the posterior glottal width, such as pathologies affecting abduction or adduction of the vocal folds. This may be due to the physical changes in tissue, which may affect the amplitude of vibration or mucosal wave, making vocal fold oscillation less efficient. Additionally, as observed in the present study, if the vocal folds are dehydrated, then they may have more difficulty coming together and may require more pulmonary effort (Finkelhor et al., 1988). This may explain why PTF increased during the dehydration trials. However, other studies have found PTF to be an unreliable measure for changes in vocal fold abduction or

hydration levels (Hoffman et al., 2012; Witt et al., 2009). This may be pertinent to the present study because flow was not a statistically reliable measure during the aerosolization treatment. It is important to note that the present study did not readjust adduction or abduction after the initial baseline values were collected. Since the air delivery system did not allow the independent regulation of pressure, and the resistance of the vocal folds was not quantified over time, flow may not be expected to change in a predictable way in response to hydration adjustments. A technical error also occurred during the study due to an incorrect gain in the WinDaq software. This error occurred because the researcher based the settings on previous studies of porcine larynges and did not expect the PTF values to exceed 50 L/min (Alipour & Jaiswal, 2008). Due to this error, the data peak clipped at about 46 L/min, and all data above this value were lost. Data may have peak clipped due to high onset pressures.

Fundamental Frequency

Several factors may influence fundamental frequency. Among those factors are viscosity levels, which may be associated with the degree of surface tissue hydration. Previous literature has observed the effects of dehydration on viscosity levels and have found that they increase with dehydration (Finkelhor et al., 1988; Jiang et al., 1999). Additionally, a mucosal wave has been observed in healthy larynges, which is thought to contribute to the optimal oscillation of the vocal folds. However, if the larynx becomes severely dehydrated, this mucosal wave may disappear, causing the vocal folds to cease vibration. The vocal folds also ceased to vibrate after an average of 10, 1-min desiccation doses. Witt et al., (2009) discussed the possible negative effects caused by altering the normal level of viscosity. This change in viscosity level may predispose the vocal folds to injury during phonation. In the present study, the vocal folds may have been injured during phonation due to the reduced elasticity caused by the changes in

viscosity. This may also have affected the F_0, as well as the subglottal pressure and flow required to oscillate the vocal folds.

Changes in vocal fold elongation may also influence the F_0. In a study comparing porcine, ovine, and cow larynges, porcine larynges phonated at an average F_0 of 220 ± 57 Hz. (Alipour & Jaiswal, 2008). However, the present study found the porcine larynges to have a higher average F_0 around 450 Hz. This may have been due to the vocal folds being elongated by attaching a suture to the anterior commissure of the thyroid cartilage. The laryngeal tension caused by elongating the vocal folds may have affected the pitch throughout the study. Hanson, et al. (2011) found the level of dehydration may impact the tissue's ability to recover after severe dehydration. Likewise, in the present study, the ability of the vocal folds to recover may have been affected by the number of 1-min desiccation doses required to dehydrate the vocal folds. For this reason, the porcine larynges phonated at a relatively lower F_0 during the aerosolization challenge than during the desiccation challenge, where they phonated at a higher F_0.

Limitations

This study was subject to several potential limitations. First, since this was a pilot study, the sample size of only five porcine larynges per group was quite small, and much variability between the porcine larynges (e.g., gender, age, size) existed. The sample size may have limited the external validity of the findings. Additionally, as is the case in many studies, a steep learning curve existed for the researchers, and there is a chance they may have improved their use of the materials and dissection techniques by the end of the study. This study required much training and attention to detail when dissecting the vocal folds and running the equipment, leaving room for researcher error. For example, dissection of the vocal folds may have been variable, and the true vocal folds may have been damaged during dissection leading to faster vocal fold

dehydration. Although the porcine larynx has many similarities to the human larynx, the vocal folds of the porcine larynx are at a 45º angle compared to the horizontal human vocal folds at 0º. Moreover, there may have been a researcher bias during the study since all the researchers were aware of the outcome and this may have influenced the perceptual judgement of phonation onset. Other equipment limitations may have included a problem with the PTF gain, which was preset to record at a low flow level, and which caused the data to peak clip after 48 L/min. Additionally, the ambient humidity levels were relatively stable during experiment days. There were also overheating difficulties with the humidifier included in this study due to overheating issues. Finally, this experiment did not take place in a sound booth, which may have allowed some reverberations or ambient noise, which may have affected the detection of phonation onset.

Implications for Future Research

Future studies should include a larger sample size to increase reliability. A future study should also include more experienced researchers for data collection and dissection. Limiting the study to using a specific gender, preferably males, due to their larger size of larynx compared to females may reduce variability between larynges. A future study may also observe sustained, onset and offset PTP and PTF. Furthermore, a study observing the effects of different dosage levels may contribute to future clinical applications. Another study may observe the optimal manner in which excised tissue should be stored in order to prevent changes in the tissue which may disqualify this study from being applied to human subjects.

Conclusion

In the present study, excised porcine larynges were dissected, dehydrated supraglottally until phonation ceased, and then were rehydrated supraglottally with aerosolized saline by way of a nebulizer. A total of 10 excised porcine larynges were used in the study, 5 larynges in each

group. Each larynx in the dehydration then aerosolization group was dehydrated supraglottally

for 1-min intervals until phonation ceased and then rehydrated supraglottally for 2-min intervals

until phonation reinitiated. The PTP and PTF measures were recorded simultaneously in

between each desiccation or aerosolization trial. Overall, PTP values decreased as hydration

increased, and increased as hydration decreased. Similar but not statistically significant trends

were noted for PTF values across trials. The present study showed that vocal fold dehydration

may be reversed through the topical application of aerosolized saline. In a physiologically

realistic excised mechanical model, this study advances important theoretical constructs related

to dehydration-related voice disorder prevention. Future research involving a more realistic

model may be necessary to determine a better form of dosage to implement into clinical settings

and recommend to patients.

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Table 1

Aerodynamic and Acoustic Raw Data at Baseline

Measure	Mean	*SD*	Median	Range
Phonation Threshold Pressure (cmH$_2$O)				
A/B	29.3	13.9	25.6	37.0
B/A*	17.4	8.5	17.2	21.0
Control*	24.6	10.9	23.0	30.1
Phonation Threshold Flow (L/min)				
A/B	11.2	7.0	12.5	17.1
B/A*	8.5	7.0	5.8	15.7
Control*	23.4	18.0	17.5	41.3
Fundamental Frequency (Hz)				
A/B	397.5	25.9	397.5	36.7
B/A*	441.6	189.8	437.7	486.9
Control*	352.2	77.4	389.2	141.0

Note. A = desiccation; B = aerosolization; SD = standard deviation; * = from Hansen (2016).

Table 2

Environmental Humidity During Each Experimental Session

Group	Session Date	Baseline Percent Relative Humidity (%)	Endpoint Percent Relative Humidity (%)
A/B			
Pig 1	02/19/16	14	13
Pig 2	02/19/16	14	13
Pig 3	02/19/16	14	13
Pig 4	03/05/16	12	12
Pig 5	03/05/16	12	12
B/A*			
Pig 1	02/20/16	13	13
Pig 2	02/20/16	13	13
Pig 3	02/26/16	12	13
Pig 4	02/26/16	12	13
Pig 5	03/05/16	12	12
Control*			
Pig 1	02/26/16	12	13
Pig 2	02/26/16	12	13
Pig 3	03/11/16	13	13
Pig 4	03/11/16	13	13
Pig 5	03/11/16	13	13

Note. A = desiccation; B = aerosolization; * = from Hansen (2016).
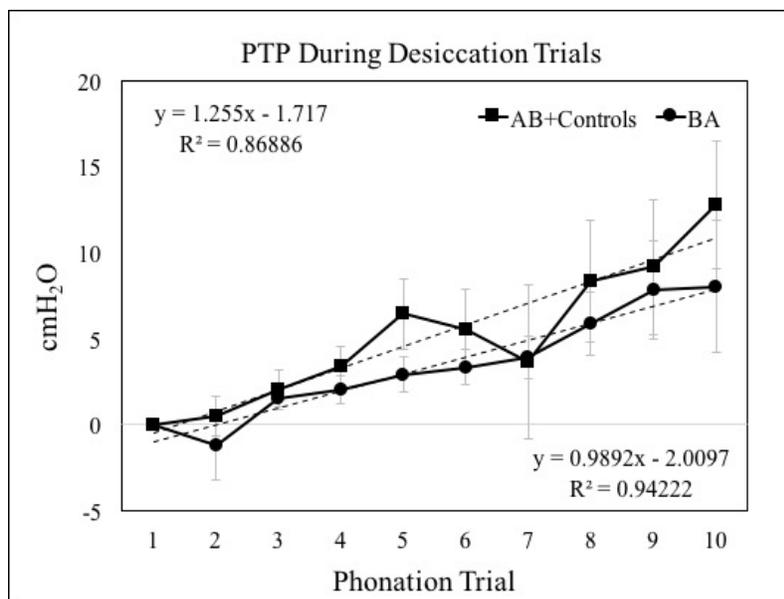
Figure 1. Normalized phonation threshold pressure (PTP) at baseline (0 on the y axis) and following each 1-min desiccation dose. Mean, standard error represented by vertical bars, linear trendlines with a > .80 criterion for fit, and R^2 values with formulas are provided. Groups include A/B (Desiccation then Saline), B/A *(Saline then Desiccation), and A* (Desiccation only Control). *From Hansen, 2016.
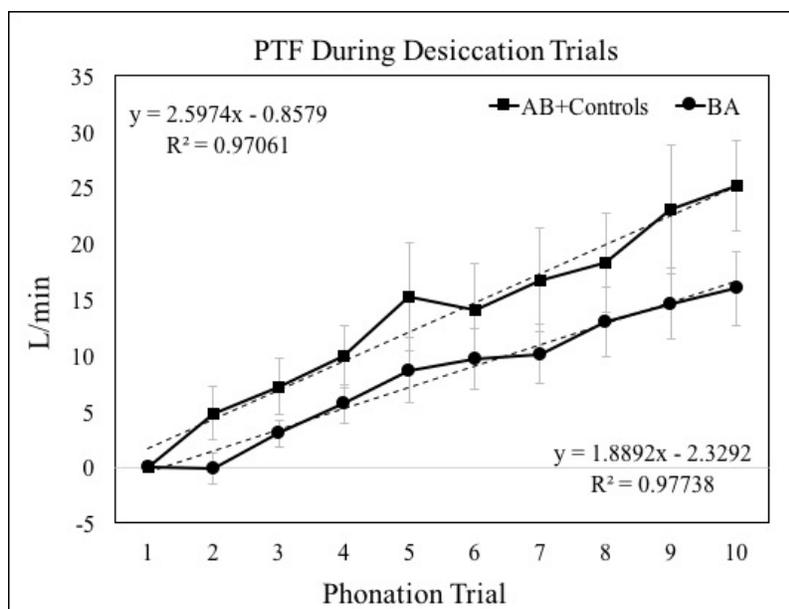
Figure 2. Normalized phonation threshold flow (PTF) at baseline (0 on the y axis) and following each 1-min desiccation dose. Mean, standard error represented by vertical bars, linear trendlines with a > .80 criterion for fit, and R^2 values with formulas are provided. Groups include A/B (Desiccation then Saline), B/A* (Saline then Desiccation), and A* (Desiccation only Control). *From Hansen, 2016.
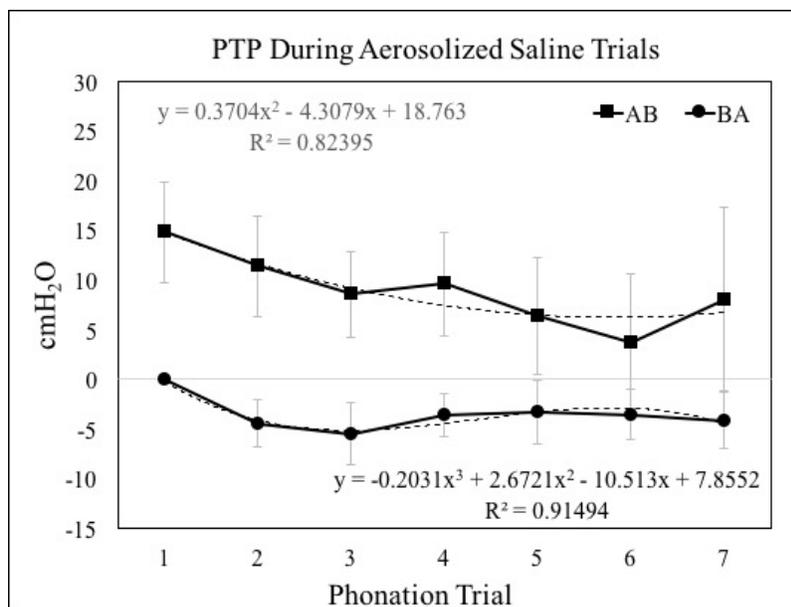
Figure 3. Normalized phonation threshold pressure (PTP) at baseline and following each aerosolized saline dose. Means, standard error represented by vertical bars, polynomial model trendlines with a > .80 criterion for fit, and R^2 values with formulas are provided. Groups include A/B (Desiccation then Saline) and B/A* (Saline then Desiccation).
*From Hansen, 2016.
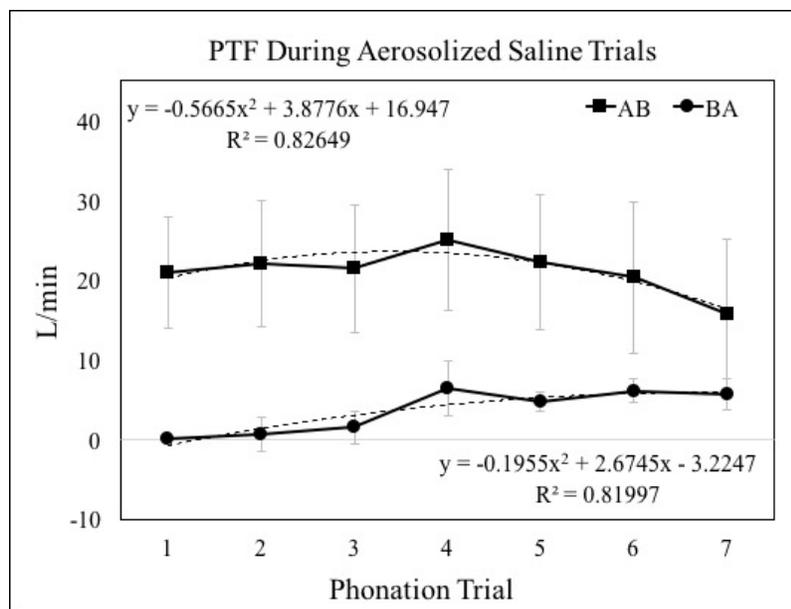
Figure 4. Normalized phonation threshold flow (PTF) at baseline and following each aerosolized saline dose. Means, standard error represented by vertical bars, polynomial model trendlines with a > .80 criterion for fit, and R^2 values with formulas are provided. Groups include A/B (Desiccation then Saline) and B/A* (Saline then Desiccation).
*From Hansen, 2016.
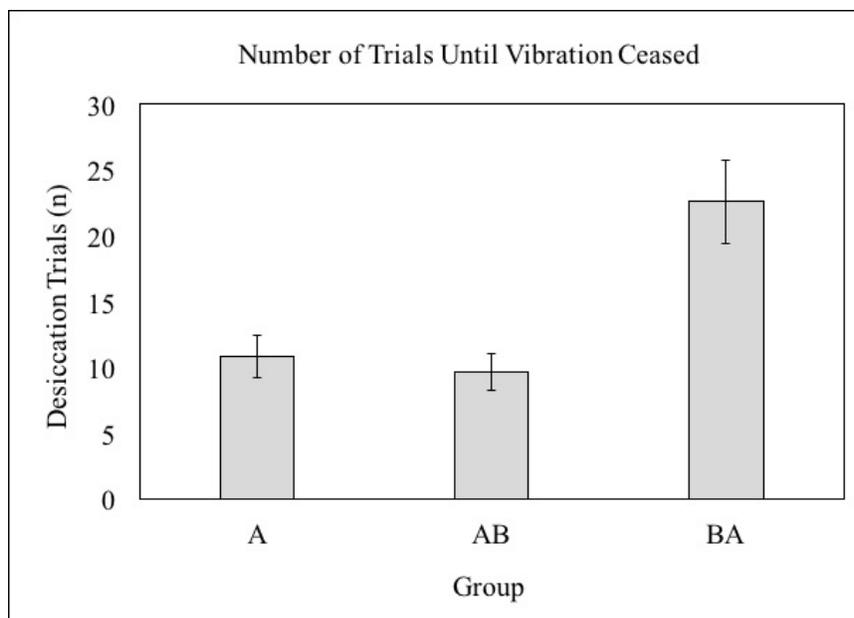
Figure 5. The number of desiccation trials required to cease vocal fold vibration for larynges in the A/B (Desiccation then Saline), B/A* (Saline then Desiccation), and A* (Desiccation only Control) groups. Standard error is represented by vertical bars. Significant differences were observed based on one-way analysis of variance and Tukey's post hoc tests (p < .002).
*From Hansen, 2016.

APPENDIX A: Annotated Bibliography

Alipour, F., & Jaiswal, S. (2008). Phonatory characteristics of excised pig, sheep, and cow larynges. *Journal of Acoustical Society of America, 123*, 4572-4581. doi:10.1121/1.2908289

Purpose of the study. The purpose of this study was to compare the similarities and differences between animal larynges (e.g., porcine, sheep or cow) and human larynges. Another purpose of this study was to possibly identify other alternatives for phonatory models of the human larynx.
Method. This study included eight porcine larynges, eight sheep larynges and six cow larynges. These larynges were bought from a butcher shop, cleaned and then slow frozen until used. When the experiment began, each larynx was thawed overnight in 0.9% saline. The epiglottis of each larynx was also removed prior to the experiments. Each excised larynx was mounted onto a tapered tube that provided pressurized, heated and humidified air. Several variables were measured such as the subglottal pressure, electroglottograph, mean flow rate, audio signal and the sound pressure level. The electroglottograph was then used to identify the fundamental frequency. Additionally, the lateral cricoarytenoid muscle was stimulated to manipulate adduction of the vocal folds. The levels of adduction were low, medium and high. In addition, each larynx underwent two pressure-flow sweeps (e.g., upward and downward). Each pressure-flow sweep included all three levels of adduction.
Results. The results gathered from this experiment found porcine larynges to have the largest range of fundamental frequency (F_0) compared to the other two animal species. Additionally, the porcine larynges produced the loudest sound. The following difference was observed between human and porcine larynges: the false vocal folds were found to participate in the production of sound in porcine larynges. This may have contributed to the loud noise produced by the porcine larynges. The highest value of the maximum frequency range was produced by the porcine larynges, and the lowest value was produced by the cow larynges. It is speculated this was due to the larger size of the cow larynges. Physically, 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 like the human larynx in comparison to the larynges of the other two animal types (e.g., cow and sheep).
Relevance to the current work. The information gained from this study supported the relationship between porcine larynges and human larynges. A study involving porcine larynges may be a more physiologically realistic model and more accurate representation of human larynges than perhaps a different study involving a different animal model.

Finkelhor, B. K., Titze, I. R., & Durham, P. L. (1988). The effect of viscosity changes in the vocal folds on the range of oscillation. *Journal of Voice, 1*, 320-325.

Purpose of the study. The purpose of this study was to determine the relationship between vocal fold dehydration and oscillation threshold pressure.
Method. A total of four excised canine larynges were used in this study. After dissection took place, the larynges were mounted onto a structure and secured by custom-designed prongs. The vocal folds were then lengthened uniformly. Additionally, the vocal folds were tested for three

different glottal widths (e.g., 0 mm, 1 mm and 2 mm). These were also tested for three dehydration levels and the threshold pressure for oscillation was also measured over a range of lengths -3 mm to +3 mm. The glottal widths were maintained by inserting a metal shim between the vocal folds during phonation. The vocal folds were also hydrated with normal saline, distilled water and 2.7% saline for 15 min each. To maintain uniformity, each vocal fold underwent the same hydration procedure. This included a rubber stopper used to plug the tubing to prevent the solution from entering the air supply system. A u-tube manometer was also used to measure the glottal threshold pressure after each larynx was hydrated.

Results. The results of this study indicated a decreased glottal threshold pressure when the vocal folds were not lengthened. A higher oscillation threshold was observed when the vocal folds were immersed in hypertonic saline and a lower oscillation threshold was observed when the vocal folds were immersed in distilled water.

Conclusions. The data from this study proved vocal fold hydration decreased the oscillation threshold and vocal fold dehydration increased the oscillation threshold. Additionally, the data collected also acknowledged the possible increase in mucosal wave activity as vocal fold dehydration increased, leading to an increased glottal oscillation threshold.

Relevance to the current work. The data collected in the previous study informed the current work about the potential effects of vocal fold dehydration. Vocal fold dehydration may have influenced the amount of breath support needed to reinitiate phonation. Increased vocal strain or excessive dehydration may have led to vocal fold pathologies.

Hanson, K. P., Zhang, Y., & Jiang, J. J. (2010). Parameters quantifying dehydration in canine vocal fold lamina propria. *Laryngoscope, 120*, 1363-1369. doi:10.1002/lary.20927

Purpose of the study. The following study quantified the liquid and solid mass and volume of excised canine vocal fold lamina propria at different levels of dehydration. The study also sought to find data supporting the biphasic theory, which explained the physiology and biomechanics of the vocal folds.

Method. Fifteen canine larynges were included in this study. Both vocal folds were extracted from each canine larynx for a grand total of 30 tissue samples. Prior to desiccation trials, the mass and volume of each vocal fold was recorded. All tissue samples were divided into three groups, which underwent trials of different levels of dehydration. The groups consisted of varying dehydration levels such as 30%, 50% and 70% dehydration. After each tissue underwent a specific trial, it was then completely dehydrated for measuring the mass and volume of the tissue's solid state.

Results. Several ratios of data were recorded such as the liquid volume and mass fractions and liquid: solid volume and mass ratios. The results indicated that the ratios were significantly different at each dehydration level except for the liquid solid volume ratios for 30% dehydration compared to 50% dehydration. Inverse linear relationships were detected for each solid to liquid ratio.

Conclusions. The results of this study supported the biphasic theory and suggested that the level of dehydration undergone by the vocal fold lamina propria may be directly identified based on the parameters of the biphasic theory.

Relevance to the current work. This study provided insight to the possible behaviors of the lamina propria during dehydration and this knowledge could help predict the reversibility of dehydration levels for vocal folds.

Hanson, K. P., Zhang, Y., & Jiang, J. J. (2011). Ex vivo canine vocal fold lamina propria rehydration after varying dehydration levels. *Journal of Voice, 25*, 657-662. doi:10.1016/j.jvoice.2010.06.005

Purpose of the study. This study used canine vocal folds to observe the recoverability of the vocal fold lamina propria after dehydration for several levels of dehydration.

Method. Ten canine larynges were involved in this study. All larynges had both vocal folds removed. The volume of each excised vocal fold was measured. Afterwards, the vocal folds were split into two groups made up of 10 vocal folds each. One group underwent 30% dehydration of its mass, and the other 10 samples were dehydrated 70% of their original mass. Following desiccation, the vocal folds were rehydrated using 0.9% saline until mass was stable.

Results. After hydration, the volume of the larynges was then recorded and compared to baseline prior to desiccation. The results showed significant differences between the vocal folds dehydrated by 30% and the ones dehydrated by 70%. The volumes of the vocal folds that had been dehydrated to 70% were further apart from the original volumes. About half of the 10 vocal folds dehydrated to 30% dehydration fully recovered after hydration, but only one of the 10 vocal folds dehydrated to 70% fully recovered to its original mass after hydration.

Conclusions. This study's findings indicated the level of potential rehydration after desiccation may be largely influenced by the level of dehydration that took place prior to rehydration. These results also supported the biphasic theory.

Relevance to the current work. The amount of dehydration the vocal folds undergo could affect the amount of rehydration that takes place after desiccation. Perhaps if the vocal folds were dehydrated to a state of no reversal, the vocal folds may not have phonated following the desiccation challenge and after the rehydration treatment.

Hartley, N. A., & Thibeault, S. L. (2014). Systemic hydration: relating science to clinical practice in vocal health. *Journal of Voice, 28*, 651-652. doi:10.1016/j.jvoice.2014.01.007

Purpose of the study. This study explored the role of systemic hydration in vocal health and function.

Method. A literature search, which included numerous professions such as speech language pathology (SLP), physiology, biomechanics, nutrition and dietetics as well as sports, medicine and exercise science, was conducted.

Results. The study found further research is necessary in the areas of reversing vocal dehydration using excised animal models as well as in determining the effects of certain substances which are thought to dehydrate the vocal folds. Additionally, more effective treatments indicating the type of fluid and the dosage to reverse or prevent laryngeal dehydration should be explored to determine the true effect of systemic hydration on the vocal mechanism. Moreover, further research should observe the effects of hypohydration and hyperhydration of the vocal folds.

Conclusions. Future research is necessary to further understand the relationship between systemic hydration and vocal quality. More appropriate research would have been multidisciplinary and would have facilitated analysis to provide more guidance towards future studies.

Relevance to the current work. This study highlighted the importance of a multidisciplinary approach. Additionally, this study did not find significant underlying mechanisms or an

effective way of treating systemic dehydration to directly influence vocal fold hydration. For this reason, the present study observed the effects of surface tissue hydration.

Hemler, R. J., Wieneke, G. H., & Dejonckere, P. H. (1997). The effect of relative humidity of inhaled air on acoustic parameters of voice in normal subjects. *Journal of Voice, 11*, 295-300.

Purpose of the study. The aim of this study was to determine whether relative humidity (RH) of inhaled air influenced voice production in normal subjects.

Method. The following study involved eight healthy humans, four women and four men. Each subject was asked to orally inhale three different conditions of air for 10 min. The conditions of air consisted of environmental air, standard environmental air humidified by evaporated water and dry air with low RH. At the end of 10 minutes, each subject was asked to produce a sustained /a/ at a controlled pitch and loudness. All subjects maintained the loudness of their voice by observing a visual feedback device. Microphones were placed 20 cm away from the subject's mouth and were used to record phonation. Data was then collected and analyzed for noise-to-harmonic parameters and perturbation.

Results. The results gathered from this experiment did not find any significant differences between standard and humidified air. Additionally, the noise-to-harmonic ratio proved to be similar in the data collected from all three conditions. However, the data collected found a decrease in RH to have a significant effect on vocal perturbation. This was due to the drastic increase in vocal perturbation measures even after minimal exposure to dry air.

Conclusions. The data collected from this experiment reflected the vocal fold's sensitivity to decreases in RH during inhalation. This may be helpful to speech pathologists who may be working with patients who have chronic dry vocal folds. Perhaps rehydrating techniques may be implemented with the purpose of improving vocal quality and reducing vocal perturbation.

Relevance to the current work. The results collected regarding the relationship between a decrease in RH and vocal perturbation may contribute to future research. For example, the knowledge of a noticeable difference in perturbation when RH is reduced may be useful when collecting data from porcine larynges which have been dehydrated supraglottally with dry air molecules. Perhaps, future studies could quantify the percent of RH found in air delivered supraglottally to animal models.

Hoffman, M. R., Rieves, A. L., Budde, A. J., Surender, K., Zhang, Y., & Jiang, J. J. (2012). Phonation instability flow in excised canine larynges. *Journal of Voice, 26*, 280-284. doi:10.1016/j.jvoice.2011.03.007

Purpose of the study. This study observed the phonation instability flow, or the amount of air flow necessary for chaotic phonation, 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 the current work. This study provided additional information about other aerodynamic measures useful for observing the biological mechanism of the vocal folds. Perhaps, future studies could include phonation instability flow and phonation flow range to detect changes in hydration levels of the vocal folds.

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

Purpose of the study. Prephonatory glottal width was varied to compare PTF and PTP in terms of physiologic changes.

Method. This study included 10 excised canine larynges. The posterior glottal width of each larynx was abducted at five different glottal widths using a metal shim of widths ranging from 0 mm – 4 mm. The onset airflow and onset pressures were each measured.

Results. According to the results evaluated by a one-way analysis of variance; PTF increased along with posterior glottal width. However, the results for PTP were not significant and did not increase with increased glottal width.

Conclusions. The data collected indicated PTF is more sensitive to changes in the posterior glottal width than PTP. Knowing this may be useful in detecting vocal fold pathologies which relate to problems with adduction.

Relevance to the current work. The PTP and PTF were both evaluated in the current study. If PTF was more sensitive to a posterior gap than PTP, then this may have been observed in the present study involving porcine larynges. Perhaps the PTF will have a greater increase as the vocal folds are more severely dehydrated compared to PTP.

Jiang, J., Ng, J., & Hanson, D. (1999). The effects of rehydration on phonation in excised canine larynges. *Journal of Voice, 13*, 51-59.

Purpose of the study. This study observed the efficiency of rehydrating desiccated vocal folds and 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 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 the current work. The current study sought to determine the effects of PTP on dehydrated vocal folds. This study supported the hypothesis of the porcine larynx model by describing that rehydrating the larynx was successful in decreasing PTP as well as improving phonation efficiency. This study followed a similar set up to the porcine larynx model because it also included a bench apparatus with a humidifier and used saline to rehydrate the vocal folds.

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

Purpose of the study. The purpose was to identify the minimal airflow required to begin phonation. This study also compared PTF to PTP.
Method. A one mass model was used to study the minimal glottal airflow necessary to initiate vocal fold vibration.
Results. The results of the study indicated that PTF depended largely upon the glottal shape. Additionally, PTF also varied 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 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 the current work. Although this study did not look at excised larynges, it included the evaluation of PTF and the factors which may play a role in influencing the changes in PTF.

Jiang, J. J., & Titze, I. R. (1993). A methodological study of hemilaryngeal phonation. *Laryngoscope, 103*, 872-882. doi:10.1288/00005537-199308000-00008

Purpose of the study. This study observed the phonation characteristics of a hemilarynx. These observations were then compared to data collected from phonation of a full larynx.
Method. The following study included nine excised larynges from large mongrel dogs. Each larynx was dissected and placed in a 0.67% saline solution for refrigeration. Before the experiment took place, each larynx was thawed and mounted onto an apparatus. Data was then collected from the whole larynx as each was phonated. Afterwards, each larynx was dissected once again in order to remove the left vocal fold. A vertical 9-mm-thick plexiglass plate was then used to replace the left vocal folds. Each plexiglass plate contained pressure transducers. Following this dissection, each larynx was phonated once again. This process included recording the PTP, sound pressure level, F_0 and the average glottal flow. Additionally, a stroboscope was used in order to record the amplitude of the vocal fold vibration of each larynx.
Results. The data collected from this experiment supported a similar PTP for hemilarynges and full larynges. Frequency ranges collected from the hemilarynges and the full larynges were also very similar. Additionally, a slight difference was observed between the full larynges and the hemilarynges in regards to the phonation instability pressure. The full larynges had slightly lower phonation instability pressure. Moreover, a slight difference was noted between the amplitude of the vocal fold vibrations in the hemilarynges and the whole larynges. The hemilarynges seemed to vibrate at half the amplitude of the whole larynges. The researchers also found the full larynges to be on average louder than the hemilarynges.
Conclusions. The results gained from this experiment supported that a hemilarynx does have

many similarities to a full larynx. This knowledge could be helpful to those patients who may undergo a partial vertical laryngectomy. The similar frequencies ranges observed in the hemilarynges and the full larynges provided additional information about phonation to patients who may be considering a partial laryngectomy. This was also essential information for SLPs and may enhance therapy techniques used for patients who have had a partial laryngectomy. **Relevance to the current work.** The results from this study contributed to the following research experiment due to the type of data collected. The PTP and frequency range were two essential measurements taken, which may expand the knowledge researchers have regarding hemilarynges and pathological vocal folds. Additionally, this experiment included canine larynges, which contributed to the idea that animal larynges may be used to accurately represent the human larynx.

Johanes, I., Mihelc, E., Sivasankar, M., & Ivanisevic, A. (2011). Morphological properties of
    collagen fibers in porcine lamina propria. *Journal of Voice, 25*, 254-257.
    doi:10.1016/j.jvoice.2009.09.006

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 the 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.

Kawaida, M., Fukuda, H., Kano, S., Shiotani, A., & Kohno, N. (1990). Dynamic movement of air tract fluid in lubrication of the larynx during phonation: A basic study using excised canine larynges and experimental air tract fluid by means of x-ray stroboscope system. *Auris Nasus Larynx, 16*, 237-243.

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 the 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.

Li, L., Zhang, Y., Maytag, A. L., & Jiang, J. J. (2015). Quantitative study for the surface dehydration of vocal folds based on high-speed imaging. *Journal of Voice, 29*, 403-409. doi:10.1016/j.jvoice.2014.09.025

Purpose of the study. The purpose of this study involved quantifying the differences of the vocal folds' mucosal wave and glottal area during phonation at three different dehydration levels.

Method. This study included 10 excised canine larynges. The study also involved three dehydration degrees consisting of 0% for normal, 25% for moderate dehydration and 75% for severe dehydration. The vocal folds were dehydrated by attaching the trachea to a pipe, which was attached to a conventional air compressor, which was then secured to the trachea with a clamp. High-speed images, and DKG image sequences were used to collect data regarding the glottal width and mucosal wave for all three dehydration levels. The glottal area measurements were divided into direct and indirect components. The direct components measured the amplitude and the indirect components measured the frequency of the glottal area. Additionally,

the frequency and amplitude data of the vocal folds was also collected through DKG imaging. The same amount of air pressure was used to dehydrate all vocal folds. The vocal folds all went through the same dehydration process. This included 1 min of blowing dry air and 2 min of rest where no air was being blown.

Results. The results from this study illustrated an increased in glottal area amplitude as the vocal folds became more dehydrated. Additionally, as the dehydration levels of the vocal folds increased, the frequency of the glottal area decreased, and the mucosal wave amplitude decreased as the vocal folds became more dehydrated. This was supported by the measurements taken from both left and right vocal folds. The mucosal wave frequency was not significantly altered as the dehydration levels increased, but instead remained relatively constant.

Conclusions. The data collected supported an increase in glottal area amplitude as dehydration increased, and possibly reflected an ability of vocal folds to restore themselves after rehydration. The lack of effect dehydration had on mucosal wave frequency should be studied further. Studies in this area could lead to a better understanding of the relationship between vocal dehydration and vocal hoarseness.

Relevance to the current work. The knowledge gained from this study could prove to be beneficial for other studies involving the effects of breathing in vocal fold dehydration. The change in glottal area amplitude with vocal fold dehydration could provide more information about the effects of vocal fold dehydration caused by inhalation and phonation.

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

Purpose of the study. This study identified the average PTP and PTF for human larynges. This study also tested the effects of gender, posterior glottal width and glottal area on human larynges. An additional objective of this study was to determine the presence of hysteresis in human vocal fold oscillation.

Method. This study included nine human larynges, which were obtained 24 hours or less, after death. These were then kept in a sealed beaker with phosphate-buffered saline. Prior to testing, the larynges were prepared by having the extrinsic laryngeal muscles and associated tissue dissected away. 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. Additionally, other factors tested were the effects of gender, posterior glottal width, and glottal area. The posterior glottal width was tested by plastic shims of different thicknesses (0.5, 1, 2, 3 mm). These were placed between the arytenoid cartilages. A total of five phonation trials were carried out for each posterior glottal width on each larynx.

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 the 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.

Meyer, J. P., McAvoy, K. E., & Jiang, J. (2013). Rehydration capacities and rates for various porcine tissues after dehydration. *PLoS One, 8*(9), e72573. doi:10.1371/journal.pone.0072573

Purpose of the study. The purpose of this study was to identify the rehydration capacities and rates for porcine tissues after dehydration took place.

Method. All tissues used in this study were donated by Schmidt's Slaughterhouse. After the tissues were collected, they were divided into six different categories (e.g., muscle, tendon, skin, fat, cartilage, and lung). These tissues were then carefully sliced into small pieces and placed in a 0.9% saline solution. These were then frozen at -12° C for later use. Additionally, 24 of these tissue samples were selected randomly from each tissue type. These samples were then dabbed with a cloth to remove excess water and were then weighed. Afterwards, these pieces were placed in a vacuum oven at 38° C. These were left in the oven until they weighed about 100% to 40% of the original weight before dehydration occurred. After these were measured, all tissues were placed in a 0.9% saline solution for an additional five hours. At the end of five hours, each tissue was dabbed with a cloth to remove excess water and then weighed three times successively every 10 min. Moreover, after the tissues were rehydrated, they were each placed back into the 38° C oven for eighteen additional hours. These were then taken out and weighed in three successive 10 min intervals. This was done to test that the mass remained steady.

Results. According to the data gathered, the capacity for a tissue to reabsorb a liquid may have depended on the amount of dehydration the tissue previously experienced. Additionally, the amount of fluid a tissue reabsorbed may have depended on the unique characteristics that made up the tissue. For example, all of the tissues absorbed fluid nonlinearly over time except for fat. Fat did not absorb as much fluid as the rest of the tissues when the dehydration percentage increased.

Conclusions. The results taught that studies involving rehydrated cartilage must pay close attention to the tissues levels of dehydration. This reasoning may be due to the possibility that the level of dehydration the tissue experienced may have affected the final mass restored upon rehydration.

Relevance to the current work. The knowledge of rehydrating capacities and rates of tissue may aid in future studies involving rehydrated larynges. Additionally, cartilage was not as affected as fat was to increased levels of dehydration when rehydrating to its original mass.

Nakagawa, H., Fukuda, H., Kawaida, M., Shiotani, A., & Kanzaki, J. (1998). Lubrication mechanism of the larynx during phonation: An experiment in excised canine larynges. *Folia Phoniatrica et Logopaedica, 50*, 183-194.

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

Method. Two solutions of different viscosities were applied to the excised canine larynges in this study. The high viscosity fluid was made up of dissolved chondroitin sulfate sodium salt and the low viscosity fluid was made up physiologic saline. The arytenoid cartilages were sealed with a suture in order to increase adduction for phonation. Prior to piping the fluid onto the vocal folds, the vocal fold's surface mucous was removed with swabs. The 0.1 ml of the fluid was then spread 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. Overall, the frame numbers depicting the opening and closing phases were larger with the higher viscosity fluids. However, for the closed phases, the frame numbers tended to be lower. Additionally, the open quotient was increased and the normalized glottal peak area was significantly decreased for the high viscosity fluid. Also, the horizontal and vertical components' amplitudes decreased with the high viscosity fluid when the image was observed from the frontal plane.

Conclusions. Due to this study, researchers may conclude that since viscosity changes affected the wave motion of the vocal fold mucosa, then changes in viscosity may cause certain disorders such as vocal hoarseness.

Relevance to the current work. Many secretory glands may be found in the ventricular folds and laryngeal ventricles. Therefore, the fluid moving from the air tract to the supraglottis made up the column on the superior surface of the vocal folds. Conversely, the contents of the fluid contributed to vocal fold vibration. In the current study, dehydrating the vocal folds may have increased the viscosity of the fluid, making it more difficult for the wave to form on the mucous membrane of the vocal folds. During the prophylaxis trials, the vocal folds were hydrated using aerosolized saline and this may have reduced the viscosity of the vocal folds, therefore increasing the column which contributed to phonation.

Regner, M. F., Tao, C., Zhuang, P., & Jiang, J. J. (2008). Onset and offset phonation threshold flow in excised canine larynges. *Laryngoscope, 118*, 1313-1317. doi:10.1097/MLG.0b013e31816e2ec7

Purpose of the study. This study sought to identify onset and offset PTF in excised canine larynges. The presence of hysteresis was also tested.

Method. This study included 10 excised canine larynges, which were not killed for the purpose of this study. These were then inspected for pathologies and trauma, and later stored in 0.9% saline solution and frozen for later use. In preparation for the study, the larynges were defrosted and mounted on a bench apparatus. A pseudolung was also attached to the inferior portion of the larynx. During the study, a subglottal flow was introduced to the larynges being tested. This subglottal flow was increased until phonation occurred. The subglottal flow was then decreased until phonation ceased. During the experiment, measurements were taken for the onset and offset of the PTP and the PTF. Additionally, after the first measurements were taken, the vocal

folds of these same larynges were elongated and PTP and PTF were recorded. This was done to determine if the same results of PTP and PTF would be obtained for vocal folds with pathologies.

Results. The results of this study confirmed the researchers' original hypothesis. 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. Additionally, as suspected a presence of hysteresis was identified due to the higher levels of PTP than PTF in the study.

Conclusions. The knowledge gained from this study may be beneficial in treating and identifying vocal fold dysfunction. The PTF measurement could potentially become an additional support in identifying weak laryngeal resistance or aerodynamic power in the vocal folds.

Relevance to the current work. Like the current study, this study involved excised animal larynges. The setup of this study may be helpful in delivering the new study. A pseudolung was also used, but the larynges were not previously frozen. The present study did not perfectly represent *in vivo* vocal fold behavior due to the lack of innervation and additional muscular support.

Roy, N., Tanner, K., Gray, S. D., Blomgren, M., & Fisher, K. V. (2003). An evaluation of the effects of three laryngeal lubricants on phonation threshold pressure (PTP). *Journal of Voice, 17*, 331-342.

Purpose of the study. The purpose of this study was to evaluate the effects of three different types of lubricants (water, Mannitol-an osmotic agent, and Entertainer's Secret Throat Relief- a Glycerin Based Product) on PTP.

Method. This study included 18 healthy female participants with typical vocal function. Their PTP was measured twice before the lubricants were applied, and four times after 2 ml of each lubricant was nebulized. Each participant was tested on three separate occasions during a three-week period. During the three weeks, each week, one different lubricant was applied and tested on each subject. Additionally, using an oral pressure flow system, PTP was measured for both high and comfortable fundamental frequency for each subject.

Results. The results indicated that the Mannitol solutions had the best effects on decreasing PTP in the higher pitches. However, the effects faded about 20 minutes after administration. The other two solutions, water and Entertainer's Secret Throat Relief, did not demonstrate any significant effects on PTP after they were each administered.

Conclusions. This study supported other clinical research done regarding vocal fold hydration and the benefits of staying hydrated. The luminal airway surface may have been affected and hydrated by using substances such as Mannitol to create more efficient phonation by decreasing PTP.

Relevance to the current work. This study supported the rationale behind using PTP to measure vocal fold efficiency. This study also found Mannitol to be an effective way to decrease PTP. Similarly, this study supported the efficiency of using a nebulizer to hydrate the vocal folds.

Sivasankar, M., & Fisher, K. V. (2002). Oral breathing increases P_{th} and vocal effort by superficial drying of vocal fold mucosa. *Journal of Voice, 16*, 172-181.

Purpose of the study. This study sought to compare and contrast oral breathing and nasal breathing relating to levels of phonation threshold and vocal effort.

Method. This study involved 20 female participants with perceptual normal speech and voice. This group was then randomly divided into two groups of 10 subjects. One group participated in the nasal breathing and the other in the oral breathing for 15 min. Additionally, a vented pneumotachograph was used in order to calibrate the amount of airflow received by the subjects. The maximum vocal pitch was then determined by having the subjects perform a pitch glide on vowel /a/. The subjects were then asked to increase their pitch from a comfortable speaking pitch to the highest pitch they could reach. The minimum vocal pitch was found in a similar manner. The subjects in this experiment were also asked to produce /pi/ five times in their lowest pitch (without whispering), in their highest pitch as well as their comfortable pitch. Moreover, vocal effort was determined by having the subjects sing "Happy Birthday" in their lowest, comfortable and highest pitches.

Results. The results concluded nasal breathing reduced phonation threshold compared to oral breathing, which increased phonation threshold. Additionally, according to 7 out of 10 subjects nasal breathing decreased vocal effort. Moreover, 6 out of 10 subjects reported oral breathing to have increased their vocal effort.

Conclusions. The data gathered from this experiment may have been helpful in clinical prevention of laryngeal dryness especially for professionals who may excessively use their voice. Superficial hydration of the vocal folds may have contributed to therapy methods that facilitated phonation.

Relevance to the current work. Data gathered from this study supported other experiments involving animals, which concluded if water is lost from the sol layer, then this may increase the viscosity of the mucosal layer, which may then lead to increased phonation effort. The knowledge of the sol layer and its relationship to the phonation threshold may have contributed to research involving animal larynges.

Sivasankar, M., & Fisher, K. V. (2007). Vocal fold epithelial response to luminal osmotic perturbation. *Journal of Speech Language Hearing Research, 50*, 886-898. doi:10.1044/1092-4388(2007/063)

Purpose of the study. This study involved measuring bidirectional transepithelial water fluxes in vocal folds. This was done in order to determine whether viable vocal fold epithelium would reduce an osmotic challenge on the lumen by generating a water flux.

Method. A total of 36 ovine vocal folds collected from a local abattoir were used in this study. In preparation for the study, all larynges were bisected along the midsagittal plane. Additionally, two horizontal, 1-cm incisions were made on each larynx (one above and one below the vocal folds). The basal lamina and superficial lamina propria of the vocal fold epithelium were then dissected from each larynx using fine-tissue forceps. The membranes, which consisted of the vocal fold's basal lamina propria and superficial lamina propria, were then placed in a Ussing chamber, which may detect transport and barrier functions of living tissue, where the tissue was soaked in Hank's Balance Salt Solution to prevent death of the tissue. The potential difference and short-circuit current of the membranes were then measured using two irreversible non-

polarizable current electrodes. After the membrane was exposed to the luminal challenge, the potential difference and the short-circuit current were recorded at 10, 20, and 30 minutes. Additionally, the ratio of the potential difference and short-circuit current were used to calculate the membrane resistance.

Results. The data gathered from this study did not support a change in potential difference for each time segment. The PD measured for the sham and Mannitol solutions appear to be very similar for each time segment. Similarly, the data showed the short-circuit current did not fluctuate with different solutions. Moreover, vocal folds exposed to the osmotic challenge did not differ in resistance from vocal folds exposed to sham. These results may have lead researchers to believe that osmotic perturbations to luminal surface fluid did not have an effect on the electrophysiological viability of ovine vocal fold epithelium. However, the results of 60% of the vocal folds exposed to the luminal osmotic challenge indicated that the luminally directed water flux did in fact increase. Furthermore, this increase in water flux only increased after the first 10 min of exposure to the osmotic challenge.

Conclusions. The results of this experiment may have been helpful in pharmacological treatment. For example, hyperosmotic Mannitol could perhaps increase surface fluid volume, aid laryngeal dehydration, and decrease mucous in the vocal tract. This could in turn have prevented laryngitis, irritable larynx syndrome, nasal obstruction as well as other vocal tract issues that may come up due to irritable particles in the air.

Relevance to the current work. The knowledge of the permeability of the vocal folds was useful in studying vocal fold hydration. The optimal solution used in the present study was isotonic saline.

Sivasankar, M., & Fisher, K. V. (2008). Vocal folds detect ionic perturbations on the luminal surface: An in vitro investigation. *Journal of Voice, 22*, 408-419. doi:10.1016/j.jvoice.2006.11.005

Purpose of the study. This study sought to determine if changes to the ionic and osmotic composition of fluid on the luminal surface could be detected by viable vocal fold epithelium. The bioelectric measure of potential difference, short-circuit current, and the resistance of the tissues were measured. Additionally, these measurements were taken before, during and after the challenge.

Method. Fifty ovine larynges, which were excised within an hour postmortem, were included in this study. The larynges were collected from adult sheep in a slaughterhouse. In preparation for the study, these larynges were bisected into two hemilarynges in the midsagittal plane. Additionally, two horizontal incisions were made, one above and another below the true vocal folds, each 1 cm long. These dissected larynges were then mounted onto a Ussing chamber to begin the challenges. A voltage clamp was also used to measure the membranes' bioelectric properties. The challenges included exposing the larynges to sham, ionic, osmotic, and combined ionic-osmotic fluid.

Results. The measurements recorded during the ionic and combined ionic-osmotic challenges illustrated a decrease in the potential difference and the short circuit current. However, the results also showed it was possible to reverse the effects of the challenge once the challenge was removed. Additionally, results taken during the sham or osmotic challenge showed no evidence of altered bioelectric parameters over time. Furthermore, the measurements gathered from this study did provide evidence supporting the researcher's hypothesis that viable ovine vocal fold

epithelia had the ability to detect ionic perturbations to the luminal surface.

Conclusions. In conclusion, the knowledge gathered from this study may be helpful in improving treatment for individuals with chronic dry throats. Since vocal fold hydration is necessary for optimal speech, the information gathered from this data about the ability of vocal fold epithelia to not only detect changes in the luminal surface, but also aid in reaching homeostasis, could have contributed to vocal treatment.

Relevance to the current work. This study was relevant to the current study because of its breathing component. This study identified an additional ability of the vocal fold epithelium, specifically the ability of the vocal fold epithelium to detect changes in the luminal surface. If this was possible, then the epithelium may have been able to detect the dehydration level of the vocal folds as well.

Tanner, K., Roy, N., Merrill, R. M., & Elstad, M. (2007). The effects of three nebulized osmotic agents in the dry larynx. *Journal of Speech Language Hearing Research, 50*, 635-646. doi:10.1044/1092-4388(2007/045)

Purpose of the study. The PTP and perceived phonatory effort (PPE) were investigated using three different solutions (hypertonic saline, isotonic saline, and sterile (hypotonic) water) following a laryngeal surface dehydration challenge.

Method. Sixty vocally healthy women were involved in this study and divided into four groups. One group served as the control trial and the remaining groups participated in the administration of one of the different nebulized solutions. The PTP and PPE were measured for each subject immediately after desiccation, and at an exact time post desiccation (5, 20, 35 and 50 min).

Results. The results showed that PTP increased significantly after the desiccation challenge for all women in the study. On average, PTP values increased about 0.5 cmH$_2$O immediately post desiccation compared to baseline measures. Overall, PTP values did not change significantly after nebulized treatments were administered. However, a temporary trend of decreasing PTP was observed for the group that participated in the isotonic saline treatment. Although unexpected, the PPE ratings decreased after the desiccation challenge and the PPE ratings did not correlate with the PTP data.

Conclusions. This study proved that temporary exposure to relatively low levels of humidity may increase PTP. However, the results of this study also indicated that PTP and PPE may not be perceptually related and the assumed relationship may be questioned. Also, none of the nebulized treatments proved to significantly decrease PTP after desiccation.

Relevance to the current work. The porcine model included isotonic saline as a form of aerosolized treatment because this solution proved to be the most effective in decreasing PTP and reducing the effort required to initiate vocal fold vibration.

Tanner, K., Roy, N., Merrill, R. M., Kendall, K., Miller, K. L., Clegg, D. O., . . . Elstad, M. (2013). Comparing nebulized water versus saline after laryngeal desiccation challenge in sjögren's syndrome. *Laryngoscope, 123*, 2787-2792. doi:10.1002/lary.24148

Purpose of the study. A laryngeal desiccation challenge (<1% relative humidity transorally) and two nebulized hydration treatments were used in this study in order to identify differences between the PTP, self-perceived vocal effort and throat dryness. Patients with chronic airway dryness were used as subjects in this study.

Method. The following study involved 11 subjects with Primary Sjögren's Syndrome. More specifically, 10 females and one male were included in this study. Each subject endured a laryngeal desiccation challenge for 15 min. This experiment was conducted once a week over a course of two weeks. After desiccation, each test subject received either a nebulized isotonic saline (0.9%) or nebulized isotonic water treatment. These were administered transorally at a given rate of 8 L/min. The factors tested were PTP, vocal effort, and self-perceived throat dryness. These were assessed before and after the treatment as well as 5, 35 and 65 min after the treatment.

Results. The data collected from this indicated a significant increase in PTP, vocal effort, and oral dryness after the desiccation challenge. Additionally, results indicated the nebulized isotonic saline treatment to be more effective for hydration than the nebulized isotonic water treatment. However, the difference between these treatments was not statistically significant.

Conclusions. This experiment caused patients with chronic dry throats to have phonatory changes after the desiccation challenge. Moreover, nebulized isotonic saline may have contributed to vocal hydration causing a decrease in PTP, vocal effort and dryness of throat or mouth.

Relevance to the current work. In relation to the present study, the knowledge of a decrease in PTP and vocal effort due to the nebulized isotonic saline treatment was helpful in conducting studies related to vocal hydration. The use of a nebulizer in this study also served to provide additional support to the validity of using such instruments when testing vocal fold hydration.

Titze, I. R., Klemuk, S., & Lu, X. (2014). Preliminary experiments to quantify liquid movement under mimetic vocal fold vibrational forces. *Logopedics, Phoniatrics, Vocology, 39*(2), 50-55. doi:10.3109/14015439.2013.848236

Purpose of the study. This study explored the movement of the superficial water layer on the vocal folds during vocal fold oscillation.

Method. The researchers in this study examined several previous studies that failed to identify the movement of the liquid during vocal fold oscillation. Hyaluronic acid (HA) and its characteristics were also explored in this paper.

Results. Vocal folds with more viscous liquid on the surface, required a larger lung pressure to initiate phonation. If the vocal folds were phonated for prolonged periods of time, then the PTP increased. This may have happened due to the time the tissues required to evenly distribute the water after phonation. Additionally, a portion of the study looked at HA and its effects on dry absorption paper. The results indicated 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. The results of this study found that much research is left to be done regarding how fluid travels through the extracellular matrix as well as how HA affects hydration of the vocal mechanism. Additionally, the described preliminary studies indicated that exposing HA to vibrational energies consistent with vocal fold vibration were not likely to break the bonds between HA and H_2O.

Relevance to the current work. This paper supported the reason for conducting the present study. Perhaps determining whether PTP was affected by reversing the desiccation process of the vocal folds may have provided clues about the movement of the water and how dehydration takes place.

Witt, R. E., Regner, M. F., Tao, C., Rieves, A. L., Zhuang, P., & Jiang, J. J. (2009). Effect of dehydration on phonation threshold flow in excised canine larynges. *Annals of Otology, Rhinology, and Laryngology, 118*, 154-159.

Purpose of the study. This study was conducted to identify the minimum airflow necessary to initiate stable vocal fold vibration in excised canine larynges (i.e., phonation threshold flow; PTF).

Method. This study involved 11 excised canine larynges, which had been sacrificed for other research purposes. Eight of these larynges were used for the dehydration trials, two were used as controls, and the remaining larynx was used for both a control and experimental trial. The manner in which these excised larynges were tested involved sequential trials. The trials included a cycle of phonation, which lasted 10 seconds, and a cycle of rest, which lasted three seconds. The larynges were mounted on a bench apparatus. During the experimental trials, dry air (non-humidified; 25% ± 3% relative humidity) was directed subglottally until phonation occurred for 10 sec. Moreover, during the control trials, the air being blown into the larynges was humidified and 0.9% isotonic saline solution was applied frequently to the vocal folds.

Results. During the experiment, the results of the larynges involved in the experimental trials demonstrated a direct relationship between PTF and the exposure to dry air. Additionally, the larynges did reach a point at which phonation was no longer possible. This was suspected to be due to the dryness of the tissue making up the vocal folds.

Conclusions. The knowledge gained through this experiment of the direct relationship between PTF and the exposure to dry air could potentially aid in clinical assessment and prevention of dehydration. Further research involving *in vivo* subjects in this area could improve current hydration therapies.

Relevance to the current work. Knowing the relationship between the exposure to dry air and PTF was helpful in conducting experiments related to vocal fold hydration and human respiration. This knowledge could be helpful to improve treatment techniques for vocal fold dryness.

Witt, R. E., Taylor, L. N., Regner, M. F., & Jiang, J. J. (2011). Effects of surface dehydration on mucosal wave amplitude and frequency in excised canine larynges. *Otolaryngology Head Neck Surgery, 144*, 108-113. doi:10.1177/0194599810390893

Purpose of the study. The purpose of this study was to identify the effects of vocal fold desiccation and hydration on mucosal wave amplitude and frequency of excised canine larynges.

Method. The study involved 10 excised canine larynges. The ages of the excised larynges are unknown. Once the larynges were excised they were immediately placed in a 0.9% isotonic saline solution and quickly frozen. The larynges were then thawed prior to being mounted onto a bench model. This system was attached to a pseudolung and placed in a triple walled sound attenuated room. The environment also included a stabilized room temperature and humidity level. High-speed video was used to record the mucosal wave of all ten larynges. Eight of the 10 excised control larynges were exposed to dehumidified air at 20 cmH_2O.

Results. The data of this experiment was analyzed using a MATLAB program and videokymography. The linear regression of the amplitude and frequency of vibration was also calculated for each individual larynx. Mann-Whitney Rank Sum tests were then used to identify a statistical relationship between the control group and the experimental larynges. Analysis of

the results indicated that the eight larynges in the experimental group indicated a statistical difference between the control group and the experimental larynges.

Conclusions. The results of this experiment indicated an inverse relationship between decreased hydration and increased amplitude and frequency of the vocal folds.

Relevance to the current work. This study laid groundwork for experimental methodology and operational procedures for the present investigation. The results from this study indicated that dehydration and hydration effects may be tested effectively in excised larynx mechanical models. This study supported the reason for using porcine larynges, which have similarities to human vocal folds, such as the vocal ligament.

Zhang, Y., Jiang, J. J., Tao, C., Bieging, E., & MacCallum, J. K. (2007). Quantifying the complexity of excised larynx vibrations from high-speed imaging using spatiotemporal and nonlinear dynamic analyses. *Chaos, 17*, 043114. doi:10.1063/1.2784384

Purpose of the study. This study sought to qualitatively describe the irregularities of vocal fold vibrations using spatiotemporal and nonlinear dynamic analyses.

Method. Twelve excised canine larynges were used and the superior portion of the vocal tract was removed for all larynges. High speed images were recorded to aid in the acquisition of data for the spatiotemporal and nonlinear dynamic analyses.

Results. The characteristics of the spatiotemporal analyses illustrated an irregular vibratory pattern for the irregular vibration. A periodic time series of the glottal area was produced by the regular vibration. However, the irregular vibration produced an aperiodic time series of the glottal area. Moreover, irregular vibrations of the vocal folds produced aperiodic glottal area series, broadband spectra, and complex spatiotemporal vibratory patterns.

Conclusions. The results from this study indicated spatiotemporal analysis and nonlinear dynamic analysis had the capacity to describe the complex dynamics of vocal fold vibrations directly from high speed imaging. Additionally, this may have been useful to evaluate disordered behaviors in biomedical laryngeal systems.

Relevance to the current work. This study also used high speed imaging to assist the analysis of the vocal fold vibration. Furthermore, this study provided benefits for using an excised larynx instead of an *in vivo* model.

Zhang, Y., Reynders, W. J., Jiang, J. J., & Tateya, I. (2007). Determination of phonation instability pressure and phonation pressure range in excised larynges. *Journal of Speech Language Hearing Research, 50*, 611-620. doi:10.1044/1092-4388(2007/043)

Purpose of the study. This study sought to identify the dynamic mechanisms of phonation instability pressure, phonation pressure range and PTP through bifurcation analysis. This study also tested the effects lengthening the vocal folds had on these three factors: phonation instability pressure, PTP, and phonation pressure range.

Method. This experiment included 10 excised canine larynges that were not sacrificed for the purpose of this study. Additionally, the experiment took place within 48 hours after collecting the larynges. In preparation for the experiment, the canine larynges were mounted onto a pipe, which was attached to a pseudolung. A metal clamp was then attached to the trachea and two, three pronged devices were also attached to the larynx for better stabilization. Regulated air then began to flow from the pseudolung to the vocal folds. 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. Moreover, PTP and phonation instability pressure were also tested during the elongation experiments. For each elongation, the experimental procedure was repeated three times for accuracy.

Results. The data collected from the excised larynges supported the use of bifurcation analysis to accurately measure phonation instability pressure, PTP and phonation pressure range. Additionally, when vocal folds were elongated, the authors identified a significant increase in the PTP, an insignificant change in the phonation instability pressure and a significant decrease in the phonation pressure range.

Conclusions. The knowledge gained from this study provided speech pathologists information about providing better treatment for individuals who have voice pathologies. Knowing bifurcation analysis did adequately determine phonation instability pressure and phonation pressure range may have clinical potential in addressing improvement after treatment. This technique may also have been helpful in diagnosing laryngeal pathologies.

Relevance to the current work. A similar setup and similar measures were used in the present study. Although, pig larynges were not used, canine larynges were used and this supported the use of animal models when testing for constants in human larynges.

APPENDIX B: Experimental Checklist

Materials for Dissection:
1. scalpels (2 different types)
2. apron
3. gloves
4. green dissection paper (to be laid on the dissection table)
5. saline spray bottle
6. 1 Ziploc bag
7. hemostats
8. sutures (1 for each larynx)
9. protective goggles
10. dissection table
11. red hazard box (rinse scalpels and then place them in this box)
12. tub-fridge drawer (to hold un-dissected larynges)
13. Clorox wipes (for clean-up)
14. Paper towels (to hold your larynx steady)
15. Mini fridge

Additional Notes:
- 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. nozzle for desiccated air
5. 2 mesh type nebulizers (filled with saline) (MicroAir OMRON NE-U22)
6. Teflon tape (used to seal edges of trachea onto the custom tubing which is attached to the pseudolung)
7. Flow meter (Aalborg mass flow meter GFM-47)—flow should be calibrated at 0, 10 and 15 cmH$_2$0
8. Medical Flow Meter- attached directly to the air tank and to the Aalborg mass flow meter GFM
9. 2 Air tanks (one will attach to the flow meter and the humidifiers; the other will be for desiccated air)
10. pressure transducer (should be plugged in from computer to inferior lateral portion of larynx or the custom tubing)
11. pressure calibrator box (should be used only to calibrate pressure transducer) calibration occurs at 0, 10 and 15 PSI
12. check all plugs
13. 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)
14. Humidifier (make sure tubing is plugged in to pseudolung and air tank)
15. High Speed video camera: Trigger should be on and plugged into the sound board
16. 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.)
17. High Speed-make sure trigger is plugged in
18. 4 Metal clamp (secure trachea onto the custom tubing which attaches to the pseudolung)
19. Metal clamps (hold flashlights & Microphone)
20. Vinegar and distilled water (for cleaning nebulizers)- follow instructions for cleaning at the end of the day of all experiments
21. Clorox Wipes
22. Paper towels
23. Metal shim (diameter 5mm)

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

Procedure for Desiccation Trials (rehydration)

- 5 pigs were included in this section
- For each larynx, baseline measures were collected and were named trial 1 (larynges were vibrated without desiccation trial) (if the larynx did not vibrate, then it was removed from the study)
- After baseline was collected, pigs were desiccated for 1- minute increments using custom tubing which was attached to one of the air tanks (a shim was held in place posterior to the true vocal folds in the interarytenoid space)
- each larynx was then vibrated following 1- minute desiccations (data was collected after each desiccation trial)
- Desiccation trials were continued until vocal folds ceased to vibrate or phonation ended

- Aerosolized trials were begun immediately after desiccation trials
- Aerosolized saline was applied directly onto the surface of the true vocal folds using a nebulizer with custom tubing attached to the mouthpiece
- Aerosolization lasted 2 minutes for 7 trials
- Each larynx attempted vibration after 2 minutes of aerosolization and data was collected for each trial. Onset was considered when the larynx began to phonate.

Procedure for Control Trials
- 5 pigs were included in this section
- For each larynx, baseline was collected (larynges were vibrated without desiccation trial) (if the larynx did not vibrate, then it was removed from the study)
- After baseline was collected, pigs were desiccated for 1 minute increments (a shim was held in place posterior to the true vocal folds in the interarytenoid region)
- each larynx was then vibrated following 1 minute desiccations (data was collected after each desiccation trial)
- Desiccation trials were continued until vocal folds ceased to vibrate or phonation ended

Measuring Humidity
- Record % humidity at the beginning of the experiment and at the end
- Make sure hygrometer is calibrated.

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

APPENDIX C: Food Handler's Permit

Utah Food Handler
Certificate of Training Completion

StateFoodSafety.com™

Presented to: **Maya Nunez**

5f672-ghgehh5

Certificate Verification Number
Verify at www.foodhandlerverification.com

Training approved by Utah Department of Health

UTAH DEPARTMENT OF
HEALTH

Oct 15, 2015

Date of Completion (**valid 30 Days**)

Employers: According to the new Utah food handler law, this certificate allows your employee to handle food for 30 days until they receive their official permit from the local health department.

President, StateFoodSafety.com