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The Effects of Laryngeal Desiccation and Nebulized Isotonic Saline in Trained Male Singers

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ABSTRACT

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in Trained Male Singers

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Vocal fold hydration is important for healthy function of the vocal mechanism. Vocal fold surface fluid protects the mucosa and facilitates efficient vocal fold oscillation. Dry air exposure, mouth breathing, insufficient intake of liquids, and behavioral factors may contribute to laryngeal dehydration. Singers are believed to be particularly at risk for voice problems related to dehydration due to environmental and voice use factors. Laryngeal desiccation and nebulized hydration treatments have been shown to influence phonation threshold pressure (PTP) and self-perceived phonatory effort (PPE) in females. However, little research exists exploring the effects of hydration in males. Additionally, few studies have examined the dose-response relationship of hydration treatments. This investigation examined the effects of a laryngeal desiccation challenge and two different doses of nebulized isotonic saline on voice production in trained male singers. In a double-blind, within-subjects repeated measures crossover investigation, 10 male singers (ages 18 to 24) received a 30 minute laryngeal desiccation challenge followed by either 3 mL or 9 mL of nebulized isotonic saline on two consecutive weeks. PTP, PPE, and self-perceived mouth and throat dryness were sampled during the following observations: pre-desiccation, post-desiccation, and at 5, 35, and 65 minutes post-nebulization. No differences in PTP were observed after desiccation or nebulized treatment. PPE, however, rose significantly after desiccation and returned near baseline after treatment. No significant differences between dosages were observed.

Keywords: isotonic saline, laryngeal desiccation, males, hydration, voice production, phonation threshold pressure, vocal effort

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I would like to thank Dr. Kristine Tanner for her incredible dedication to both research and my education. Her mentorship not only made a difficult task possible, but also provided me with valuable clinical and research experience. I am so grateful for her time and sacrifice on my behalf. I would also like to thank my parents for their example of how a professional can achieve clinical excellence, produce worthwhile research, and balance family and personal life. Their constant support, as well as the support of my sister, Amanda, made this project possible. In addition, I would like to thank all the members of my committee for their time and effort.

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Description of Structure and Content

The body of this thesis was written as a manuscript suitable for submission to a peer-reviewed journal in speech-language pathology. This thesis is part of a larger collaborative project, portions of which may be submitted for publication, with the thesis author being one of multiple co-authors. Experimental protocol is contained in Appendix A, an annotated bibliography is presented in Appendix B and Appendix C contains the participant consent form.

Introduction

Vocal fold hydration is essential to optimal function of the voice. Vocal fold surface fluid consists of mucus and water layers that are believed to protect the mucosa and allow the folds to oscillate efficiently (Labiris & Dolovich, 2003; Leydon, Sivasankar, Falciglia, Atkins, & Fisher, 2009; Sivasankar & Fisher, 2007; Widdicombe, 1997). Unfortunately, there are many environmental and behavioral factors that may dehydrate the vocal mechanism. Dry air exposure (Hemler, Wieneke, & Dejonckere, 1997), mouth breathing (Sivasankar & Fisher, 2002; 2003), insufficient intake of liquids (Verdolini, Min, & Titze, 2002), and voice use factors (Solomon & DiMattia, 2000; Solomon, Glaze, Arnold, & van Mersbergen, 2003) may contribute to this phenomenon. Therefore, it is important for optimal vocal performance to take measures to ensure that the vocal mechanism is properly hydrated. This may be important particularly for occupational voice users and performers who may have greater vocal demands. Professional voice users such as singers require optimal vocal health and may be at risk for voice problems due to dehydration. For example, frequent airplane travel, hotel stays, and changing performance schedules and venues may place singers at increased risk for dehydration-related voice problems. For this reason, various humidifiers, sprays and drinks exist ostensibly for the purpose of improving hydration for optimal voice function (Leydon et al., 2009).

The two chief biological mechanisms that mediate hydration of the vocal mechanism include systemic and surface hydration. Systemic hydration consists of maintaining normal cell volume through homeostatic regulation of extracellular fluids throughout the body. A lack of systemic hydration can negatively affect voice production (Fisher, Ligon, Sobecks, & Roxe, 2001; Fisher, Telser, Phillips, & Yeates, 2001). Surface hydration refers to the vocal fold surface fluid, consisting of a superficial mucus blanket and deeper water layer, which allows the vocal folds to oscillate optimally (Labiris & Dolovich, 2003; Sivasankar & Fisher, 2007; Widdicombe, 1997). Vocal fold surface fluid seems to be regulated by transepithelial water flux via active transport mechanisms (Sivasankar & Fisher, 2007). Surface hydration may be treated with humidifiers, whereas systemic hydration can generally be addressed through increased ingestion of fluids. While the precise mechanisms regulating vocal fold hydration remain largely unknown, both systemic and surface hydration seem to play an important role in optimal vocal fold functioning (Roy, Tanner, Gray, Blomgren, & Fisher, 2003; Verdolini et al., 2002).

As noted above, surface hydration of the vocal folds is accomplished by the maintenance of vocal fold surface fluid. Adequate surface hydration is key to optimal vocal fold oscillation because moist vocal fold mucosa requires less subglottic air pressure to oscillate than when the system is dry. The minimum amount of subglottic air pressure necessary to initiate vocal fold oscillation is referred to as phonation threshold pressure (PTP; Titze, 1994). Generally it is believed that lower PTP reflects less stress and effort required from the vocal mechanism (Verdolini-Marston, Titze, & Druker, 1990). Dryness of inhaled air affects PTP, presumably due to its relationship with vocal fold surface fluid (Sundarrajan, Erickson-Levendoski, & Sivasankar, 2012; Witt, Taylor, Regner, & Jiang, 2011). To improve voice production after dry air exposure, several studies have examined possible nebulized hydration treatments and their effects on voice function. Roy et al. (2003) found that certain nebulized treatments lowered PTP, particularly at high pitches. Participants received a laryngeal desiccation challenge followed by one of three nebulized treatments, including liquid Mannitol, water, or Entertainer's Secret Throat Relief™, on three consecutive weeks. It was found that Mannitol reduced PTP, indicating that nebulized treatments with certain ionic properties might improve voice function and increase hydration of the vocal mechanism.

Another investigation examined the effects of three nebulized treatments on PTP following laryngeal desiccation (Tanner, Roy, Merrill, & Elstad, 2007). In a double-blind, randomized experimental trial, 60 females with normal voices were randomized to one of four conditions, including 3mL of nebulized isotonic saline (0.9% $Na^{+}Cl^{-}$), hypertonic saline (7% $Na^{+}Cl^{-}$), sterile water, or no treatment. The results showed significant worsening of PTP and vocal effort after a 15-minute desiccation challenge, followed by improved function after nebulized isotonic saline and nebulized water treatments. In a similar study, Tanner, Nelson, Merrill, Muntz, Houtz, Elstad and Wright-Costa (2010) studied the effects of nebulized saline versus nebulized water or no treatment in classically trained sopranos. Nebulized isotonic saline again showed potential as a laryngeal lubricant, possibly aiding surface hydration. This research has important implications because singers require their vocal mechanisms to perform at a higher level and are therefore at high risk for vocal problems if the vocal apparatus is not functioning optimally.

Although these studies have been useful in shaping current understanding of both surface hydration and its effects on PTP, it is important to note these data have been primarily collected from female participants (e.g., Roy et al., 2003; Tanner et al., 2010). A few studies suggest that the effects of surface tissue dehydration may affect males differently than females. Sivasankar and Erickson (2008) observed no significant differences for males with normal voices and males with vocal fatigue before and after a 25-minute oral breathing challenge. Similarly, effects of a systemic dehydration challenge, combined with vocally fatiguing tasks, had mixed effects on PTP and vocal effort in male speakers (Solomon et al., 2003). There are several factors that suggest that men might respond to laryngeal desiccation challenges and hydration treatments differently than women. The lower incidence of vocal disorders in men suggests that the male vocal apparatus may be less sensitive to poor functioning conditions including dehydration. This may be due to the increased mass, thickness, and length of the male vocal folds that occurs during puberty (Titze, 1994). It is possible that differences in the viscoelastic properties of the vocal folds and/or the biochemical properties of vocal fold surface fluid may be different in males versus females (Sivasankar & Erickson, 2008). Male vocal folds also contain significantly more collagen than female vocal folds. This allows the male apparatus to reach higher levels of stiffness with less elongation. There are also possible chemical factors, which could explain why men may not respond to a desiccation challenge in the same manner as women. Hyaluronic acid (HA) aids in the development of the vocal tissues and is found throughout the body in areas where shock absorption occurs. The male vocal apparatus has a consistent distribution of HA throughout the system, whereas the female apparatus has a lower level on the surface lamina putting the females at higher risk for damage (Ward, Thibeault, & Gray, 2002).

It should also be noted that the results of past studies have differed when the participants were singers. For example, Tanner et al. (2010) found that while laryngeal desiccation and nebulized treatments did not produce statistically significant changes in PTP in classically-trained sopranos, perceived phonatory effort (PPE) changed significantly. The authors theorized that trained vocalists might be aware of changes in the vocal effort related to dehydration, but were able to compensate for these effects to some extent. Or perhaps the singers were more sensitive to dehydration changes that were not necessarily experimentally or clinically significant. It is clear that further investigation is warranted regarding surface hydration and its effects on PTP and PPE in men, both with and without vocal training.

The results of studies reporting the effects of surface tissue dehydration in males have been somewhat mixed, and no studies have examined the effects nebulized hydration treatments in men. Additionally, the dose-response relationship between laryngeal desiccation and nebulized treatments is largely unknown. Individuals with voice training may be more at risk for voice problems that accompany vocal fold surface tissue dehydration. The present study was therefore undertaken to answer the following research questions: What is the effect of laryngeal desiccation challenge on vocally-healthy male singers? Does nebulized isotonic saline improve voice function following laryngeal desiccation? How does isotonic saline dose influence this effect?

Method

Participants

Participant inclusion criteria included a chronological age of 18 to 25 years and being accepted to the Brigham Young University (BYU) School of Music in vocal training programs. The upper age range was limited to 55 to prevent any age-related changes from affecting the results of the study. The lower range was set at 18 to ensure that the vocal mechanisms of participants were relatively mature. Participants reported they were free of any history of a voice disorder for which they sought medical attention. Participants were asked to reschedule if they were suffering from any upper respiratory illness or allergy symptoms at the time of their participation in the study. Participants were non-smokers and reported no history of hearing loss, asthma, or other lung disease. Participants were all currently enrolled in the Brigham Young University School of Music, majoring in vocal performance, music education (with an emphasis in voice), or music dance theater programs. Participant identification and recruitment procedures were accomplished in agreement with the BYU institutional review board (#F 130071).

Experimental Design

This investigation employed a double-blind, within-subjects repeated measures crossover design. Participants took part in two experimental sessions. Both included a 30-minute laryngeal desiccation challenge involving transoral breathing of extremely low humidity air (< 1%). Participants then either received 3 mL or 9 mL of nebulized isotonic saline (0.9%). The nebulizer well was filled by a second examiner and covered with tape to blind both the primary examiner and the participant to dose. The order of nebulized treatment dose was counterbalanced to avoid order effects. We attempted to schedule sessions at the same time of day to ensure that the participants' voices would be in similar conditions during both sessions. Participants were instructed to maintain similar hydration conditions and vocal behaviors prior to each experimental session.

Dependent variables included PTP, PPE, self-perceived mouth dryness, and self-perceived throat dryness. Observations occurred immediately before desiccation, immediately following desiccation, and at 5, 35, and 65 minutes after nebulization. During the pilot phase of the investigation it was determined that each session would require approximately two hours for data collection, and an additional 30 minutes for PTP training and pitch range establishment during the first session, resulting in total session times of 2.5 and 2.0 hours for each session, respectively (see Appendix A).

Procedure

Experimental procedures were completed as follows, beginning with pitch establishment in order to determine pitch targets for the PTP task.

Pitch establishment. Prior to the PTP task, maximum and minimum fundamental frequency (F_0) was identified. Participants were asked to glide to the highest pitch they could sustain for 3 seconds on the vowel /i/. They were then asked to glide to the lowest pitch they could sustain for 3 seconds on the same vowel. Participants were then asked to perform the same task as softly as possible (practice attempts were permitted). The pitch range for each participant was subsequently calculated in Hz using a piano keyboard. The pitch range was then used to find the 80th and 10th percentiles of the F_0 range; rounding to the next whole unit was used when necessary.

PTP signal acquisition. Participants were trained to perform the PTP task at 80th and 10th percentiles. After a demonstration from the examiner, participants were asked to say /pi/ syllables in strings of five, three times each (Smitheran & Hixon, 1981) at a rate of approximately 3.5 syllables per second (Aeroview Manual, 2012). Participants performed the task at suprathreshold, subthreshold, and finally at threshold level (i.e., just above a whisper). Training was repeated until participants were able to perform the task consistently at the correct pitch and loudness level as judged by the examiner. Participants were then asked to repeat these sounds into the airflow mask attached to the pressure and flow measurement system (Glottal Enterprises Aeroview Phonatory Aerodynamics System, v1.4.9, Syracuse, New York). A nose clip was worn during pressure and flow signal acquisition. The examiner monitored the participants' productions for accuracy in terms of pitch and loudness level as well as pressure and flow output. When the participants were able to perform the task correctly, baseline PTP was recorded for high and low F_0. Prior to each data collection session, all airflow equipment was calibrated per manufacturer specifications (see Appendix B).

PPE acquisition for speaking. Immediately following each PTP signal acquisition, participants rated their level of self-perceived vocal effort during speaking. For the speaking task, participants read the Rainbow Passage and sustained the /ɑ/ vowel at a comfortable pitch and loudness level for a minimum of 5 seconds. This task was chosen in order to give the results more clinical relevance. Immediately following this task, they rated their levels of vocal effort on a 10 cm visual analog scale (VAS), with the far left of the scale representing no effort and the far right representing extremely high effort. Participants were permitted to view their previous ratings from within the session when making their VAS ratings.

PPE acquisition for singing. For the singing task, participants sang a messa-di-voce exercise, which consisted of a five note scale sung on the word "amore." The exercise began on the tonic note of the scale, shifted to the dominant note and descended through the scale to the tonic. The tonic was sung forte and the dominant was sustained for two to three seconds, starting pianissimo and swelling to forte. For tenors the exercise was performed from A3 to E4 and for baritones the exercise was performed from F3 to C4. These pitches were chosen because they were well within the singers' ranges, but high enough to require some effort and technique. Again after the singing task, a VAS was completed with the far left representing no vocal effort and the far right representing extreme vocal effort.

Self-perceived dryness. Dryness ratings were also made following PPE measurement. Participants were instructed to rate their level of perceived mouth and throat dryness using 10 cm VASs with the far left representing no mouth or throat dryness and the far right representing extreme dryness.

Reliability

Ten percent of oral pressure samples were reanalyzed and PTP recalculated by the original examiner and a second examiner. Similarly, 10% of visual analog scale ratings were re-measured. Spearman correlations of 1.0 ($p < .01$) and 1.0 ($p < .01$) were obtained for intrajudge and interjudge reliability estimates, respectively.

Statistical Analysis

Descriptive statistics were used to evaluate and summarize the effects of the desiccation challenge and saline nebulized treatment doses on PTP, PPE, and throat dryness. Freidman nonparametric tests for related samples were used to evaluate main effects. Post hoc comparisons were made using the Wilcoxon Signed Ranks Tests, with a Bonferroni correction for multiple comparisons. Statistical analyses were completed using IBM SPSS Statistics, version 21.

Results

Environmental Humidity

Relative humidity (RH) was recorded before and after each session using the HygroSet II Digital Hygrometer (Model DHYG-Round), calibrated using the Humidi-Pak Calibration Kit (Quality Importers). For session 1, the mean RH at baseline was 15.6% (*SD* = 3.4%; range 11.0% to 21.0%); mean RH at 65 min post nebulization was 14.9% (*SD* = 3.0%; range 10.0% to 18.0%). For session 2, the mean RH at baseline was 17.3% (*SD* = 6.7%; range 10.0% to 26.0%); mean RH at 65 min post nebulization was 18.0% (*SD* = 6.7%; range 10.0% to 27.0%). RH was not significantly different from session one to session two at baseline, based on the Wilcoxon Signed Rank Test for related samples ($p = .972$). The average absolute difference in RH at baseline was 6.5%. Additionally, RH did not change significantly within experimental sessions, based on the Wilcoxon Signed Rank Test for related samples ($p = .358$). The average absolute difference in RH from baseline to 65 min post nebulization was 1.65%.

Participants at Baseline

Mean participant age was 21 years (*SD* = 2.3; range 18 to 24 years). Videolaryngostroboscopy was performed on all participants at baseline to evaluate the vocal folds for any non-normal laryngeal findings. Exams were reviewed by a board-certified laryngologist with over 20 years of experience in laryngeal imaging. No significant mucosal changes, lesions, vibratory anomalies, or vocal fold mobility limitations were observed for the participants. At baseline, the mean time since participants last ate was 183 min (*SD* = 208 min; range 0 to 675 min); the mean time since participants last drank fluids was 58 min (*SD* = 150 min; range 0 to 675 min).

PTP

The 10% F_0 mean was 131.57 Hz (*SD* = 25.43 Hz; range 99.79 Hz to 180.97 Hz). The 80% F_0 mean was 397.98 Hz (*SD* = 83.01 Hz; range 263.58 Hz to 574.99 Hz). No statistically significant differences in PTP were observed at baseline across sessions for 10% F_0 ($Z = -.051$, $p = .959$) or 80% F_0 ($Z = -1.070$, $p = .285$), based on the Wilcoxon Signed Ranks Test for related samples. Mean PTP increased 0.43 cmH_20 following the laryngeal desiccation challenge at 80% F_0. Mean PTP decreased 0.16 cmH_20 following the laryngeal desiccation challenge at 10% F_0. The main effect of time was not statistically significant for PTP at 80% F_0 ($\chi^2(4) = .96$, $p = .916$) or 10% F_0 ($\chi^2(4) = 3.37$, $p = .499$) based on the Freidman Test for related samples. No statistically significant differences were observed between 3mL and 9mL saline doses. PTP at each observation for 3mL and 9mL are displayed in Figure 1 for 80% F_0 and Figure 2 for 10% F_0.

PPE for speaking

For speaking PPE, the main effect of time was statistically significant based on the Friedman Test for related samples, $\chi^2(4) = 9.56$, $p < .049$. Post hoc comparisons were pursued using a Bonferroni correction and an adjusted alpha level of 0.0125. A statistically significant desiccation effect was observed between baseline and post desiccation observations, based on a Wilcoxon Signed Ranks Test, $Z = -2.929$, $p < .003$. A treatment effect at 5 min post

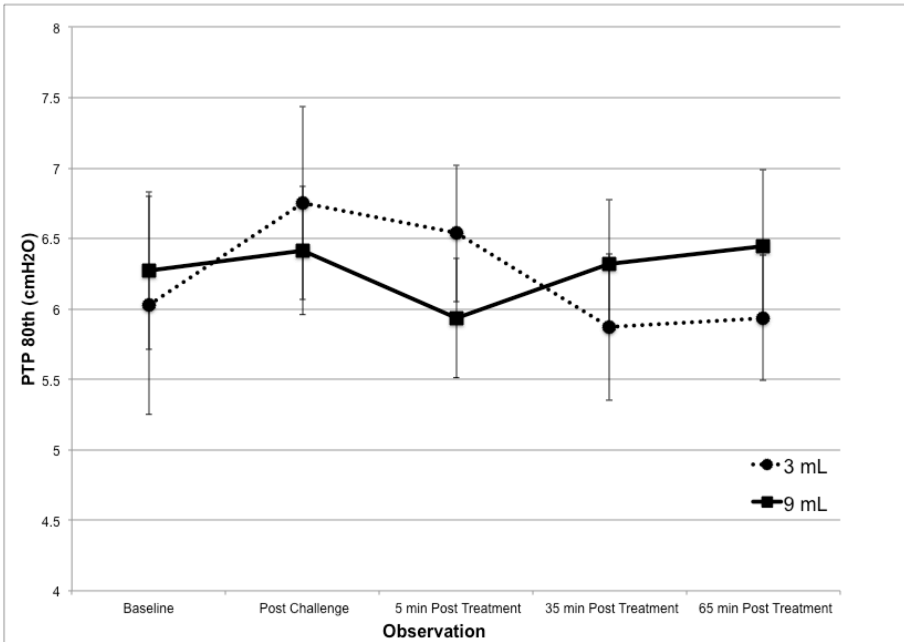

Figure 1. Means and standard errors for PTP at each observation for 3mL and 9mL at 80% F_0.

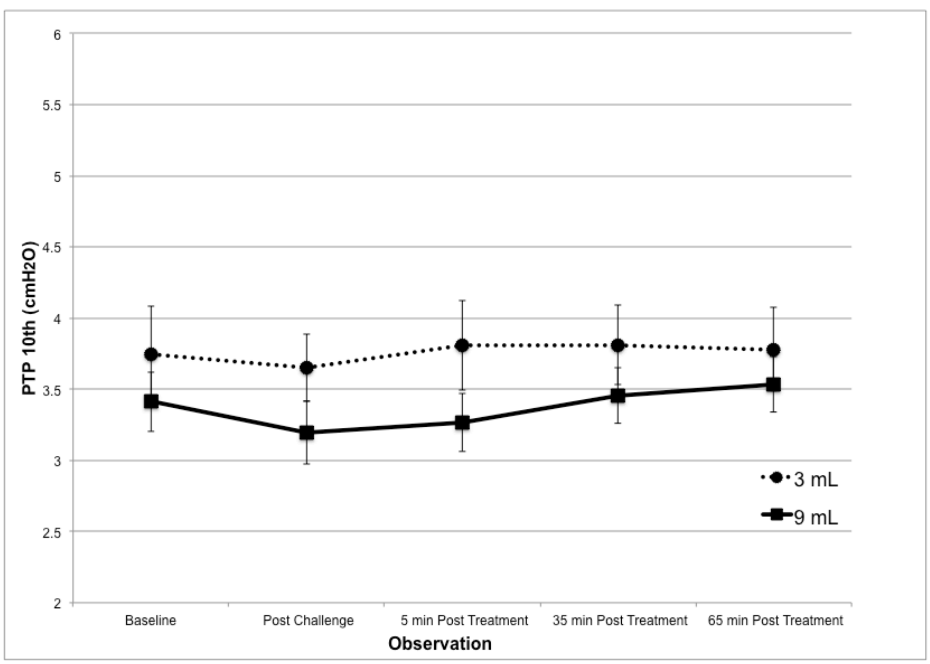

Figure 2. Means and standard errors for PTP at each observation for 3mL and 9mL at 10% F_0.

nebulization approached statistical significance, $Z = -2.420$, $p < .016$. No other significant effects for time were observed. No statistically significant differences were observed for 3mL versus 9mL. Figure 3 illustrates the effects of desiccation and saline, which were observed in speaking PPE ratings.

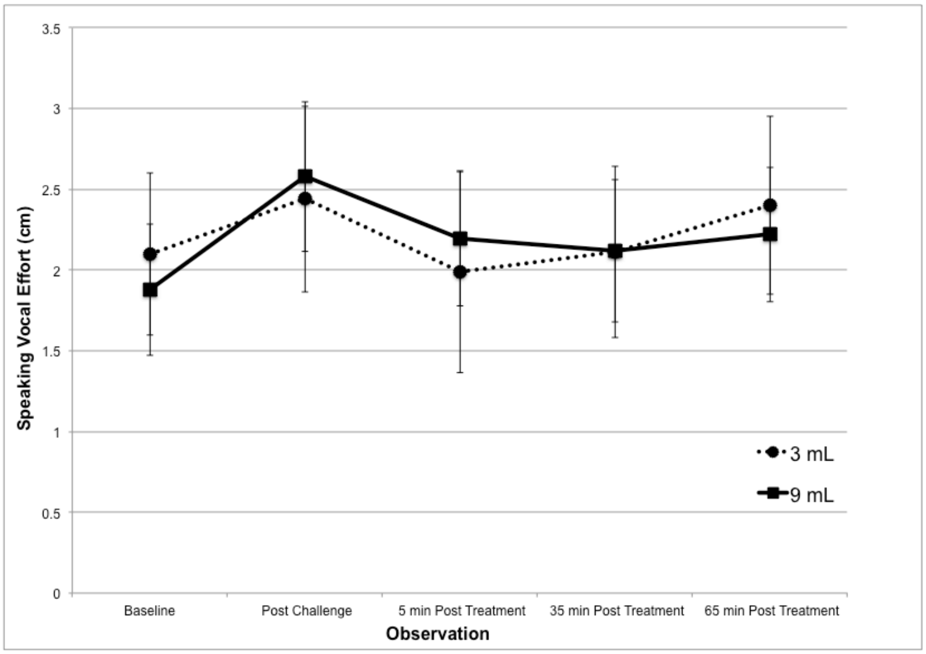

Figure 3. Means and standard errors for PPE ratings for speaking for 3mL and 9mL dosages at baseline and subsequent observations.

PPE for singing

For singing PPE, the main effect of time was statistically significant based on the Friedman Test for related samples, $\chi^2(4) = 9.856$, $p < .043$. Post hoc comparisons were made using a Bonferroni correction and an adjusted alpha level of 0.0125. A desiccation effect between baseline and post desiccation observations approached statistical significance, based on a Wilcoxon Signed Ranks Test, $Z = -2.335$, $p < .020$. A treatment effect at 5 min post nebulization was statistically significant, $Z = -2.877$, $p < .004$. No other significant effects for time were observed. No statistically significant differences were observed for 3mL versus 9mL.

Figure 4 illustrates the effects of desiccation and saline, which were observed in singing PPE ratings.

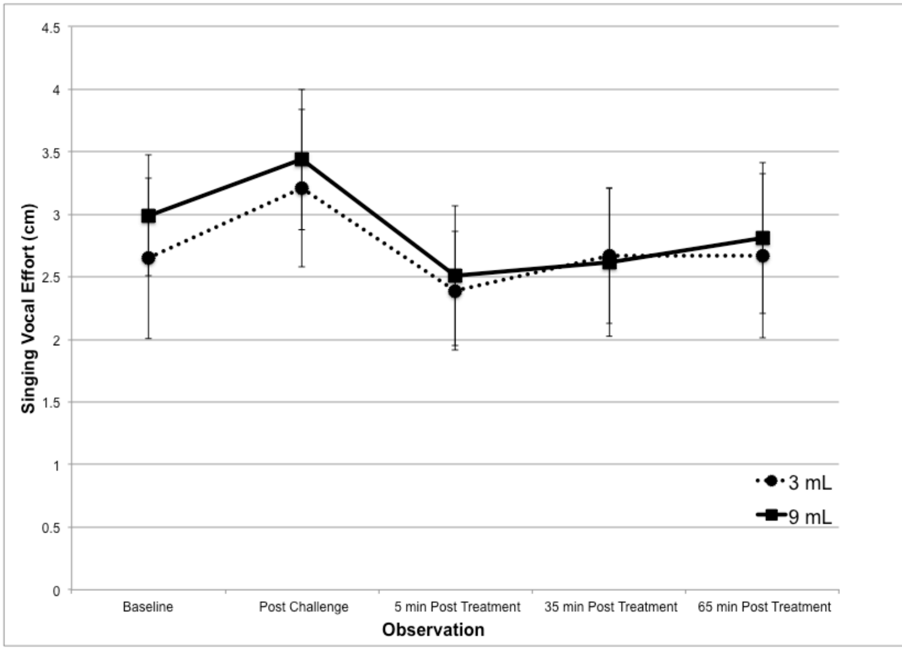

Figure 4. Means and standard errors for PPE ratings for singing for 3mL and 9mL dosages at baseline and subsequent observations.

Throat dryness

For throat dryness, the main effect of time was statistically significant based on the Friedman Test for related samples, $\chi^2(4) = 23.117$, $p < .001$. Post hoc comparisons were pursued using a Bonferroni correction and an adjusted alpha level of 0.0125. A statistically significant desiccation effect was observed between baseline and post desiccation observations based on a Wilcoxon Signed Ranks Test, $Z = -3.549$, $p < .001$. Statistically significant treatment effects were observed at 5 min ($Z = -3.268$, p < .001), 35 min ($Z = -2.940$, $p < .003$), and 65 min ($Z = -2.536$, $p < .011$). No statistically significant differences were observed for 3mL versus 9mL. The effects of desiccation and saline treatment on throat dryness are illustrated in Figure 5.

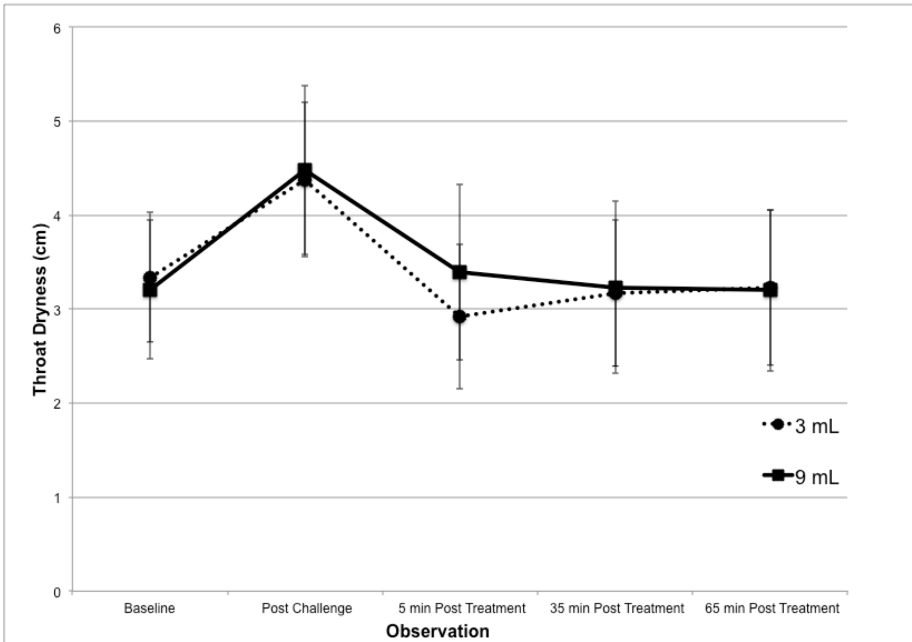

Figure 5. Means and standard errors for perceived throat dryness ratings for 3mL and 9mL dosages at baseline and subsequent observations.

Mouth dryness

For mouth dryness, the main effect of time was statistically significant based on the Friedman Test for related samples, $\chi^2(4) = 15.501$, $p < .004$. Post hoc comparisons were pursued using a Bonferroni correction and an adjusted alpha level of 0.0125. A statistically significant desiccation effect was observed between baseline and post desiccation observations based on a Wilcoxon Signed Ranks Test, $Z = -2.810$, $p < .005$. Treatment effects at 5 min approached statistical significance, $Z = -1.955$, $p < .051$. No other statistically significant treatment effects were observed. No statistically significant differences were observed for 3mL versus 9mL. The effects of desiccation and saline treatment on mouth dryness are illustrated in Figure 6.

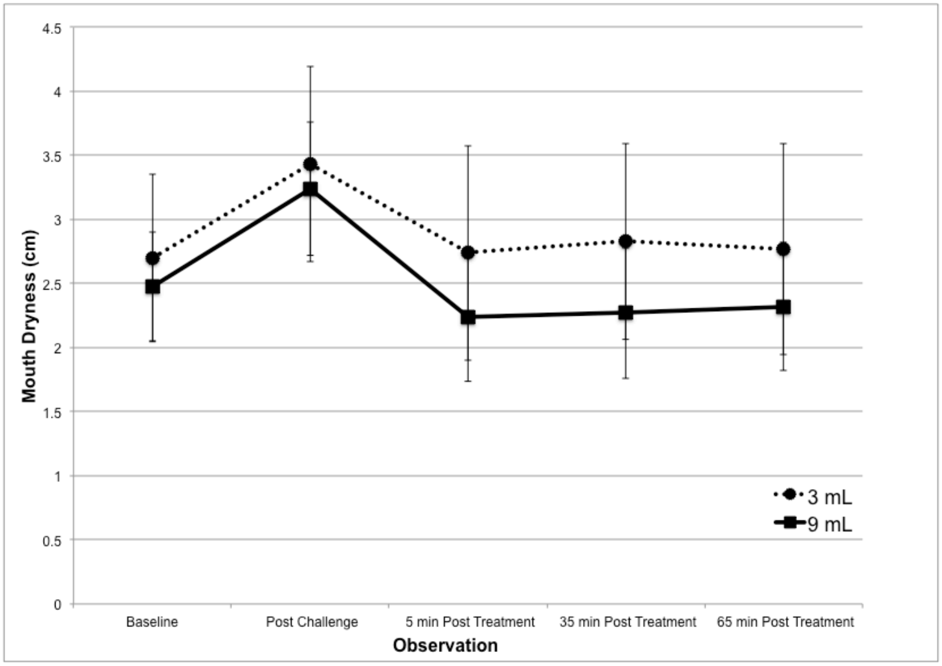

Figure 6. Means and standard errors for perceived mouth dryness ratings for 3mL and 9mL dosages at baseline and subsequent observations.

Discussion

This study sought to determine the effects of a laryngeal desiccation challenge and subsequent nebulized hydration treatments in healthy male singers. Previously studies involving female nonsingers found that desiccation increased PTP and VAS ratings for PPE, throat dryness, and mouth dryness (Tanner et al., 2007; 2013). However, these findings differed for trained female singers (Tanner et al., 2010). Specifically, PPE increased significantly following laryngeal desiccation but PTP did not. This was perhaps related to singers being better able to compensate for the effects of desiccation, despite the fact that they were perceptually sensitive to dehydration changes in the larynx. In the present study, it was hypothesized that similar differences may exist in male singers. It was unknown, however, how the anatomical and physiologic differences between the male and female larynx might ultimately influence the study outcome. Previous studies have found that nebulized isotonic saline has shown promise as an effective method of hydrating the larynx (Tanner et al., 2007). PTP has been somewhat responsive to isotonic saline in females, and PPE has decreased significantly after saline treatments, particularly when compared with control groups who have received no treatment. While it has been demonstrated that nebulized isotonic saline may potentially be beneficial as a laryngeal lubricant, there is little literature pertaining to what the most efficacious dosages of saline might be. In order to determine if dosage had an effect on treatment results, this study examined the effects of both a 3 mL and a 9 mL dosage of nebulized isotonic saline over a 65-minute period after laryngeal desiccation.

In the present study, no statistically significant changes in PTP were observed after the desiccation challenge. However, PTP at 80% F_0 did increase by 0.43 cmH_20 post desiccation. Generally, this negative finding would support the findings of Tanner et al. (2010) that trained singers might respond differently than nonsingers to the laryngeal desiccation challenge. It would also confirm that the male larynx might be more resilient to desiccation and the effects of mouth breathing. The absence of a statistically significant desiccation effect is particularly striking given that the challenge was twice the duration of those in previous studies involving females (Tanner et al., 2007; 2010; 2013). PTP at 80% F_0 returned to baseline values after nebulized saline treatments, but these findings were also not statistically significant. Given the lack of statistical significance, caution is warranted when interpreting these findings. It is valuable to note, however, that these short-lived reductions in PTP with nebulized isotonic saline are similar to other studies involving nebulized saline to treat laryngeal dehydration (Tanner et al., 2007; 2010; 2013).

In general, PTP measurements taken at 80% F_0 were more varied than those observed at 10% F_0, although there were no statistically significant differences. The differences between 80% and 10% F_0 were similar for both 3 mL and 9 mL. This finding is consistent with previous research indicating that PTP at higher F_0 seems to be more sensitive to changes in laryngeal dehydration (Roy et al., 2003). High F_0 is routinely used when evaluating PTP in clinical and research populations (Plexico, Sandage, & Faver, 2011). It is possible that the PTP task at 10% F_0 is particularly insensitive to hydration changes in vocally trained males due in part to the ease with which the task was performed. Similarly, it is also possible that higher F_0 taxes the vocal mechanism such that laryngeal dehydration changes are more easily detected. Perhaps one reason for the lack of significant PTP change is the use of falsetto phonation in performing the high F_0 PTP task. Falsetto phonation is known to involve changes in vocal fold vibratory features (Echternach, Dippold, Sundberg, Arndt, Zander, & Richter, 2010), perhaps resulting in differences in PTP production. Regardless, the results from this investigation indicate that future studies should continue to include high F_0 in examining PTP.

Despite the minimal changes in PTP observed in the present investigation, changes in VAS ratings for PPE during speaking, PPE during singing, throat dryness, and mouth dryness did occur. A statistically significant increase in PPE during speaking was observed immediately following desiccation. This finding was reversed after nebulization for both 3 mL and 9 mL dosages of isotonic saline. For 3 mL, PPE returned to baseline and then rose again at the 35 and 65-minute post nebulization observations. For the 9 mL dosage, PPE approached baseline, decreasing significantly after nebulization; this effect remained through the 35 and 65-minute markers. Effects were similar for VAS ratings of PPE in singing, but were greater in magnitude for the singing versus speaking tasks. This may be because singing is a more complex and demanding task than conversational speech. It is possible that the effects of saline and desiccation were particularly evident when the system was taxed. It could be that taxing the voice in other ways, such as more difficult or longer periods of singing, would make these results more evident. VAS ratings for throat and mouth dryness also followed this basic pattern. Both throat and mouth dryness rose significantly after desiccation and fell after treatment. No significant differences between dosages were observed. This may mean that additional treatment after 3 mL does not have any additional benefit for the male larynx. The changes in throat and mouth dryness are similar to recent research involving nebulized treatments in clinical populations (Tanner et al., 2013). Changes in self-perceived throat dryness were not unexpected, given that this was the primary outcome variable being manipulated in this study. However, it is somewhat interesting that mouth dryness changes were so similar to throat dryness changes. Perhaps a significant amount of the nebulized particles remain in the oropharynx after treatment, thus increasing perceived mouth hydration.

Despite the fact that no significant changes were observed in PTP, these VAS findings are valuable clinically. Desiccation of the larynx caused a perceived increase in PPE in singers who were trained to use their voices efficiently. It is likely that if it was perceived that phonation became more effortful following desiccation, that these individuals would be at increased risk for excess tensions or fatiguing over time. Individuals who suffer from lack of laryngeal hydration would not likely follow through with a regimen that they did not perceive was helping the problem. As has been shown in previous studies, these VAS ratings would indicate that nebulized isotonic saline showed promise as a method of decreasing PPE, throat dryness, and mouth dryness, as participants did perceive that treatment helped (Tanner et al., 2010; 2013).

No significant differences were observed between the 3 mL and the 9 mL dosages of saline. There are, however, other potential variables that should be explored in the future. The concentration level of the isotonic saline, for example, may influence the effectiveness of treatment. Isotonic saline has been shown to be more effective than sterilized water or several other popular osmotic agents at hydrating the larynx (Tanner et. al, 2007), but it is unknown what role the concentration level plays. It is therefore possible that transepithelial water flux across the vocal fold epithelia could change if the concentration level of the hypertonic agent were changed. While the mechanics of this phenomenon are largely unknown, it could be that the hyperosmolarity of the liquid on the laryngeal epithelia would result in increased transepithelial water fluxes that improve vocal fold surface fluid properties. This could in turn result in increased airway and laryngeal hydration, improved mucociliary clearance (Reeves & McElvaney, 2012) and increased laryngeal and vocal fold health.

These findings also reinforce the idea that PTP and PPE may not be strongly correlated measures. Previous studies have also found that PTP and PPE ratings do not seem to be related (Tanner et al., 2007; Verdolini et al., 1994). There have been several suggested explanations for this lack of correlation. Verdolini et al. (1994) suggested that it may be due to the fact that participant and examiner biases often exist during PTP collection. Tanner at al. (2010) suggested that PTP may not be sensitive to the sensory effects of throat and mouth dryness, while PPE, which is a psychological measure, is more sensitive to these changes. McHenry, Evans, and Powitzky (2013) also found that PTP and PPE were not correlated in singers regardless of amount of vocal training or gender. They proposed that this could be due in part to differences in terminology between researchers and singers. Additionally, it has been suggested that there may be a practice effect as participants get more proficient at performing the PTP task.

One of the issues with the PTP task is the possibility of a practice effect. That is, it might be possible to train participants to perform the syllable repetitions such that increased task familiarity results in an improvement in production. In this study, PTP did not decrease with time during experimental sessions, suggesting that no practice effect occurred within sessions.

Similarly, if there were a practice effect between sessions, one would expect to see PTP improve between baseline measurements of session one and two. Current findings would argue that there was no practice effect between sessions as baseline PTP measures were not significantly different between sessions. Therefore some confidence exists in the double-blind, within-subjects experimental design employed in this study. Future studies should consider minimizing the effects of PTP variability and examiner bias by incorporating a double-blind, within-subjects design. Within-subjects designs have historically been useful in detecting laryngeal dehydration changes (Sivasankar & Fisher, 2002; 2003).

There are several potential limitations to the current study which warrant mention. First, the sample included in this study was a small group of young, vocally healthy, singers. It may be that age, vocal health, and/or vocal training influence susceptibility to laryngeal dehydration. Second, there was no control or comparison group and no placebo treatment or condition. VAS ratings may have been influenced by participants' expectation that treatment would improve vocal hydration and decrease vocal effort. Examiners did their best to prevent bias during experimental procedures, but it is possible that a bias did exist. Lastly, ambient humidity levels fluctuated slightly between sessions. Although humidity levels were recorded, there was no independent control of ambient humidity. It is possible that changes in humidity may have influenced susceptibility to the desiccation challenge and subsequent treatments. It is unlikely, however, that these small fluctuations in humidity would have had a significant effect on the general trends in PTP which were observed. Future studies may include high-speed imaging of the vocal folds or acoustic analysis of speaking tasks to see if there were differences post-desiccation, which were not seen in PTP but may still exist. Additional studies should include a more challenging or longer singing task, in order to determine if singers are more sensitive to laryngeal hydration when they are more challenged vocally. Despite its limitations, this is the first study to examine laryngeal desiccation and subsequent nebulized treatments in healthy male singers. It will serve as a foundation for future research in this area.

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Appendix A

Experimental Protocol

Participant Code_________
Session #________
Date _________
Examiner _________
%RH Pre _________
%RH Post _________

REMINDER CALL: No Upper respiratory Symptoms ____
No eating/drinking ____

PRE-SESSION 1 CHECKLIST:
1. Airflow Equipment on and calibrated.
2. Obtain nebulized treatment. ____
3. Consent Document signed. ____
4. Participant List completed ____
5. Mask and nose clip sterilized. ____
6. Videostroboscopy equipment ready. ____
7. Recording equipment ready. ____
8. Video Recording (Rainbow passage & ____
sustained /a/ vowel > 5 sec).
9. Stroboscopy ____

PITCH PROTOCOL: (exclude glottal fry)
1. Glide to the highest note able to sustain for 3 sec. on /i/, then soft. ____ Hz
2. Glide to the lowest note able to sustain for 3 sec. on /i/, then soft. ____ Hz
3. Calculate pitch range in Hz, divide by 5, subtract from highest Hz. ____ Hz
Round up to find note at 80% pitch level.
4. Calculate 10% pitch level by dividing by 10, find the first 10th.
5. Model and practice /pi pi pi pi pi/ "just above a whisper" [SPOKEN]
(<5 practice trials) at the 80% pitch level. (Avoid pitch drop-offs, avoid complete whisper and be sure syllables are connected, on 1 breath, x 5 reps). (3.5 syllables/sec) METRONOME 180.

____
6. Read the following instructions to the participant:

PTP
"Now you will please repeat the /pi pi pi pi pi/ syllables into this mask. In just a minute, you will place your face into the mask with the plastic tube just inside your lips. Be sure to swallow before you put the tube in your mouth, and try not to allow saliva to drain into the tube. You can see a hole in the tube. Try not to press on the hole with your tongue or cheek.

"Please repeat /pi pi pi pi pi/ in 5-syllable strings, 3 times each, like this. (Demo, breathe in between 3 productions of 5 syllables each). Remember to do this as softly as possible, but still using your voice, at the pitch we just practiced. Just speak the syllables; don't sing them. Let's try it." (Be sure the participant is at the same pitch, prompt higher or lower if necessary).

Let them practice (<5 times). Be sure there are no leaks around the mask.

When they're ready, sample. You can always stop and restart, and don't save until you have 3 sets of 5 strings at approximately even peaks.

Participants should not be talking after the first observation. They may clear their throat or vocalize briefly prior to PTP repetitions & VAS tasks.

NO eating or drinking during the session.

<u>Vocal Effort</u>
Record "The Rainbow Passage" & sustained /a/ vowel > 5 seconds.

Rate **<u>Vocal Effort</u>** and **<u>Mouth and Throat Dryness</u>** using paper rating forms.

Record % Relative Humidity ____

<u>Observation 1:</u> **<u>Baseline</u>**

- PTP with nose clip (save data) ____
- <u>Record Passage & /a/ vowel (>5sec)</u> ____
- Vocal Effort rating (from Rainbow Passage) ____
- Mouth and Throat Dryness rating ____
- *Singing Task ____
- *Vocal effort rating (from singing) ____

Breathe medical grade dry air (Oxygen mask, nose plugged) for 30 min ____

<u>Observation 2:</u> **<u>Immediately following dry air:</u>**

- PTP with nose clip (save data) ____
- <u>Record Passage & /a/ vowel (>5sec)</u> ____
- Vocal Effort rating (from Rainbow Passage) ____
- Mouth and Throat Dryness rating ____
- *Singing Task ____
- *Vocal effort rating (from singing) ____

Nebulization substance (10 min) ____

Start timer immediately following Nebulization ____

Observation 3: **5 minutes after Nebulization:**

PTP with nose clip (save data) ____
Record Passage & /a/ vowel (>5sec) ____
Vocal Effort rating (from Rainbow Passage) ____
Mouth and Throat Dryness rating ____
*Singing Task ____
*Vocal effort rating (from singing) ____

Observation 4: **35 minutes after Nebulization:**

PTP with nose clip (save data) ____
Record Passage & /a/ vowel (>5sec) ____
Vocal Effort rating (from Rainbow Passage) ____
Mouth and Throat Dryness rating ____
Singing Task ____
Vocal effort rating (from singing) ____

Observation 5: **65 minutes after Nebulization:**

PTP with nose clip (save data) ____
Record Passage & /a/ vowel (>5sec) ____
Vocal Effort rating (from Rainbow Passage) ____
Mouth and Throat Dryness rating ____
*Singing Task ____
*Vocal effort rating (from singing) ____

Record % Relative Humidity ____

Confirm Session 2 (session 1 only) ____

Appendix B

Annotated Bibliography

Fisher, K. V., Ligon, J., Sobecks, J. L., & Roxe, D. M. (2001). Phonatory effects of body fluid removal. *Journal of Speech, Language and Hearing Research, 44,* 354-367. doi: 10.1044/1092-4388(2001/029)

Purpose of the study:

This study examined whether body fluid reduction, in the absence of dehydration, increases PTP, causes additional perceived vocal effort and worse vocal quality.

Method:

A single subject design was used to examine six adults with advanced renal disease. Each subject had a measured volume of body fluid removed. Vocal quality, perceived effort, PTP, heart rate and blood pressure were longitudinally examined. Two control subjects were included to rule out confounding variables.

Results:

In four of the six subjects participants, PTP was seen to increase with fluid volume reduction and then returned to its initial level when fluids were replaced. Heart rate and blood pressure were correlated with PTP for both treatments and the placebo.

Conclusions:

This study documented significant extracellular volume depletion in the absence of body dehydration caused voice symptoms. It is possible that this effect was under autonomic nervous control. The researchers suggested that the water flux of the vocal folds is likely controlled by a mechanism within the folds themselves.

Hemler, R. J. B., 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. jvoice.org

Purpose of the study:

This study sought to demonstrate that the relative humidity of the environmental air produces deleterious effects on vocal functioning.

Method:

Eight participants were asked to inhale three different types of air (dry, normal and humid) before producing a sustained /a/ vowel at a specific pitch and loudness. This was then examined for noise-to-harmonic and perturbation parameters.

Results:

It was found that perturbation measures were virtually identical between humid and normal air but perturbation increased in dry air conditions. There were no significant findings relating to noise-to-harmonic parameters between the three air types.

Conclusions:

It was concluded that the human voice is affected by decreases in relative humidity. This study showed that perturbation increased in participants even after short exposure to dry air. This study laid the groundwork for the laryngeal desiccation challenge in the present study.

Hunter, E. J., Tanner, K., & Smith, M. E. (2011). Gender differences affecting vocal health of women in vocally demanding careers. *Logopedics Phoniatrics Vocology,* 2011; Early Online, 1–9. doi: 10.3109/14015439.2011.587447

Purpose of the study:

This article serves as a survey of current literature regarding the anatomical differences between the vocal apparatuses of men and women. These differences are examined to review why women are more prone to suffer from vocal disorders.

Summary:

Changes caused by puberty make the male vocal folds longer and thicker than in females. The larynx also becomes larger and the thyroid angle more prominent in males. These anatomical differences cause males to have a lower fundamental frequency than females. These differences also cause the male vocal folds to oscillate less frequently than the female vocal folds. Endocrine differences have also been observed between the male and female vocal apparatus. Hormones excreted during menses have been found to increase vocal fatiguing, lower levels of vocal endurance, decrease vocal range and other vocal problems. Menopause has also been shown to have negative effects on the female vocal apparatus. There are also differences between the male and female respiratory systems. The female system requires a higher percentage of air volume in order to function. The male vocal folds also contain more hyaluronic acid, which has been shown to promote healing. There are also possible factors involving the nervous system, digestive system, and whole body hydration as well as other possible psychological factors which may play a role.

Conclusions:

Women have been shown to suffer more frequently from vocal health issues than men. This may be related to differences in laryngeal anatomy and physiology, hormone differences, general anatomical characteristics and other non-physiological characteristics. The voice would appear to be closely connected to the physiology and anatomy of much of the body. Further investigation into the differences between the male and female vocal apparatus and the risk factors for vocal maladies in women is needed.

Kitch, J. & Oates, J. (1994). The perceptual features of vocal fold fatigue as self-reported by a group of actors and singers. *Journal of Voice*, *8*, No. 3, 207-214. jvoice.org

Purpose of the study:

This study sought to determine how vocal fatigue is perceived in actors and singers. Singers and actors were asked to fill out a questionnaire detailing the vocal changes they perceived when vocally fatigued.

Summary:

Ten singers and ten actors completed a survey detailing the vocal changes they perceived when vocally fatigued. Both groups reported similar frequency patterns and perceived symptoms of vocal fatigue. Actors reported being most vocally effected in "power" features of the voice (i.e., projection), while singers reported being most effected in vocal dynamic range (i.e., pitch range) when fatigued vocally. The reported causes of vocal fatigue included high vocal demands (particularly high pitched and increased in loudness), overuse and feeling "run down".

Conclusions:

This study would suggest that actors (and possibly untrained speakers) tend to feel the effects of vocal fatigue in a loss of vocal power (i.e., reduced loudness or volume), while singers tend to feel a loss in their dynamic range (i.e., pitch range). Further research is needed in order to fully understand the causes of vocal fatigue and its effects on professional voice users and normal speakers.

Leydon, C., Sivasankar, M., Falciglia, D. L., Atkins, C., & Fisher, K. V. (2009). Vocal fold surface hydration: A review. *Journal of Voice, 23*, 658-665. doi: 10.1016/j.jvoice.2008.03.010

Purpose of the study:

This study suggests that clinical practices, which are aimed at facilitating vocal fold epithelial ion and fluid transport, benefit healthy voice users as well as those with vocal disorders or risk of vocal disorders.

Summary:

Surface hydration of the vocal folds is partially sustained by salt and water fluxes across the epithelium. A transcellular pathway is proposed to be one method of hydrating the vocal folds. This pathway consists of sodium–potassium pump, sodium–potassium–chloride cotransporter, epithelial sodium channels, cystic fibrosis transmembrane regulator chloride channels, and aquaporin water channels. In vitro and in vivo studies involving hydration challenges and the current understanding of ion fluid transport explain how clinical protocol and environmental and behavioral challenges may affect vocal surface hydration. The paper also provides an overview description of the in vitro and in vivo studies performed on surface hydration.

Conclusions:

Clinical practices aimed at facilitating vocal fold epithelial ion and fluid transport benefit healthy voice users as well as those with vocal disorders or who are at risk of vocal disorders. Individuals suffering from systemic and/or superficial dehydration may experience particular benefit from therapeutic hydration treatments. While there may be other sources of hydration, functional ion and water transport proteins found in vocal fold epithelial cells would suggest that these cells may play a part in maintaining vocal fold hydration. It is possible that vocal fold hydration is also managed by laryngeal gland secretion, paracellular ion-coupled water fluxes, and mucociliary clearance. Further study is required to better understand the mechanisms that maintain laryngeal hydration.

McHenry, M., Evans, J. & Powitzky, E. (2013). Singers' phonation threshold pressure and ratings of self-perceived effort on vocal tasks. *Journal of Voice*, *27*, No. 3, 295-298. http://dx.doi.org/10.1016/j.jvoice.2012.12.013

Purpose of the study:

This study examined the relationship between perceived vocal effort and PTP in singers.

Method:

Forty-eight trained singers produced /pi/ syllable strings to evaluate PTP. Following this, each singer performed four vocal tasks including a staccato "ah" in chest and head voice and singing the song "Happy Birthday" in head voice. The singers then recorded their perceived vocal effort for each task.

Results:

No correlation was found between perceived effort and PTP in these subjects. The data were examined in several ways taking into account gender, amount of voice training, and other possible covariates; no significant correlations were found.

Conclusions:

Perceived effort ratings do not appear to be a good predictor of PTP. This could be due to the differences in terminology or concepts used by singers versus speech pathologists or researchers. It may be that self-perceived vocal effort could be used to predict vocal health. More research is needed regarding the relationship between PTP and perceived vocal effort.

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. doi: 10.1067/S0892-1997(03)00078-X

Purpose of the study:

This study was designed to determine whether three different nebulized lubricants would lower PTP in 18 healthy females.

Method:

Eighteen healthy females ranging from twenty to thirty five years of age participated. Participants reported no history of vocal problems. Participants were tested three times over a three-week period. Each time participants breathed two milliliters of either sterile water, 12.5% Mannitol, or Entertainer's Secret Throat Relief. PTP was measured twice before nebulization and four times after nebulization.

Results:

To ascertain if any of the nebulized substances improved PTP, a within-subjects, repeated measures design was utilized. A Wilcoxon Signed Ranks test showed that Mannitol was the only substance which reduced PTP after it was administered. However, these effects were marginally statistically insignificant (p<.071). These results were seen five minutes after nebulization but were not observed twenty minutes after, suggesting that the effects wore off in less than twenty minutes.

Conclusions:

Nebulized sterile water and Entertainer's Secret Throat Relief produced no effect on PTP in vocally healthy females. Mannitol slightly lowered PTP immediately after it was administered. It is possible that Mannitol slows water absorption, which in turn leaves more water in the bronchiotracheal mucus. It is not clear, however, how Mannitol lowered PTP. It is also unclear whether the effect of Mannitol on PTP will prove to be clinically significant since the difference only approached statistical significance. Further investigation of the phenomenon is warranted.

Sivasankar, M., & Fisher, K. V. (2002). Oral breathing increases PTH and vocal effort by superficial drying of vocal fold mucosa. *Journal of Voice*, *16*, 172–181. doi: 10.1016/S0892-1997(02)00087-5

Purpose of the study:

This study sought to determine the effects of short-term nasal and oral breathing on perceived vocal effort and PTP.

Method:

Twenty vocally healthy female subjects participated in this study. They were divided into two groups – one group for oral breathing and one for nasal breathing. Each subject performed a /pi/ task (/pi/ in strings of five) and sang Happy Birthday at various pitch and loudness levels. PTP and perceived effort were calculated after these measures. After a pretreatment sample, participants either breathed orally or nasally for fifteen minutes. Then the tasks were performed again and measurements were taken a second time.

Results:

It was found that PTP decreased for those subjects who breathed nasally between measures and that perceived effort decreased in 7 of 10 participants. Oral breathing was found to increase PTP at most pitches and increased perceived effort in 6 of 10 subjects.

Conclusions:

It was concluded that, for vocally healthy subjects, oral breathing put speakers at increased risk for the symptoms of abnormally high vocal effort. The superficial hydration brought about by nasal breathing seemed to prevent laryngeal dryness.

Sivasankar, M., & Fisher, K. V. (2003). Oral breathing challenge in participants with vocal attrition. *Journal of Speech, Language, and Hearing Research*, *46*, 1416-1427. doi: 10.1044/1092-4388(2003/110)

Purpose of the study:

This study sought to determine if female students with a history of temporary vocal fatigue and minor dryness or discomfort suffered increased vocal attrition due to oral breathing.

Method:

Eighteen females with healthy voices and eighteen females with symptoms typical of vocal attrition participated in this study. Participants then took part in either a nasal or oral breathing challenge for periods of fifteen minutes.

Results:

Oral breathing was found to increase PTP in both populations examined, but more significantly in those with symptoms of vocal attrition. This effect was seen consistently in those with vocal attrition. Nasal breathing lowered PTP in all healthy participants and in many participants with vocal attrition, but not all.

Conclusions:

It was speculated that oral breathing puts people at greater risk for vocal attrition. Also, it is thought that those already suffering from vocal attrition are less able to compensate for superficial laryngeal dryness. Further research is need in order to understand exactly why this is and if this can be effectively treated.

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

Purpose of the study:

This study investigated whether vocal fold epithelium would create a water flux in order to lessen an osmotic challenge on the lumen. Vocal folds were subjected to luminal osmotic perturbations and bidirectional transepithelial water fluxes were analyzed.

Method:

Isosmotic or luminal hyperosmotic perturbations were examined in thirty-six ovine vocal folds. At baseline and thirty minutes after the challenge, vocal fold viability and water fluxes in the direction of the lumen and into the mucosa were analyzed.

Results:

Throughout the osmotic perturbation vocal fold electrophysiological viability was preserved. During this exposure, water fluxes bound in the direction of the lumina increased in sixty percent of the vocal folds examined. This increase was short in nature and did not negate the osmotic gradient.

Conclusions:

Perturbations to the luminal surface were recognized by ovine vocal fold epithelia. Homeostatic regulation of vocal fold surface liquid during osmotic perturbations is possibly affected by the ability to detect surface composition changes.

Sivasankar, P., & Erickson-Levendoski, E. (2012). Influence of obligatory mouth breathing, during realistic activities, on voice measures. *Journal of Voice*, *26*, 813.e9-813.e13. doi:10.1016/j.jvoice.2012.03.007

Purpose of the study:

This study sought to examine whether obligatory mouth breathing during everyday tasks would effect superficial vocal fold hydration in a manner that would change PTP and perceived vocal effort.

Method:

Sixty-three vocally healthy adults took part in this study. Subjects were divided into two groups, those who reported having experienced vocal fatigue after heavy voice use and those who reported no such symptoms (31 with symptoms and 33 without). Subjects participated in a fifteen minute exercise task and a fifteen minute loud reading task in either low or high humidity conditions. PTP and self-perceived vocal effort were measured before and after each task.

Results:

Forced mouth breathing increased PTP significantly during a loud reading exercise. This effect was seen in both high and low humidity conditions for both participant groups. No statistically significant changes were observed in perceived vocal effort.

Conclusions:

This study would suggest that mouth breathing has a negative effect on PTP. Further research is needed with a wider range of tasks, longer tasks and a more varied participant pool in order for this effect to be better understood.

Sivasankar, M., Erickson, E., Scheider, S., & Hawes, A. (2008). Phonatory effects of airway dehydration: Preliminary evidence for impaired compensation to oral breathing in individuals with a history of vocal fatigue. *Journal of Speech, Language, and Hearing Research*, *51*, 1494-1506. doi: 10.1044/1092-4388(2008/07-0181)

Purpose of the study:

This study examined the effects of short-term mouth breathing on subjects with a history of vocal fatigue and on subjects with no symptoms. These effects were observed in environments with low, moderate, and high humidity.

Method:

Eight females with a history of vocal fatigue and eight females with no history of vocal problems participated in three sessions. Both a nasal breathing challenge and an oral breathing challenge were administered. PTP and perceived vocal effort were measured pre and post challenges.

Results:

In the high humidity condition, no significant changes were observed after oral breathing in either participant group. In the low and moderate humidity conditions, participants with a history of vocal fatigue experienced a greater increase in PTP than their vocally healthy counterparts. Self-perceived effort and PTP were not strongly correlated.

Conclusions:

This study would indicate that individuals with a history of vocal fatigue may be more susceptible to the dehydrating effects of mouth breathing than those with no history of fatigue. This may be because these individuals are less capable of compensating for the airway dehydration induced by oral breathing. Further research is needed in order to fully explain this effect.

Smitheran, J. R., & Hixon, T. J. (1981). A clinical method for estimating laryngeal airway resistance during vowel production. *Journal of Speech and Hearing Disorders*, *46*, 138–146. doi: 10.1044/jshd.4602.138

Purpose of the study:

This publication detailed a non-invasive method to estimate laryngeal airway resistance.

Method:

Fifteen males participated in this study. Participants were asked to produce the sound /pi/ repeatedly at a rate of 1.5 syllables each second. Correct production was modeled for the subjects and they were asked to repeat this measure until three correct strands of seven /pi/ utterances were produced. Laryngeal airway resistance was then computed by dividing the translaryngeal pressure by the translaryngeal flow.

Results:

The criterion scores of the string of /pi/ utterances were found to be reliable estimates of resistance.

Conclusions:

The proposed method of measuring laryngeal airway resistance was found to be reliable and accurate when compared with more invasive measurement methods. Further research is needed to determine the effectiveness of this method on disordered voices, but the results in normal speakers were promising.

Solomon, N. P., & DiMattia, M. S. (2000). Effects of a vocally fatiguing task and systemic hydration on phonation threshold pressure. *Journal of Voice*, *14*, 341–362. doi: 10.1016/S0892-1997(00)80080-6

Purpose of the study:

This study examined whether PTP in four female voice users was affected by a two-hour reading task at above average volume. The study also examined the effects of systemic hydration (drinking) and small periods of vocal rest on this phenomenon.

Method:

Four women with untrained voices participated in four sessions in which they were asked to read for two hours at an above average volume and at varying pitches (conversational pitch, 10%, 50% and 80% F_0). Several measures were taken to ensure that conditions were similar for all four participants. Diet, time awake before the session, and time from participants' menstrual cycle were all considered.

Results:

PTP increased after the reading task at all pitches, but particularly at the 80%. Systemic hydration (drinking water) slowed this fatiguing effect in three of the four subjects. It was also observed that fifteen minutes of vocal rest slowed this effect as well.

Conclusions:

Although further research is needed, these findings would indicate that increased intake of water will delay fatiguing in untrained speakers after long periods of loud phonation.

Solomon, N. P., Glaze, L. E., Arnold, R. R., & van Mersbergen, M. (2003). Effects of a vocally fatiguing task and systemic hydration on men's voices. *Journal of Voice*, *17,* 31–46. doi:10.1016/S0892-1997(03)00029-8

Purpose of the study:

This study examined whether the same patterns of vocal fatigue previously seen in women during a reading task would be observed in men under the same conditions.

Method:

Four male, untrained voice users participated in five sessions each where they were subjected to normal hydration conditions and high and low hydrated conditions. Participants read at normal pitch and loudness from Harry Potter for ten minutes to warm up. They then read for a one-hour period and a two-hour period at loud volume with limited liquids.

Results:

The four subjects in the study demonstrated increased PTP levels after speaking loudly for an hour. This effect was magnified after two hours of speaking loudly. High pitches made the changes in PTP greater.

Conclusions:

Past literature has supported the idea that the male vocal folds are more protected from fatiguing due to hyaluronic acid than female folds. It could therefore by hypothesized that he male system would take longer to fatigue than the female system. The findings of this study contradict this idea due to the fact that men were seen to fatigue similarly to women. Further research is needed to learn what causes these results to differ from what might be expected.

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

Purpose of the study:

This study sought to investigate the results of nebulized hypertonic saline, isotonic saline and sterile water on PTP and perceived vocal effort following a dehydration challenge.

Method:

Sixty vocally healthy women participated in this double-blind study. Subjects were randomly assigned to one of four groups. After a desiccation challenge, participants were administered hypertonic saline, isotonic saline, sterile water or no treatment. PTP and perceived vocal effort ratings were taken at baseline, immediately following desiccation, 5, 20, 35, and 50 minutes after desiccation.

Results:

PTP was elevated for all subjects after a desiccation challenge. None of the administered treatments seemed to reverse this effect and return PTP to baseline. Self-perceived vocal effort unpredictably declined following the desiccation challenge. PTP and perceived vocal effort did not seem to be correlated.

Conclusions:

These results indicated that a desiccation challenge raised PTP in healthy female participants. None of the treatments administered were effective in significantly reversing this effect and PTP measures did not seem to be correlated with perceived effort rating. Further research is needed in order to evaluate if the nature of the relationship between PTP and perceived effort is different then generally supposed.

Tanner, K., Roy, N., Merrill, R., Muntz, F., Houtz, D., Sauder, C…Wright-Costa. J. (2010). Nebulized isotonic saline versus water following a laryngeal desiccation challenge in classically trained sopranos. *Journal of Speech, Language and Hearing Research*, *5*, 1555-1566. doi: 10.1044/1092-4388(2010/09-0249)

Purpose of the study:

This study examined the effects of nebulized isotonic saline and sterile water on PTP and perceived vocal effort in classically trained sopranos after a desiccation challenge.

Method:

Thirty-four subjects participated in three sessions where they completed a desiccation challenge followed by one of three treatments. These treatments included either nebulized saline, sterile water or no treatment. PTP and PPE were measured at baseline and every fifteen minutes following the desiccation challenge.

Results:

PPE was elevated after the desiccation challenge for all treatment conditions. The isotonic saline was the only treatment which lowered perceived effort. After the sterilized water treatment and the absence of treatment, perceived effort continued to rise. PTP did not demonstrate any significant changes after treatment or desiccation.

Conclusions:

After a desiccation challenge, classically trained sopranos experienced increases in perceived vocal effort, but little to no change in PTP. Isotonic saline treatment reversed this effect and perceived effort returned to baseline. The other treatments had no effect and perceived effort continued to increase during the observed time frame. Although further research is needed, isotonic saline showed potential as a treatment for perceived vocal dehydration.

Tanner, K., Roy, N., Merrill, R., Kendall, K., Miller, K., Clegg, D…Elstad, M. (2013). Comparing nebulized water versus saline after laryngeal desiccation challenge in Sjogren's syndrome. *The Laryngoscope, 123(11),* 2787-2792. doi: 10.1002/lary.24148

Purpose of the study:

This study sought to determine the effects of laryngeal desiccation on PTP, perceived effort and throat dryness in participants with Sjogren's syndrome. The study also examined two potential nebulized treatments, water and saline solution, and their effect on the same measures.

Method:

Eleven participants with Sjögren's syndrome participated in this study. Each participant took part in a fifteen minute desiccation challenge followed by 3ml of either nebulized water or saline solution. PTP vocal effort, and throat dryness were measured at baseline, immediately following desiccation, and 5, 35, and 65 minutes after treatment. Double blinding was used to remove patient or examiner bias.

Results:

A significant rise in PTP, throat dryness, and vocal effort was observed after the desiccation challenge. Neither water nor saline solution demonstrated a significant treatment effect. Saline produced more of a treatment effect than water, but this effect was still not statistically significant. PTP and throat dryness were more correlated than PTP and vocal effort.

Conclusions:

Sjogren's patients were affected by dry air exposure. Throat dryness, vocal effort, and PTP were all negatively impacted. It is possible that nebulized saline has some worth as a treatment for dry air exposure in this population. More research is needed to determine dosage and to determine if saline is the best possible treatment.

Verdolini-Marston, K., Titze, I. R., & Druker, D. G. (1990). Changes in phonation threshold pressure with induced conditions of hydration. *Journal of Voice*, *4*, 142–151. jvoice.org

Purpose of the study:

This study examined the effects of hydration level on PTP in six adult participants.

Method:

Six adults between the ages of 25 and 46 years, three male and three female were chosen. All six had no history of vocal disorders and several participants had various levels of vocal training. Participants produced consonant-vowel-consonant sequences as quietly as possible at low to high pitches at baseline, hydrated, and dehydrated conditions. Average oral pressure was calculated to estimate minimal subglottal pressures required for phonation.

Results:

Dry conditions produced the highest PTP levels and the lowest PTP levels occurred during wet conditions. The Results were found to be more sensitive at high pitches.

Conclusions:

It was concluded that vocal fold viscosity is closely related to PTP and to vocal effort. This relationship seems to be particularly strong at high pitches. More research is needed regarding this phenomenon.

Verdolini, K., Min, Y., Titze, I. R., Lemke, J., Brown, K., van Mersbergen, M…Fisher, K. (2002). Biological mechanisms underlying voice changes due to dehydration. *Journal of Speech, Language, and Hearing Research*, *45*, 268–281. doi: 10.1044/1092-4388(2002/021)

Purpose of the study:

This study sought to find if systemic dehydration or secretory dehydration play a role in the increases in PTP that occur after dehydration treatments.

Method:

Four vocally healthy adults participated in this study – two male and two female. Each participant attended three sessions where they were administered either diuretic, Lasix™, diphenhydramine hydrochloride, or a placebo. PTP measures were taken following treatments. Both researchers and participants were blinded to avoid any bias.

Results:

The study found that systemic dehydration was induced by Lasix™ and PTP was seen to increase after administration of this drug. There was no sign that diphenhydramine hydrochloride made any difference to either secretory dehydration or PTP.

Conclusions:

This study found that systemic dehydration did cause PTP increases. Further research is needed to know if secretory dehydration affects PTP.

Ward, P. D., Thibeault, S. L., & Gray S. D. (2002). Hyaluronic acid: Its role in voice. *Journal of voice 16*, 303-9. doi:10.1016/S0892-1997(02)00101-7

Purpose of the study:

This article reviewed the functions and biological importance of hyaluronic acid to the human voice. The pharmaceutical applications of hyaluronic acid were also discussed.

Summary:

Hyaluronic acid plays a major role in the process of modulating cellular function and behavior in the vocal apparatus, particularly concerning shock absorption and tissue viscosity. Research indicates that the structure and viscosity of the vocal folds is greatly affected by hyaluronic acid. Hyaluronic acid controls water content, which in turn can change the biomechanical properties of the vocal folds. Vocal fold viscosity is of particular importance to vocal production because it greatly affects the initiation and maintenance of fold oscillation. Greater tissue viscosity results from larger concentrations of hyaluronic acid. Research has shown that in general, the concentration and distribution of hyaluronic acid seems to be gender specific, with men having a higher concentration and more evenly distributed hyaluronic acid than women.

Conclusions:

Hyaluronic acid serves as a shock absorber and a regulator of viscosity. It plays a crucial role in the functioning of the vocal folds. This article suggests that hyaluronic acid may be able to play a future role in the treating voice disorders.

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:

This study's purpose was to ascertain whether mucosal wave frequency and amplitude are affected by vocal fold surface hydration.

Method:

Eight larynges were placed on an excised larynx phonation system and hung on a pseudolung in a temperature and humidity controlled room. Video of the larynges was recorded during exposure to desiccated air (20 cm H_20). Both a control group and a group of dehydrated larynxes was included.

Results:

In most cases, decreased levels of hydration were correlated with decreased amplitude and frequency. In the control group there was a correlation between trial time and both amplitude and frequency. This effect was magnified in the dehydrated larynges.

Conclusions:

Vocal fold surface dehydration was shown to cause a decrease in mucosal wave frequency and amplitude. Examining excised larynges after severe surface dehydration demonstrated this phenomenon in a quantitative manner.

Yiu, E. & Chan, R. (2003). Effect of hydration and vocal rest on the vocal fatigue in amateur karaoke singers. *Journal of Voice*, 17(2), 216-227. doi:10.1016/S0892-1997(03)00038-9

Purpose of the study:

This study sought to determine if vocal fatigue in amateur karaoke singers was reduced by regular hydration (drinking water) and small periods of vocal rest.

Method:

Ten male and ten female amateur karaoke singers participated in this study. Half of the participants were provided drinking water and took regular short breaks to rest their voices, while half the participants sang continuously.

Results:

Singers with water and vocal rest sang longer than their counterparts who sang continuously. The participants who sang continuously experienced increased jitter and decreased pitch range as they sang, while those who drank and rested showed little to no change in acoustic or perceptual measures.

Conclusions:

These results would indicate that regular hydration and vocal breaks slow the onset of vocal fatigue in amateur karaoke singers. Additional information regarding this phenomenon in professional voice users, men versus women and additional research regarding the causes and effects of vocal fatiguing are needed.

Appendix C

Consent to be a Research Subject

Introduction

This research study is being conducted by Kristine Tanner, Ph.D., Arden Hopkin, D.M.A., and Ray M. Merrill, Ph.D. at Brigham Young University, M. Preeti Sivasankar, Ph.D. at Purdue University, and Katherine Kendall, MD, and Mark Elstad, MD at the University of Utah, to determine the effects of throat dryness and saline mist on voice production in males. You were invited to participate because you are a male, between the ages of 18 and 55, with or without singing voice training, with no history of voice problems.

Procedures

If you agree to participate in this research study, the following will occur:

You will participate in 2 experimental sessions lasting approximately 2¾ and 2¼ hours during 2 consecutive weeks (total of 5 hours). You will be asked not to eat, drink, or speak extensively during the session. Both sessions will occur in the John Taylor Building at Brigham Young University.

The researchers will view your vocal folds using video recording system similar in shape to a toothbrush. This procedure is brief and not painful or difficult. The small scope is placed in the back of your mouth but does not go down. The procedure takes approximately 30 seconds. Only your vocal folds will be recorded.

You will participate in pitch glide tasks to obtain your pitch range and target pitches. The researchers will assist you with this process.

You will say “pea pea pea” into a mask with a swimmers’ nose plug, read a paragraph, and if you are a singer, perform a singing task. This will be performed five times during the session. We will record you reading the paragraph.

You will rate your vocal effort and dryness throughout the study.

You will breathe dry air for 30 minutes during both experimental sessions with your nose plugged. Afterward, you will breathe either 3 mL or 9 mL of saline mist for approximately 7-10 minutes, but you will not know which amount you receive on which day until the conclusion of the study.

Risks/Discomforts

There are minimal risks for participating in this study. You may feel uncomfortable during the video of your vocal folds, however this procedure is brief (30 seconds), performed routinely and is not typically reported to be significant or difficult. You may pause or ask questions at any time during this procedure. You may feel that your throat is dry during some portions of the study. You may cough initially when you breathe the saline mist, but this typically subsides quickly (within one minute). The researcher will help you pause from inhaling the saline mist if you cough. The researcher will also ensure you rest your voice during the study to minimize any discomfort associated with the sensation of throat dryness.

Benefits

If you participate in this study, there will be no direct benefits to you. It is hoped, however, that through your participation researchers may learn about the effects of dryness on voice production and the potential of saline mist to offset these effects.

Confidentiality

The research data will be kept in a secure laboratory with restricted access on password-protected computer, and only the researcher will have access to the data. At the conclusion of the study, all identifying information will be removed and the data will be kept in the researcher's locked file cabinet in her private office. All de-identified data will be stored on a password-protected computer.

Compensation

You will receive $40 cash at the conclusion of your participation in the study. No prorating for partial participation is offered.

Participation

Participation in this research study is voluntary. You have the right to withdraw at any time or refuse to participate entirely without jeopardy to your class status, grade, or standing with the university.

Questions about the Research

If you have questions regarding this study, you may contact Kristine Tanner, Ph.D., at (801) 422-7045 for further information.

Questions about Your Rights as Research Participants

If you have questions regarding your rights as a research participant contact IRB Administrator at (801) 422-1461; A-285 ASB, Brigham Young University, Provo, UT 84602; irb@byu.edu.

Statement of Consent

I have read, understood, and received a copy of the above consent and desire of my own free will to participate in this study.

Name (Printed):_____________________Signature:____________________Date:__________