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The Effects of Acidic Foods on Vocal Quality of Vocally Healthy Individuals

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EFFECTS OF ACIDIC FOODS ON VOCAL QUALITY OF VOCALLY HEALTHY INDIVIDUALS

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by

Melissa Barbieri

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To: Dean Oral Strickland
College of Nursing and Health Sciences

This thesis, written by Melissa Barbieri, and entitled Effects of Acidic Foods on Vocal Quality of Vocally Healthy Individuals, having been approved in respect to style and intellectual content, is referred to you for judgment.

We have read this thesis and recommend that it be approved.

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The thesis of Melissa Barbieri is approved.

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Vice President for Research and Economic
Development and Dean of the University Graduate School

Florida International University, 2021

DEDICATION

This thesis is dedicated to my family, friends, and community that supported me throughout this program. Your presence and encouragement have made all the difference.

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ABSTRACT OF THE THESIS

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Professor Balaji Rangarathnam, Major Professor

The purpose of the current study was to examine the relationship between the consumption of acidic liquids on the vocal quality of vocally healthy individuals. Acidic foods and liquids are known to be possible causes of common voice disorders because of their putative effect on systemic hydration of the vocal folds impacting their viscoelastic properties and eventually affecting vocal quality. This study investigated the effects of acidic foods on vocal quality in comparison to the effects of non-acidic/alkaline beverages in vocally healthy individuals.

Participants were provided with 4 oz of either an acidic or alkaline drink and voice measures were analyzed at baseline, immediately after and 1-hour post consumption. Results demonstrate that acidic beverages negatively impact vocal measures immediately after the consumption of acidic beverages, particularly in females. This research is significant in providing objective information for preventative care and vocal hygiene education.

Keywords: vocal folds, reflux, dehydration, vocal quality

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CHAPTER I

INTRODUCTION:

The purpose of the current study was to examine the relationship between the consumption of acidic liquids on the vocal quality of vocally healthy individuals. This research is significant in providing objective information for preventative voice care, and vocal hygiene education. Acidic foods and liquids are known to be possible causes of common voice disorders because of their putative effect on systemic hydration of the vocal folds. Acidity and dehydration impact their viscoelastic properties and eventually affect vocal quality, as they are found in over 50% of dysphonic patients (Karkos et al., 2007). Furthermore, acidic foods can also potentially cause gastroesophageal reflux disease (GERD) which occurs when gastric contents travel up the esophagus, and in some cases, into the pharynx, described as laryngopharyngeal reflux (LPR) (Mendell & Logemann, 2002). Reflux occurs due to several underlying lower esophageal sphincter (LES) disorders. A minority of people will experience decreased LES pressure (LESP), but generally, the cause of reflux is found in frequent transient LES relaxation (TLESR) (Kahrilas, 2003). Previous studies have reported that LPR is associated with several laryngeal diagnoses, including but not limited to, posterior vocal fold edema and erythema, contact ulcers and granulomas, subglottic stenosis, and even cancer of the larynx or esophagus (Mendell & Logemann, 2002, Sataloff et al, 2010).

The effects of acidic foods on vocal quality immediately after and 1-hour post consumption of acidic beverages in comparison to the effects of non-acidic/alkaline beverages were investigated in vocally healthy individuals. Extant literature has demonstrated two mechanisms of pathophysiology of the impact of acidic foods on the

laryngeal mucosa and, consequently, voice production: 1. The possibility of reduction in the systemic hydration of the laryngeal structures and 2. The possibility of exposure of lower pH levels in the larynx due to reflux of gastric content. Both these pathophysiological mechanisms have been demonstrated to negatively impact the vocal fold histology.

CHAPTER II

REVIEW OF THE LITERATURE

Vocal Fold Histology

Vocal folds are made up of three primary layers, which begins with an inner muscular layer, being the thyroarytenoid muscle (Zhang, 2016). This is followed by a soft tissue layer called the lamina propria, followed by the final, outer epithelial layer (Weiss et al., 2016; Xue et al., 2011). The lamina propria itself can be divided into three layers: a superficial layer which consists of elastin and collagen fibers, an intermediate layer mostly made of elastin fibers, and finally, a deep layer mainly consisting of dense, collagen fibers (Titze, 2000; Zhang, 2016). These layers are often condensed into a body-cover structure in which the body layer includes the thyroarytenoid muscle and deep layer of the lamina propria, and the cover includes both the intermediate and superficial lamina propria, in addition to the epithelial layer (Zhang, 2016).

Of importance, the epithelium and superficial lamina propria serve two primary purposes: 1. Protect the connective tissue from damage and 2. Provide flexible movement to aid in vocal fold vibration (Novaleski et al., 2017; Teller et al., 2012). The viscoelastic properties of the epithelium are what makes it such a unique and valuable asset to the vocal folds, especially in the presence of high-intensity voice demands (Teller et al., 2012). In addition to its pliability, the epithelium is also able to rapidly reproduce cells in the event

of damage (Novaleski et al., 2017). This rapid cell turnover allows the vocal folds to quickly recover from environmental and external irritants (Novaleski et al., 2017).

In addition to rapid cell turnover, the epithelium is composed of approximately 5-10 epithelial cell layers, only reinforcing its ability to withstand changes (Levendoski et al., 2014). Epithelial cells transport ions and water and respond to a variety of environmental challenges such as phonotrauma and reflux (Levendoski et al., 2014). This occurs at the level of the cell junctions, in which the epithelium creates a barrier comprised of protein complexes (Levendoski et al., 2014). This barrier not only protects the innermost layers of the epithelium, but also binds the cells together to prevent irritants from entering the paracellular space (Levendoski et al., 2014). Thus, this barrier serves to prohibit water, ions, and large solutes from freely passing through (Marchiando et al., 2010). In addition, both collagen and elastin permeate the lamina propria, which contributes to the vocal fold strength, elasticity, and elongation; while fibroblasts, myofibroblasts, and macrophages make up the folds at the cellular level (Walimbe et al., 2017). These macrophages and myofibroblasts help repair any vocal fold damage, like phonotrauma, to a certain threshold (Walimbe et al., 2017). Collagen present in the vocal folds not only aid in stability and movement, but shaping of new, growing tissue, and as a result, repair (Hahn et al., 2006b). The quantity of collagen fibers depends on age and gender, and as a result, females are more likely to develop scarring or other vocal fold pathologies because they have less collagen fibers (Hahn et al., 2006b). In addition to the elastic fibers found in the lamina propria, hyaluronic acid (HA) is present to contribute to vocal fold hydration, angiogenesis, wound repair, and cell proliferation (Hahn et al., 2006a).

Repair of the vocal folds is not a simple resolution, however, as the tissue's response to injury will be expressed as scarring. When the vocal folds are scarred, the extracellular matrix involving the mucosal lining will become stiff and mucosal waves will be restricted during phonation due to the increased viscosity and collagen depositions of the vocal folds (Hirano et al., 2009; Walimbe et al., 2017), thereby affecting vocal quality. Vocal scarring can be rooted in a handful of etiologies, and are not necessarily iatrogenic in nature, such as through chronic inflammation from chemical or mechanical injury (from acidic chemicals or vocal misuse) (Hirano et al., 2009, Mattei et al., 2017). Vocal fold scarring occurs in 3 stages: beginning in inflammation, proliferation, and finally, remodeling (Mattei et al., 2017). Inflammation occurs in the first 4 to 8 hours after injury and cells with fibroblastic cells are drawn to the area of injury and differentiate into myofibroblasts. This is followed by the breaking down of collagen and elastin fiber bundles, and finally fine, type III collagen is replaced by type I between day 5 and 7, which is associated with fibrosis (Karsdal, 2016; Mattei et al., 2017).

When a voice is heavily loaded upon, the superficial lamina propria carries the weight of the trauma in the form of scarring, which as a result has a negative impact on the viscoelastic properties of the vocal folds (Teller et al., 2012). This scarring leads to a diminished mucosal wave formation due to the increased viscosity of the vocal folds (Walimbe et al., 2017). These "microtraumas" set forth a vicious cycle worsening and potentially causing long-term impairments in vocal quality (Lechien et al. 2020). The most commonly attributed vocal quality characteristic perceived by patients suffering from reflux is hoarseness, but this could subsequently cause secondary laryngeal muscular tension (Lechien et al., 2017). That is, when the mucosal wave and its ability to repair itself

is put at risk when overwhelmed by acidic, extraneous agents (ex., reflux) and/or phonotrauma, laryngeal pathologies such as nodules, polyps, and Reinke's edema become more likely (Lechien et al., 2020).

For healthy vocal fold histology, it is also critical for the tissues to be constantly hydrated, both systemically and superficially (Sivasankar & Leydon, 2010). Systemic hydration is characterized by hydration found within the body, whereas superficial hydration is fluid coating the surface of the vocal folds and the laryngeal lumen (Sivasankar & Leydon, 2010). Systemic dehydration can be caused by a simple reduction in water consumption, or water loss through diarrhea or emesis (Cannes do Nascimento et al., 2020). Vocal folds are hydrated by consuming the required quantity of water throughout the day (approximately 64 fl. oz.), reducing ingesting dehydrating agents, such as caffeine and alcohol, and using nebulizers/steam inhalation (Hartley & Thibeault, 2014). When vocal folds are dehydrated, the minimum subglottal pressure to initiate vocal fold vibration known as phonation threshold pressure is increased, thus impairing vocal fold viscosity, and consequently, vocal fold vibratory properties (Sivasankar & Leydon, 2010). Furthermore, superficial hydration serves as a protective barrier against tissue damage (Leydon et al., 2009). Maintaining this ideal hydration homeostasis is critical for shielding the vocal folds from vocal loading agents such as phonotrauma or other contributing factors including speaking environment, hydration etc. (Leydon et al., 2009). A few studies have demonstrated a causal link between acidity and dehydration (e.g. Dworkin-Valenti et al., 2015; Heil, 2010).

Effects of Dehydration on Vocal Fold Histology:

Dehydration negatively affects vocal fold tissue morphology and consequently vocal quality. Oftentimes, the effects of vocal fold dehydration can manifest as laryngeal inflammation in which reflux could be a major underlying cause (among many others) (Dworkin-Valenti et al., 2015). Dehydrating/acidic agents, such as alcohol (which serves as a diuretic) cause vocal folds to develop mucous which can be exacerbating to vocal fold vibration (Landman, 2018). In addition, this dehydration leads to less lubrication; dry, unlubricated substances do not vibrate as easily as those which are lubricated (Landman, 2018). Especially in instances of prolonged phonation, vocal fold lubrication is necessary for healthy vibratory patterns (Heman-Ackah et al., 2008). In an earlier study, however, Erickson-Levendoski and Sivasankar (2011) investigated if caffeine ingestion would be detrimental to voice production, and if it could exacerbate negative phonatory effects of vocal loading, with conflicting results. Vocal outcome measures such as phonation threshold pressure (PTP) and perceived phonatory effort (PPE) were investigated (Erickson-Levendoski & Sivasankar, 2011). The authors reported that significant ingestion of caffeine did not impair PTP or PPE within the timeline that was examined. Similarly, Hartley and Thibeault (2014) found that though caffeine, alcohol, and diuretics are known to be dehydrating agents, current literature has not revealed a correlation between these substances and voice quality. In contrast, Akhtar et al. (2007) reported that caffeine did in fact produce perceivable vocal quality alterations. Due to these conflicting results across studies, it is important that different voice measures should be used and quantities of beverages in order to develop a greater understanding of the effects of dehydrating agents on voice (Akhtar et al., 2007; Erickson-Levendoski & Sivasankar, 2011).

In relation to systemic dehydration, data suggests that the laryngeal epithelial barrier can be damaged due to the lack of hydration caused by phonotrauma and LPR, which consequently leads to damaged tissue and thus, abnormal function (Cannes do Nascimento et al., 2020). Phonation threshold pressure (PTP) is directly related to vocal fold viscosity, and viscosity is hypothesized to be inversely proportional to hydration—suggesting that PTP is also inversely related to hydration (Solomon & DiMattia, 2000). On that premise, it is possible that increased water consumption could serve as an internal irrigation system to optimize vocal fold viscosity (Solomon & DiMattia, 2000). In fact, in a vocally fatiguing activity, participants who consumed significant amounts of water before the task showed elevation of PTP to a lesser extent in comparison to those who were not as systemically hydrated (Solomon & DiMattia, 2000). However, there is presently scarce statistically remarkable data connecting the effects of hydration on the voice due to methodological differences between investigations that render the studies impossible to compare and generalize (Hartley & Thibeault, 2014). In order to gain a better understanding on how the hydration spectrum interacts with phonation, muscular involvement should also be considered. Because muscular function and movement can be reduced and fatigued due to dehydration, it can be theorized that both intrinsic and extrinsic laryngeal muscles could also be impaired, thus contributing to possible phonatory deficits (Hartley & Thibeault, 2014). In addition, the reduced viscoelastic characteristics of articulatory cartilages has been linked to dehydration, suggesting that the arytenoid cartilages could be vulnerable to dehydration as well (Hartley & Thibeault, 2014).

Laryngopharyngeal Reflux and Voice Pathology

Laryngopharyngeal reflux (LPR) is defined as a fluid movement of gastric contents up to the oral cavity and/or pharynx (Richter & Rubenstein, 2018). Patients who present with reflux conditions report heartburn, regurgitation, chronic cough, and chronic laryngitis (Richter & Rubenstein, 2018). The most common cause of reflux is related to transient lower esophageal sphincter relaxation (TLESRs), which is characterized by the relaxation of the LES separate from swallowing or peristalsis (Kahrilas, 2003). When TLESRs occur, they can last between 10-60 seconds and can be present in healthy individuals (Argyrou et al., 2018). Triggers to TLESR episodes include drugs/medications (ex., antidepressants), hormones (ex., progesterone), foods (ex., chocolate, high fat foods), and certain habits, such as drinking alcohol, caffeine, and smoking (Argyrou et al., 2018). TLESR episodes do not always result in reflux reaching the upper esophageal sphincter (UES) because generally, peristalsis will return the gastric contents back into the stomach (Kahrilas, 2003). However, most patients suffering from GERD or LPR do so because of the higher frequency of TLESR in comparison to nonpathologic episodes (Kahrilas, 2003). Though GERD and LPR share many characteristics, it appears that the key pathology with GERD is found mainly in lower esophageal dysfunction and dysmotility, whereas patients with LPR will also be experiencing upper esophageal sphincter dysfunction as the gastric contents reach the pharynx (Koufman et al., 2002). GERD generally occurs after the ingestion of spicy, fatty, and citric foods, as well as chocolate and alcohol (Richter & Rubenstein, 2018). In addition, carbonated drinks may also decrease LES pressure, allowing gastric contents to more easily retrograde into the esophagus (Martinucci et al., 2013).

The esophagus does have four preventative reflux measures, however, the first of which is the lower esophageal sphincter (Rubin et al., 2014). Should the LES allow stomach contents to flow in retrograde, esophageal acid clearance would occur, which is the process to return to normal pH levels after a reflux episode (Rubin et al., 2014). Esophageal acid clearance occurs with multiple swallows of salivary bicarbonate and is completed in approximately 5 minutes (Rubin et al., 2014). The next form of defense is esophageal epithelial resistance. When the esophagus is inflamed from a pepsin-induced tissue injury, the esophageal mucosa will release bicarbonate to help balance the pH (Rubin et al., 2014). Unfortunately, this line of defense does not occur at the level of the laryngeal epithelium. The fourth and final anti-reflux defense in the upper digestive tract is the upper esophageal sphincter, though like the LES, this can also be impaired (Rubin et al., 2014).

When a reflux event occurs, pepsin is bound to tissues in the larynx and esophagus. However, proteolytic activation occurs through the introduction of an acid, generally through the consumption of acidic foods or liquids (Rubin et al., 2014). Because pepsin injury to the pharyngoesophageal structures caused by reflux (commonly mistaken to be acid injury) is caused by a combination of pepsin *and* acid, acid suppression medications do not fully address the causes of reflux damage (Rubin et al., 2014). Before pepsin has been activated by acid, it is considered pepsinogen. The introduction of acid (dietary and/or bile acid) to pepsinogen initiates the autocatalytic process, which will then continue to break down the tissue, even when the acid/initial activator is no longer present—up to approximately pH 8 (Bardhan et al., 2011). Even so, pepsin has the ability to activate itself through endocytosis, even without reacidification (Bardhan et al., 2011). Though there are mechanisms for defense against reflux, this chronic and escalating damage will impair the

body's defenses, as well (Rubin et al., 2014). In addition, because the core function of a proton-pump-inhibitor (PPI) is to reduce acidic contents in the stomach, the gastric volume is reduced as well, and consequently, the volume of pepsin increases (Bardhan et al., 2011). Because PPIs do not reduce reflux events, refluxate will continue to reach the pharyngoesophageal structures, but primarily pepsin in concentration—thus, it is more damaging (Bardhan et al., 2011). As a result, dietary modifications are fundamental in reducing reflux events and halting the snowball effect of damage to tissue and reflux defenses (Rubin et al., 2014). Though reflux and reflux triggers vary greatly from patient to patient, there are general recommendations in order to suppress symptoms. Generally, this begins as a detoxification diet in which foods which tend to be associated with allergies/sensitivities are removed and slowly re-introduced. This can help identify a reflux trigger in a patient's diet. This may involve removing dairy, gluten, nuts, not eating within 4 hours of sleep, and drinking alkaline water (Rubin et al., 2014).

Some studies have broadly investigated the effects of acidic foods on vocal quality. Vocal loading in the form of acidic foods, for example, not only weakens the epithelium's ability to regenerate, but leads to a form of chemical phonotrauma that can inflame the vocal fold tissue (Lechien et al., 2020). Specifically, the presence of pepsin from reflux plays a major role in inflaming the vocal folds by employing the spread and growth of inflammatory cells. With inflamed vocal folds, it is common to observe muscular tension during phonation as the individual attempts to push their voice, unknowingly at times past their thresholds, leading to amplified phonotrauma (Lechien et al., 2020). The stimulation of the esophagus during reflux events has been known to cause a reflexive laryngeal contraction (Angsuwarangsee & Morrison, 2002). In addition, the common symptom of

reflux known as globus pharyngeus is also associated with increased tension in the pharynx due to the refluxate (Angsuwarangsee & Morrison, 2002). This is in large due to the body's airway protection reflex, including glottic closure and constriction of the laryngopharyngeal muscles, which can be triggered with refluxate (Angsuwarangsee & Morrison, 2002).

When LPR occurs regularly, it can be associated with several different kinds of laryngeal pathologies. More commonly, LPR is related to hoarseness, excessive throat clearing, coughing, and globus pharyngeus (Çekin et al., 2012). Esophageal acid exposure is related to the amount of time, within a 24-hour period, that the pH level of the esophagus is lower than 4 (Kahrilas, 2003). Injury to the esophagus is determined by the duration of the acid exposure, as well as the degree (Kahrilas, 2003). Acid exposure is determined by the percent of time during a 24-hour day where the pH levels within the esophagus are less than 4 (Kahrilas, 2003). LPR is also correlated with functional dysphonia, subglottic stenosis, laryngospasm, interarytenoid pachydermia, vocal leukoplakia, vocal fold carcinoma, localized or diffuse laryngeal edema, ventricular obliteration, posterior laryngeal erythemia/hyperemia, posterior commissural hypertrophy, and vocal fold granulation (Çekin et al., 2012, Li et al, 2014, Kuo et al, 2020; Sataloff et al., 2010). The possible pathophysiology relates to the negative impact of the acidic/pepsin contents on increasing vocal fold viscosity, thereby making them stiffer and consequently affecting vibratory parameters such as the mucosal wave. Acoustically, jitter, shimmer, and NHR will be impaired; this is perceived as hoarseness and roughness of the voice (Lechien et al., 2016).

In summary, the vocal folds are an intricate structure composed of three layers that sophisticatedly balance vibration, length, and tension to arrive at an individual's unique voice. Any disequilibrium of the vocal folds via systemic dehydration, reflux, vocal misuse/loading, or acidic foods can lead to major dissonance in its vibratory properties. These changes can be reflected both in acoustic and/or perceptual measures of voice. However, further data is necessary in order to substantiate the relationships between acidic foods and voice quality in order to provide better rehabilitative and preventative care to individuals with voice disorders and mitigate the effects of LPR on voice.

Statement of Need

A very common practice in clinical voice pathology relative to vocal hygiene education is to intake copious amounts of water and avoid foods that can potentially cause reflux. Although there is a wide body of literature to support how the viscoelastic properties of the vocal folds can be affected because of vocal loading and phonotrauma, the immediate effects of acidic foods on clinical voice measures have not been investigated.

Even though there are several recommendations that are believed to be beneficial to prevent acid reflux, there is still a great deal of uncertainty and lack of data to confirm a causational relationship between a diet/lifestyle modification and inhibiting reflux (Martinucci et al., 2013). In order to improve patient care, treatment for reflux must be both corroborated with data and standardized, but it is first vital to identify specific clinical effects of reflux on voice, and whether or not individual diet choices have a significant, causational relationship with impaired vocal quality. Professional voice users such as actors, singers, teachers, lawyers, journalists, physicians, and clergy are all moderately or highly at risk of developing occupational voice disorders due to high vocal demand

(Vilkman, 2000). There are some simple steps to promote vocal health, such as hydration, reducing background noise, and using voice amplifiers, but how to reduce consequences of reflux on vocal quality is still yet to be resolved (Vilkman, 2000). Determining what lifestyle changes are necessary to prevent reflux is imperative to providing preventative/rehabilitative care and vocal hygiene education for a large population in need of evidence-based treatment options.

Specific Aim and Hypothesis

Specific Aim. To determine the specific effects of the consumption of acidic beverages on the perceptual and acoustic measures of voice between vocally healthy, self-identifying and assigned at birth males and females in comparison with the consumption of a non-acidic/alkaline beverage.

Hypothesis. Participants who consume the acidic beverage will demonstrate negative changes in perceptual and acoustic measures of voice one-hour post consumption when compared to the group consuming the alkaline beverage.

CHAPTER III

METHODOLOGY

Participants

All study procedures were conducted after the approval of the Florida International University's Institutional Review Board (IRB). The study recruited 100 participants through community outreach via word of mouth. The 100 participants consisted of 50 persons who identify as female and were assigned female at birth, and 50 persons who identify as male and were assigned male at birth, between the ages of 18 and 59 randomly assigned to two groups: Group 1, that consumed 4oz of a predominantly acidic beverage –

cranberry juice; and Group 2, which consumed a measured quantity of a predominantly alkaline beverage – caffeinated green tea. Participants were provided their randomly selected beverage in order to control for beverage type/brand and quantity. The acidic beverage was a 4 oz Ocean Spray cranberry juice cocktail. The alkaline drink was a 4 oz serving of One Organic Green Tea Instant Powder. Each group was comprised of 25 males and 25 females. Exclusion criteria included a history of LPR, GERD, or other voice disorders. English speaking males and females of all races, ethnicities, and socio-economic statuses, meeting the inclusionary criteria were enrolled in the study. Exclusion criteria was screened via participant report on the Intake Form (See Appendix). All participants provided written informed consent prior to participating in the study.

Procedures

The participants' voices were recorded using PRAAT software (Boersma & Weenink, 2021) with the clinician via Zoom (Zoom Video Communications Inc., 2020). The participants connected with the clinician via Zoom, and the clinician recorded the voice samples directly onto PRAATT. There were no controls for microphone/distance from microphone for participants to maintain consistency between previously gathered female data and new male data. Data collection efforts were modified to comply with social guidelines for the COVID-19 pandemic. The voice recordings consisted of sustained phonation of the vowels /a/, /i/, and /u/ for 3 seconds, reading 6 sentences from the Consensus Auditory Perceptual evaluation of Voice (CAPE-V), 2 minutes of spontaneous speech, rainbow passage, and counting 1-10. Participants provided these samples at three time points: baseline, immediately after consumption of the beverage and 1 hour after the consumption of the beverage. Participants were requested not to consume any food or

liquids at least 2 hours prior to the baseline recording and during the 1-hour interim period between the second and third time points. There were no voice restrictions for participants during the 1-hour interim, again, in an effort to maintain consistency. Both objective and subjective voice evaluations were acquired and analyzed for jitter, shimmer, NHR, and auditory-perceptual characteristics. Two clinicians gathered voice samples and randomly organized recordings by participant number, but without clarifying the data collection time point or drink pH group. A different, double-blinded clinician analyzed the auditory-perceptual voice characteristics.

Device used by participants was not controlled in this study, but if the participants were connecting to the Zoom call via a computer, the clinician instructed participants on how to disable Zoom's noise cancellation feature as it impacted data collection for sustained phonation. If participants connected via a cellular device, headphones with microphones were highly encouraged as the feature cannot be disabled on the cellular device application for Zoom. In the event that the full, sustained phonation could not be collected in sample over at least 3 attempts due to the noise cancellation feature, participant's recordings were combined to arrive at the complete 3 second sample. This occurred for 9 different male participants.

Data Analysis

To address the specific aim of this study, a mixed model analysis of variance (ANOVA) was performed to understand the main effects of the acidic vs alkaline beverage and the interaction effects of time points, gender, and group representation.

CHAPTER IV

RESULTS

The results are presented in the following sub-headings:

1. Comparison Across Acidic Beverage in Comparison to Alkaline Beverage:

A two-way mixed ANOVA was conducted to investigate the impact of drink across three timepoints. For the comparisons across the two groups, a statistically significant difference was observed for fundamental frequency in the reading task, $F(2, 190) = 3.08$, $p = .048$ (Table 1). Specifically, in the acidic group the difference occurs between baseline and immediately after consumption (increase by 3.327 Hz) and in the alkaline group between baseline and post 1 hour consumption (decrease by 4.673 Hz). No other measures yielded statistically significant differences. Whereas statistical significance was not observed for other parameters, qualitative comparisons are suggestive of clinically relevant trends (Figures 4-6). Specifically, it appears that both groups of participants demonstrated an increase in jitter that fairly remained at higher levels one-hour post consumption of the beverages. Shimmer and NHR were observed to be higher in the acidic group at the latter two time points which was not apparent in the alkaline group.

2. Comparison Between Gender Assigned at Birth and Type of Beverage Across Three Time Points:

Comparisons between male and female samples across the three time points and group representation did not yield statistically significant differences. Because there were no significant interactions across genders, separate ANOVA were performed in males and females to observe changes in each gender. For male participants, statistically significant differences across time points were observed for shimmer on sustained /a/, $F(2, 94) = 3.15$,

p = .048, jitter for the rainbow passage, $F(2, 94) = 3.17$, p = .046, and the F0 for the 2-minute spontaneous conversation, $F(2, 94) = 3.49$, p = .035 (Table 5). However, though they were statistically significant, they are not consistently observed across the voice parameters in the way that they were for the female voice measures. Male measures on the reading task also showed an increased jitter in the acidic group but decreased in the alkaline group over the three data collection points. For the female participants, significant differences were observed for fundamental frequency for the reading task, $F(1, 94) = 4.02$, p = 0.05 (Table 6). Qualitative comparisons are presented in figures 1-3. As can be seen from the graphical representations, it appears that female participants showed trends consistent with more impact than male participants.

3. Auditory-Perceptual Voice Changes:

The CAPE-V perceptual voice measures were analyzed by a double-blinded clinician and no notable changes were perceived between male and female participants, drink pH, or data collection time points.

A summary of outcome measures that showed significant changes across time points, gender and beverage type is depicted in Table 7.

CHAPTER IIV

DISCUSSION

This study investigated the effects of an acidic beverage on the voice quality of healthy individuals in comparison to alkaline beverage immediately after and one hour after consumption. The results demonstrate that acidic beverages do yield acoustically apparent differences in voice, even though perceptual parameters did not seem to be

impaired. These changes seem to impact females assigned at birth more than males assigned at birth. Thus, our hypothesis was partially supported. It is noteworthy, however, that a handful of participants in the acidic group reported some discomfort/changes in their voice one hour after consumption. In the initial analysis comparing male and female voice measures, drink pH level (type of beverage) was related to changes in fundamental frequency during the reading task. Furthermore, when drink and time were analyzed, the acidic group showed an increase in fundamental frequency, and contrastingly, the alkaline group had a decrease. Another positive correlation between impaired voice quality and drink acidity occurs within male subjects while reading the rainbow passage (jitter is increased). Though the sustained phonation of /a/ was not affected by time or gender in males, it did increase jitter, shimmer, and NHR of the female participants, more apparently in the group that consumed the acidic beverage. These findings collectively suggest that female voices may be more vulnerable to changes in vocal quality after consuming acidic beverages. Additionally, the acidic drink was related to increased jitter, shimmer, and NHR during the sustained phonation of /a/ immediately after consumption and slightly decreased one hour after consumption, suggesting that voice quality potentially recuperated over a relatively short period of time. Variations in jitter, shimmer, and F0 were more consistently related to drink acidity, especially during continuous speech more than sustained phonation. This is possibly due to the fact that reading a passage is more consistent with normal speech patterns than a sustained phonation.

The connection between time and gender may suggest that participant's vocal quality decreased as the study progressed, potentially due to vocal fatigue or other external factors. Whereas tissue analyses were not completed and it would be premature to make

sweeping conclusions with the limited data, it does appear that chemical agents in the form of acidic beverage impair vocal fold tissue structure, possibly impacting mucosal wave as evidenced by changes in voice perturbation measures. Additionally, histological differences in females and males have also been documented (Hahn et al., 2006b). In particular, male vocal folds have been shown to possess more collagen and elastin in vocal ligament as well as the cover (Chan et al., 2007). These histological differences perhaps make the male vocal folds less susceptible to damage because of extraneous agents such as acidic beverages.

The data have a bearing on providing preventative care measures for vocal hygiene, especially for those suffering from reflux. In addition to the literature reviewed that stated that the introduction of acidity can activate pepsin in the larynx, the data reveal that acidity itself can cause changes in voice production, even for those who have not been diagnosed with reflux (much like the participants in this study). The data also showed some reductions in the negative impact one-hour post consumption of acidic liquids. It remains to be investigated if this trend continues across time. It may be possible that voices get better for smaller quantities of acidic food, however continued consumption could potentially lead to a "chemical phonotrauma". This is perhaps more noticeable in individuals with a diagnosis of LPR. As a result, to prevent further damage for individuals with reflux, it is imperative that consumption of acidic drinks should be avoided or at least lessened. Acidic drinks should also be avoided before demanding speech activities, especially for professional voice users, as the data revealed that acidic drinks impair voice at the sentence level.

The data should be interpreted with caution because of certain study limitations. There were several extraneous factors that could not be controlled due to the nature of the study and the extenuating circumstances that arose because of the COVID-19 pandemic. The study was conducted via an online software with video and audio capabilities (Zoom). New updates on the software for background noise removal made collecting sustained phonation difficult as the software would remove the sustained phonation. Other extraneous variables include connectivity issues, background noise, the use (or lack thereof) and quality of headphones, and whether or not participants were using their cellular devices or a computer to participate. None of these factors were controlled during the study and could be potential factors in the results acquired. Nine participants' voice recordings had to be re-recorded between 1-3 times and combined due to the aforementioned extraneous variables, though generally due to Zoom's background noise cancellation.

Nonetheless, as it was previously mentioned, and as these data reflect, the question at hand still remains: how and why do data amongst and between other researchers regarding this topic vary so drastically? It is possible that had more variables been controlled, there would have been more noteworthy changes in vocal quality. It is important that research continue in order to concretely determine whether or not acidic drinks can impair voice quality, and why are they notoriously known to do so if data is not always supportive of that notion.

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Conflict of interest statement

The authors declare no conflicts of interest in preparing this article.

Table 1

Univariate Tests Within-Subjects Male & Female

Source	Measure		Type III Sum of Squares	df	Mean Square	F	Sig.
Time	a_F0	Sphericity Assumed	242.994	2	121.497	.455	.635
	a_Jitter	Sphericity Assumed	27470.559	2	13735.280	121.488	.000
	a_Shimmer	Sphericity Assumed	2.830	2	1.415	.849	.429
	a_NHR	Sphericity Assumed	.003	2	.001	.168	.846
	Rainbow_F0	Sphericity Assumed	205.241	2	102.620	.796	.453
Time x Drink	a_F0	Sphericity Assumed	532.457	2	266.229	.998	.371
	a_Jitter	Sphericity Assumed	19.710	2	9.855	.087	.917
	a_Shimmer	Sphericity Assumed	4.881	2	2.441	1.465	.234
	a_NHR	Sphericity Assumed	.029	2	.015	1.931	.148
	Rainbow_F0*	Sphericity Assumed	795.735	2	397.868	3.084	.048
Time x Gender	a_F0	Sphericity Assumed	169.514	2	84.757	.318	.728
	a_Jitter	Sphericity Assumed	27928.992	2	13964.496	123.516	.000
	a_Shimmer	Sphericity Assumed	4.694	2	2.347	1.408	.247
	a_NHR	Sphericity Assumed	.026	2	.013	1.695	.186
	Rainbow_F0	Sphericity Assumed	53.728	2	26.864	.208	.812

Time x Drink x Gender	a_F0	Sphericity Assumed	18.074	2	9.037	.034	.967
	a_Jitter	Sphericity Assumed	12.013	2	6.007	.053	.948
	a_Shimmer	Sphericity Assumed	3.215	2	1.607	.965	.383
	a_NHR	Sphericity Assumed	.020	2	.010	1.314	.271
	Rainbow_F0	Sphericity Assumed	502.849	2	251.425	1.949	.145

* Statistical significance

Table 2

Tests of Between-Subjects Effects Male & Female

Source	**Measure**	**Type III Sum of Squares**	**df**	**Mean Square**	**F**	**Sig.**
Intercept	a_F0	8150911.936	1	8150911.936	3769.011	.000
	a_Jitter	59008.266	1	59008.266	336.648	.000
	a_Shimmer	2528.616	1	2528.616	301.868	.000
	a_NHR	1.371	1	1.371	96.056	.000
	Rainbow_F0	9291013.267	1	9291013.267	9160.116	.000
Drink x Gender	a_F0	420.038	1	420.038	.194	.660
	a_Jitter	30.558	1	30.558	.174	.677
	a_Shimmer	10.568	1	10.568	1.262	.264
	a_NHR	.003	1	.003	.245	.622
	Rainbow_F0	6633.457	1	6633.457	6.540	.012

Table 3

Estimated Marginal Means Male & Female: Time x Gender

Measure	Gender	Time	Mean	Std. Error	95% Confidence Interval	
					Lower Bound	Upper Bound
a_F0	Male	1	121.896	4.181	113.595	130.196
		2	121.213	4.213	112.850	129.575
		3	122.231	4.452	113.392	131.070
	Female	1	207.672	4.138	199.457	215.888
		2	209.503	4.169	201.225	217.780
		3	211.618	4.407	202.869	220.366
a_Jitter	Male	1	.633	.058	.517	.749
		2	.444	2.221	-3.966	4.853
		3	.487	1.805	-3.097	4.071
	Female	1	.392	.058	.277	.507
		2	42.552	2.198	38.188	46.916
		3	40.078	1.787	36.531	43.625
a_Shimmer	Male	1	.728	.216	.300	1.156
		2	.700	.290	.124	1.276
		3	.635	.329	-.018	1.289
	Female	1	4.835	.213	4.411	5.259
		2	5.306	.287	4.736	5.876
		3	5.305	.326	4.658	5.952
a_NHR	Male	1	.112	.014	.084	.140
		2	.093	.012	.070	.116
		3	.085	.016	.053	.117
	Female	1	.031	.014	.003	.060
		2	.036	.012	.013	.059
		3	.050	.016	.018	.082
Rainbow_F0	Male	1	150.896	3.050	144.841	156.951
		2	153.252	2.905	147.485	159.019
		3	150.589	2.870	144.893	156.286
	Female	1	202.462	3.019	196.469	208.455
		2	202.762	2.875	197.054	208.470
		3	201.422	2.840	195.784	207.061

Table 4

Estimated Marginal Means Male & Female: Drink x Gender x Time

Measure	Drink	Gender	Time	Mean	Std. Error	95% Confidence Interval	
						Lower Bound	Upper Bound
a_F0	Acidic	Male	1	119.313	5.852	107.695	130.932
			2	119.466	5.896	107.761	131.172
			3	123.342	6.232	110.969	135.714
		Female	1	203.267	5.852	191.649	214.886
			2	205.464	5.896	193.758	217.169
			3	209.706	6.232	197.334	222.079
	Alkaline	Male	1	124.478	5.973	112.620	136.336
			2	122.959	6.018	111.012	134.906
			3	121.121	6.361	108.494	133.748
		Female	1	212.077	5.852	200.458	223.695
			2	213.542	5.896	201.836	225.247
			3	213.529	6.232	201.157	225.901
a_Jitter	Acidic	Male	1	.532	.082	.369	.695
			2	.478	3.109	-5.694	6.650
			3	.487	2.527	-4.529	5.503
		Female	1	.364	.082	.202	.527
			2	43.532	3.109	37.360	49.704

			3	40.984	2.527	35.968	46.000
	Alkaline	Male	1	.734	.084	.568	.900
			2	.410	3.173	-5.890	6.709
			3	.487	2.579	-4.633	5.606
		Female	1	.420	.082	.258	.583
			2	41.572	3.109	35.400	47.744
			3	39.172	2.527	34.156	44.188
a_ Shimmer	Acidic	Male	1	.660	.302	.061	1.260
			2	.744	.406	-.062	1.551
			3	.649	.461	-.266	1.563
		Female	1	5.120	.302	4.520	5.719
			2	5.994	.406	5.188	6.800
			3	5.455	.461	4.540	6.369
	Alkaline	Male	1	.795	.308	.184	1.407
			2	.656	.415	-.167	1.479
			3	.622	.470	-.312	1.555
		Female	1	4.551	.302	3.951	5.150
			2	4.618	.406	3.812	5.424
			3	5.156	.461	4.241	6.070
a_NHR	Acidic	Male	1	.092	.020	.052	.131
			2	.114	.016	.081	.146
			3	.083	.023	.038	.128
		Female	1	.030	.020	-.010	.070
			2	.040	.016	.008	.072
			3	.066	.023	.021	.111
	Alkaline	Male	1	.133	.020	.092	.173
			2	.073	.017	.040	.106
			3	.087	.023	.041	.133
		Female	1	.033	.020	-.007	.073
			2	.032	.016	.000	.064
			3	.034	.023	-.011	.079
Rainbow_F0	Acidic	Male	1	152.534	4.269	144.059	161.009
			2	158.740	4.066	150.667	166.812

			3	159.356	4.017	151.382	167.330
		Female	1	198.129	4.269	189.654	206.604
			2	198.088	4.066	190.015	206.160
			3	197.962	4.017	189.988	205.935
	Alkaline	Male	1	149.258	4.357	140.608	157.907
			2	147.765	4.150	139.526	156.004
			3	141.823	4.099	133.685	149.961
		Female	1	206.794	4.269	198.319	215.269
			2	207.436	4.066	199.364	215.509
			3	204.883	4.017	196.909	212.857

Table 5

Univariate Tests Within-Subjects Male

Source	Measure		Type III Sum of Squares	df	Mean Square	F	Sig.
Time	a_Jitter	Sphericity Assumed	.966	2	.483	4.641	.012
	a_Shimmer	Sphericity Assumed	.222	2	.111	2.143	.123
	a_NHR	Sphericity Assumed	.019	2	.009	1.096	.339
	Rainbow_ Jitter	Sphericity Assumed	.941	2	.470	2.933	.058
	Rainbow_ Shimmer	Sphericity Assumed	.093	2	.046	5.317	.006

	Rainbow_NHR	Sphericity Assumed	.035	2	.017	1.984	.143
	Counting_F0	Sphericity Assumed	258.886	2	129.443	.548	.580
	Counting_Jitter	Sphericity Assumed	.577	2	.288	1.096	.338
	Counting_Shimmer	Sphericity Assumed	.056	2	.028	2.931	.058
	Counting_NHR	Sphericity Assumed	.015	2	.007	3.120	.049
	Convo_F0	Sphericity Assumed	171.758	2	85.879	.523	.594
	Convo_Jitter	Sphericity Assumed	.323	2	.162	1.155	.320
	Convo_Shimmer	Sphericity Assumed	.031	2	.015	2.453	.092
	Convo_NHR	Sphericity Assumed	.004	2	.002	2.402	.096
	CAPEV_F0	Sphericity Assumed	242.282	2	121.141	.682	.508
	CAPEV_Jitter	Sphericity Assumed	1000.201	2	500.101	1.034	.359
	CAPEV_Shimmer	Sphericity Assumed	.006	2	.003	.238	.789
	CAPEV_NHR	Sphericity Assumed	.003	2	.001	.551	.578
Time x Drink	a_Jitter	Sphericity Assumed	.484	2	.242	2.328	.103
	a_Shimmer	Sphericity Assumed	.326	2	.163	3.147	.048
	a_NHR	Sphericity Assumed	.041	2	.020	2.371	.099
	Rainbow_Jitter*	Sphericity Assumed	1.018	2	.509	3.172	.046
	Rainbow_Shimmer	Sphericity Assumed	.040	2	.020	2.283	.108
	Rainbow_NHR	Sphericity Assumed	.025	2	.012	1.412	.249

	Counting_F0	Sphericity Assumed	129.285	2	64.642	.274	.761
	Counting_Jitter	Sphericity Assumed	.089	2	.045	.170	.844
	Counting_Shimmer	Sphericity Assumed	.034	2	.017	1.793	.172
	Counting_NHR	Sphericity Assumed	.006	2	.003	1.223	.299
	Convo_F0*	Sphericity Assumed	1145.856	2	572.928	3.489	.035
	Convo_Jitter	Sphericity Assumed	.285	2	.142	1.017	.365
	Convo_Shimmer	Sphericity Assumed	.033	2	.016	2.642	.077
	Convo_NHR	Sphericity Assumed	.004	2	.002	2.399	.096
	CAPEV_F0	Sphericity Assumed	478.266	2	239.133	1.346	.265
	CAPEV_Jitter	Sphericity Assumed	1000.840	2	500.420	1.035	.359
	CAPEV_Shimmer	Sphericity Assumed	.037	2	.019	1.471	.235
	CAPEV_NHR	Sphericity Assumed	.003	2	.001	.576	.564
Error (Time)	a_Jitter	Sphericity Assumed	9.778	94	.104		
	a_Shimmer	Sphericity Assumed	4.866	94	.052		
	a_NHR	Sphericity Assumed	.811	94	.009		
	Rainbow_Jitter	Sphericity Assumed	15.078	94	.160		
	Rainbow_Shimmer	Sphericity Assumed	.820	94	.009		
	Rainbow_NHR	Sphericity Assumed	.825	94	.009		
	Counting_F0	Sphericity Assumed	22197.058	94	236.139		

Counting_Jitter	Sphericity Assumed	24.731	94	.263
Counting_Shimmer	Sphericity Assumed	.904	94	.010
Counting_NHR	Sphericity Assumed	.222	94	.002
Convo_F0	Sphericity Assumed	15435.390	94	164.206
Convo_Jitter	Sphericity Assumed	13.157	94	.140
Convo_Shimmer	Sphericity Assumed	.587	94	.006
Convo_NHR	Sphericity Assumed	.082	94	.001
CAPEV_F0	Sphericity Assumed	16706.236	94	177.726
CAPEV_Jitter	Sphericity Assumed	45450.245	94	483.513
CAPEV_Shimmer	Sphericity Assumed	1.197	94	.013
CAPEV_NHR	Sphericity Assumed	.224	94	.002

* Statistical significance

Table 6

Test of Between-Subject Effects Female

Source	Measure	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Intercept	a_F0	6589661.771	1	6589661.771	2404.955	.000	.980
	a_Jitter	114877.984	1	114877.984	331.365	.000	.873
	a_Shimmer	3976.511	1	3976.511	242.344	.000	.835
	a_NHR	.230	1	.230	29.098	.000	.377
	Rainbow_F0*	6133660.200	1	6133660.200	9517.634	.000	.995
Drink	a_F0	1787.052	1	1787.052	.652	.423	.013
	a_Jitter	57.541	1	57.541	.166	.686	.003
	a_Shimmer	20.985	1	20.985	1.279	.264	.026
	a_NHR	.006	1	.006	.710	.404	.015
	Rainbow_F0	2590.767	1	2590.767	4.020	.051	.077
Error	a_F0	131521.706	48	2740.036			
	a_Jitter	16640.716	48	346.682			
	a_Shimmer	787.609	48	16.409			
	a_NHR	.379	48	.008			
	Rainbow_F0	30933.706	48	644.452			

* Statistical significance

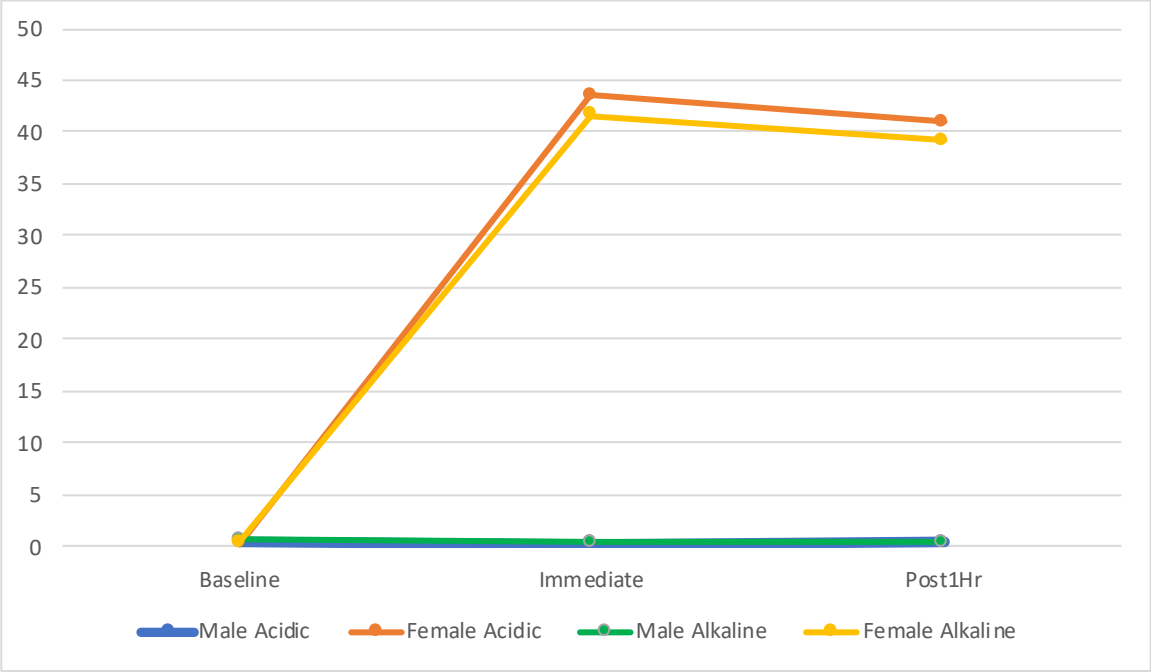
Table 7

Data Summary of Sustained Phonation /a/

	Baseline	Immediate	1 hour post
Between All Participants		• Increased jitter • Decreased shimmer	• Decreased jitter • Increased shimmer
Male		• Decreased jitter, shimmer, & NHR	• Increased jitter • Decreased shimmer • Decreased NHR
Female		• Increased jitter, shimmer, & NHR	• Decreased jitter • Increased shimmer (by .001) • Increased NHR
Acidic Group		• Increased jitter, shimmer, & NHR	• Decreased jitter, shimmer, & NHR
Alkaline Group		• Increased jitter • Decreased shimmer & NHR	• Decreased jitter • Increased shimmer & NHR

Figure 1

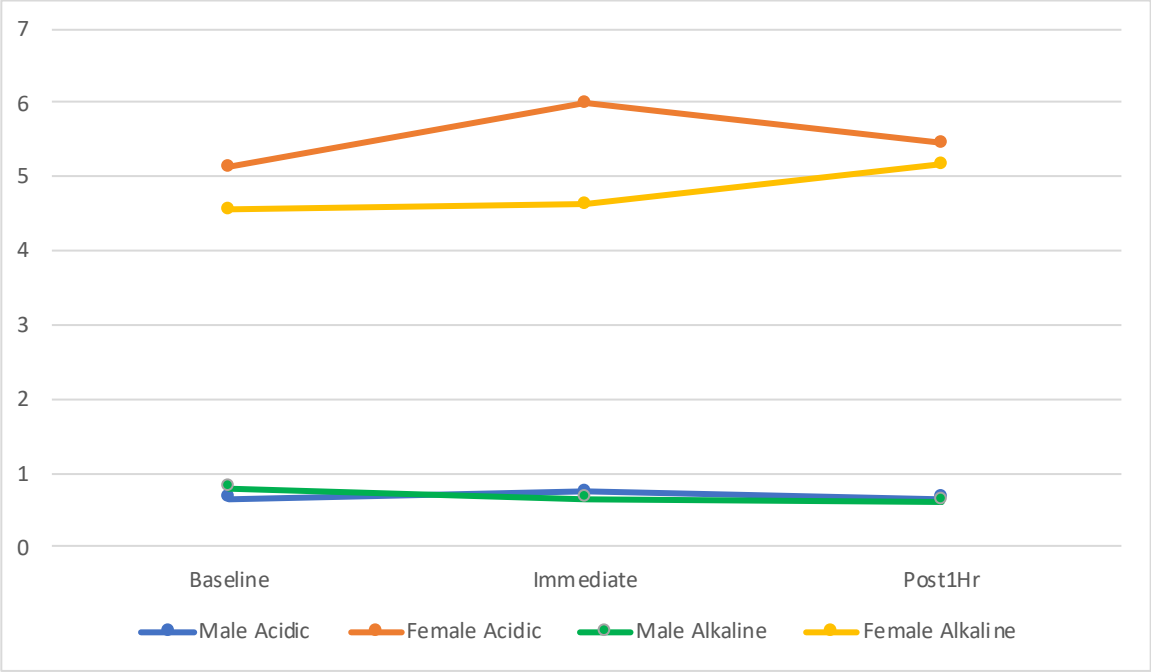
Male and Female Jitter /a/



Note. Female /a/ significantly increased immediately after consumption, and slightly decreased post 1-hour, whereas male /a/ maintained fairly stable results.

Figure 2

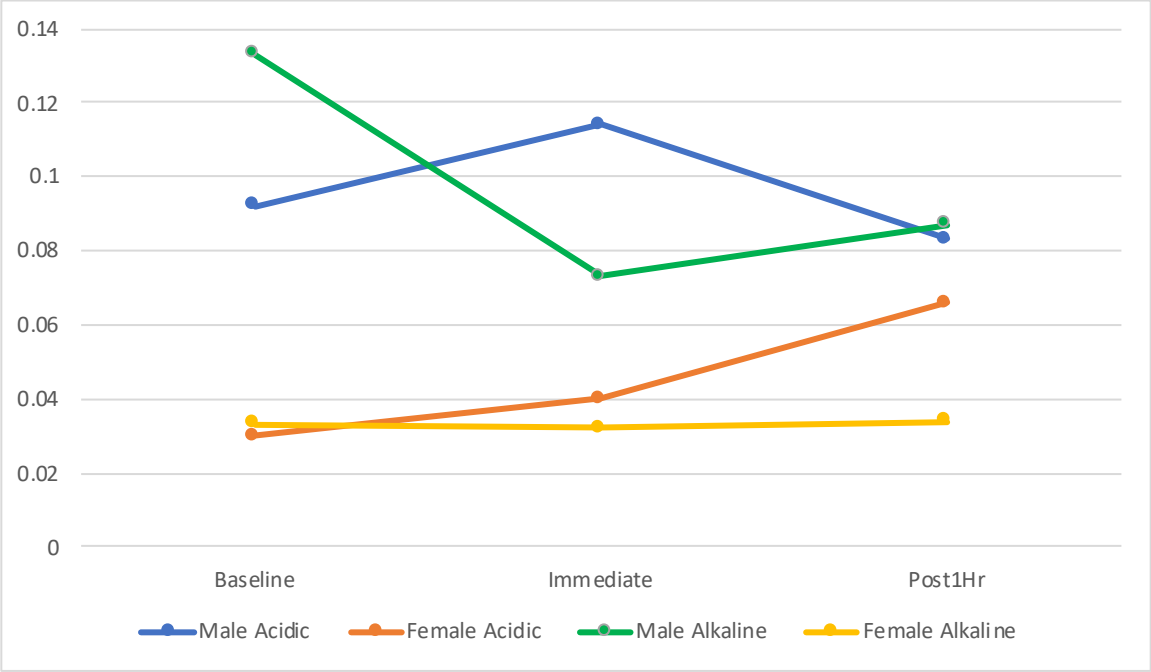
Male and Female Shimmer /a/



Note. Female /a/ significantly increased immediately after consumption, and slightly decreased post 1-hour, though maintained a more elevated result when compared to baseline. Male /a/ maintained fairly stable results.

Figure 3

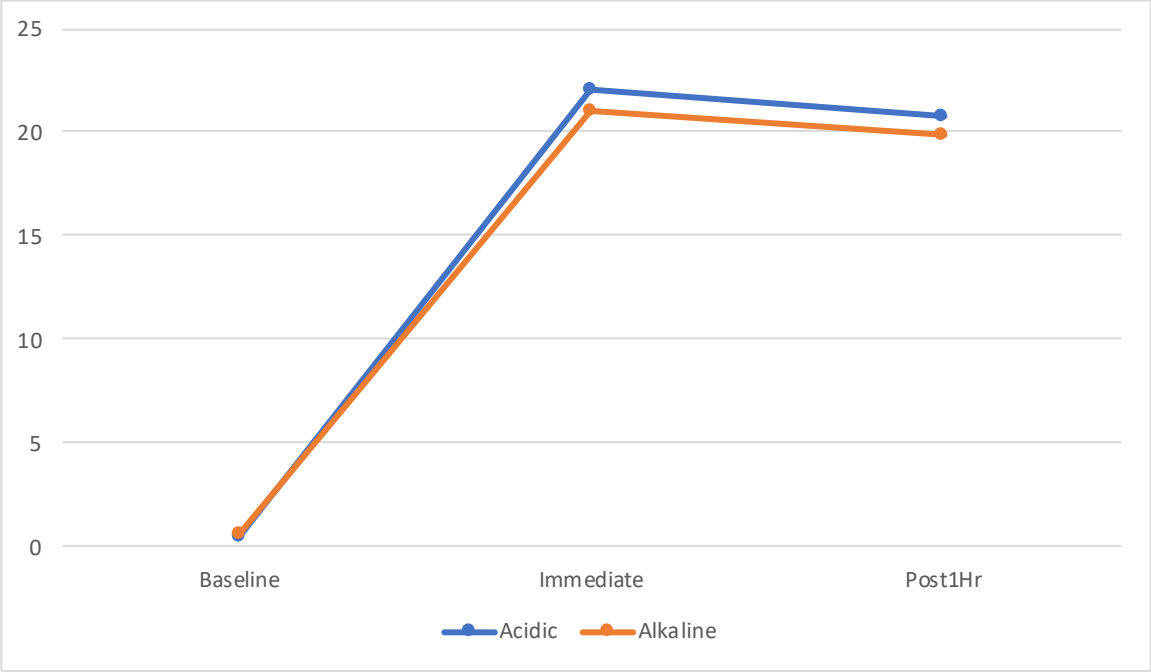
Male and Female NHR /a/



Note. Female /a/ acidic data significantly increased at the latter two time points though the alkaline female group remained fairly constant. The male acidic group increased immediately after consumption, but then decreased past baseline scores post 1-hour. The male alkaline group significantly decreased immediately post consumption, but slightly increased at post 1-hour.

Figure 4

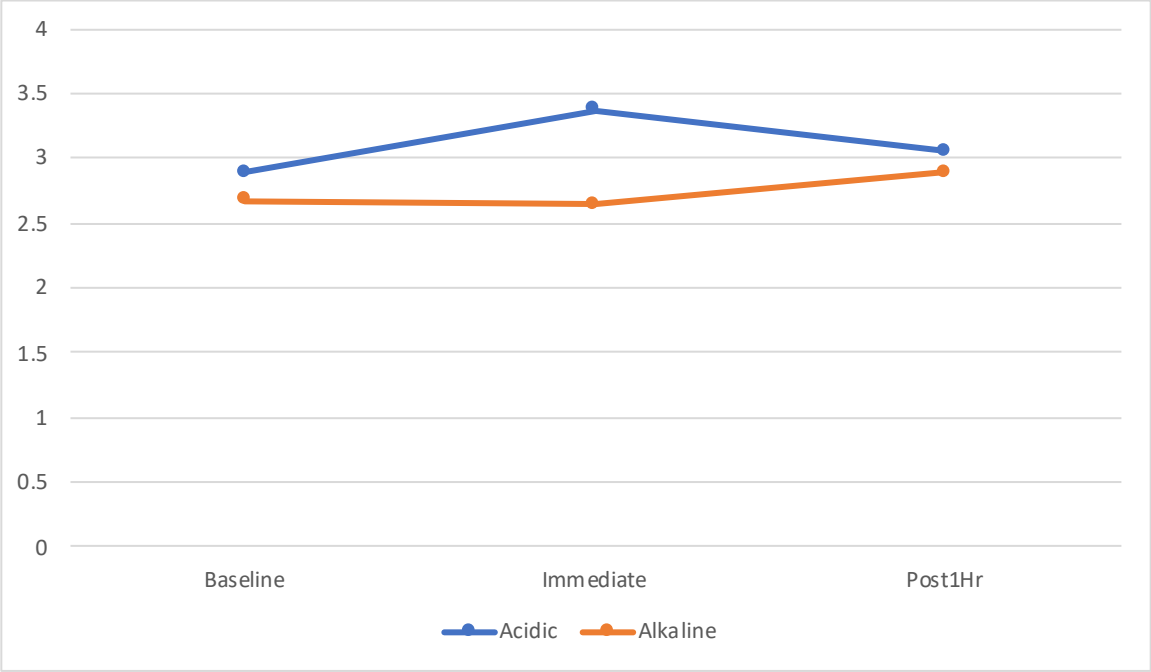
Acidic and Alkaline Jitter /a/



Note. The acidic and alkaline group both significantly increased immediately post consumption and slightly decreased post 1-hour.

Figure 5

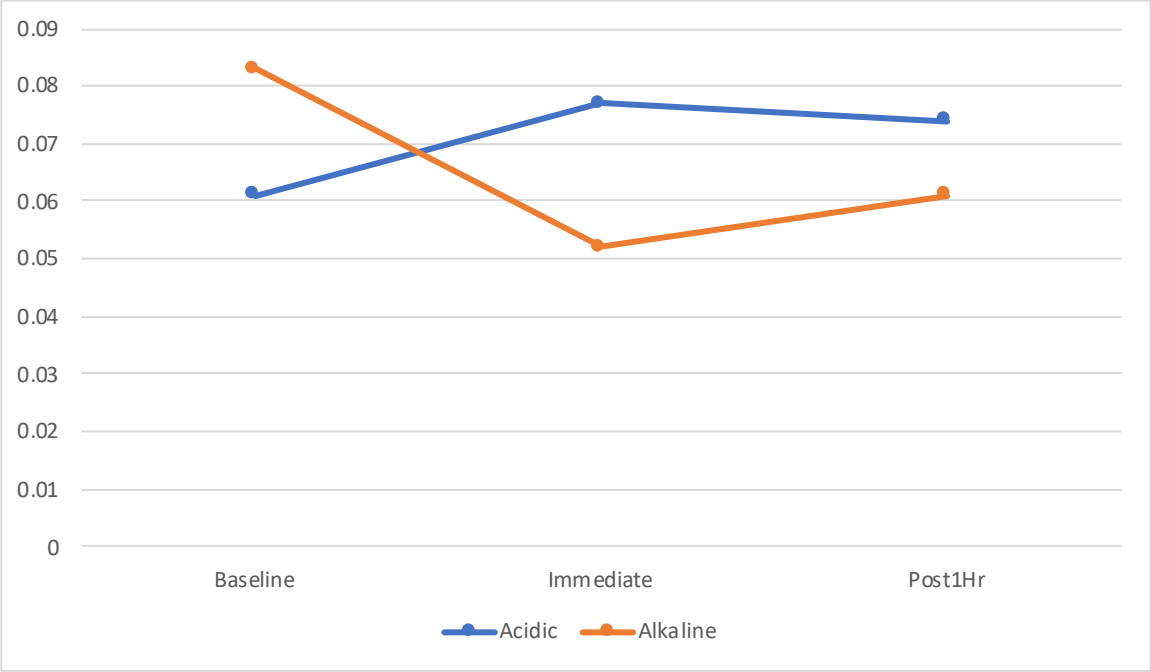
Acidic and Alkaline Shimmer /a/



Note. The acidic group slightly increased immediately post consumption and then slightly decreased post 1-hour, whereas the alkaline group remained fairly stable in the first two time points and increased post 1-hour.

Figure 6

Acidic and Alkaline NHR /a/



Note. The acidic group increased immediately post consumption and then slightly decreased post 1-hour, whereas the alkaline group significantly decreased immediately post consumption and then increased post 1-hour.

LIST OF REFERENCES

Aithal, V. U., Bellur, R., Sunila, J. S., Varghese, C., & Guddattu, V. (2011). Acoustic analysis of voice in normal and high pitch phonation: A comparative study. *Folia Phoniatrica Et Logopaedica, 64*(1), 48-53. doi:http://dx.doi.org.ezproxy.fiu.edu/10.1159/000333255

Akhtar, S., Wood, G., Rubin, J., O'Flynn, P., & Ratcliffe, P. (1999). Effect of caffeine on the vocal folds: A pilot study. *The Journal of Laryngology & Otology, 113*(4), 341-345. doi:10.1017/S0022215100143920

Angsuwarangsee, T. & Morrison, M. (2002). Extrinsic laryngeal muscular tension in patients with voice disorders. *Journal of Voice, 16*(3), 333-343. https://doi.org/10.1016/S0892-1997(02)00105-4

Argyrou, A., Legaki, E., Koutserimpas, C., Gazouli, M., Papaconstantinou, I., Gkiokas, G., & Karamanolis, G. (2018). Risk factors for gastroesophageal reflux disease and analysis of genetic contributors. *World journal of clinical cases*, *6*(8), 176–182. https://doi.org/10.12998/wjcc.v6.i8.176

Bardhan, K.V., Strugala, V., & Dettmar, P.W. (2011). Reflux revisited: Advancing the role of pepsin. *International Journal of Otolaryngology, 2012.* https://doi.org/10.1155/2012/646901

Boersma, Paul & Weenink, David (2021). Praat: Doing phonetics by computer [Computer program]. Version 6.1.21, from http://www.praat.org/

Campagnolo, A., Priston, J., Thoen, R., Medeiros, T., & Assunção, A. (2014). Laryngopharyngeal reflux: Diagnosis, treatment, and latest research. *International Archives of Otorhinolaryngology, 18*(2), 184-191. https://doi.org/10.1055/s-0033-1352504

Cannes do Nascimento, N., Dos Santos, A. P., Sivasankar, M. P., & Cox, A. (2020). Unraveling the molecular pathobiology of vocal fold systemic dehydration using an in vivo rabbit model. *PloS one*, *15*(7), e0236348. https://doi.org/10.1371/journal.pone.0236348

Cekin, E., Ozyurt, M., Erkul, E., Ergunay, K., Cincik, H., Kapucu, B., & Gungor, A. (2012). The association between helicobacter pylori and laryngopharyngeal reflux in laryngeal pathologies. *Ear, Nose, & Throat Journal, 91*(3), E6–E9. https://doi.org/10.1177/014556131209100314

Chan, R. W., Fu, M., Young, L., & Tirunagari, N. (2007). Relative contributions of collagen and elastin to elasticity of the vocal fold under tension. *Annals of biomedical engineering*, *35*(8), 1471-1483.

Clarrett, D. M., & Hachem, C. (2018). Gastroesophageal reflux disease (GERD). *Missouri Medicine, 115*(3), 214–218.

de Boer, B. (2010). Modelling vocal anatomy's significant effect on speech. *Journal of Evolutionary Psychology, 8*(4), 351-366. doi:http://dx.doi.org.ezproxy.fiu.edu/10.1556/JEP.8.2010.4.1

Dworkin-Valenti, J., Sugihara, E., Stern, N., Naumann, I., Bathula, S., & Amjad, E. (2015). Laryngeal inflammation. *Annals of Otolaryngology and Rhinology, 2*(9):1058

Erickson-Levendoski, E., & Sivasankar, M. (2011). Investigating the effects of caffeine on phonation. *Journal of Voice, 25*(5), e215-e219. doi:http://dx.doi.org.ezproxy.fiu.edu/10.1016/j.jvoice.2011.02.009

Gates, R., Forrest, L. A., & Obert, K. (2013). *The owner's manual to the voice: A guide for singers and other professional voice users*. Oxford: Oxford University Press.

Hahn, M. S., Kobler, J. B., Starcher, B. C., Zeitels, S. M., & Langer, R. (2006a). Quantitative and comparative studies of the vocal fold extracellular matrix i: Elastic fibers and hyaluronic acid. *Annals of Otology, Rhinology & Laryngology*, *115*(2), 156–164. https://doi.org/10.1177/000348940611500213

Hahn, M. S., Kobler, J. B., Zeitels, S. M., & Langer, R. (2006b). Quantitative and comparative studies of the vocal fold extracellular matrix ii: Collagen. *Annals of Otology, Rhinology & Laryngology, 115*(3), 225–232. https://doi.org/10.1177/000348940611500311

Hartley, N. A., & Thibeault, S. L. (2014). Systemic hydration: Relating science to clinical practice in vocal health. *Journal of Voice: Official Journal of The Voice Foundation, 28*(5), 652.e1–652.e20. https://doi.org/10.1016/j.jvoice.2014.01.007

Heil, D.P. (2010) Acid-base balance and hydration status following consumption of mineral-based alkaline bottled water. *Journal of the International Society of Sports Medicine,* 7(29) https://doi.org/10.1186/1550-2783-7-29

Heman-Ackah, Y., Sataloff, R., Hawkshaw, M., & Divi, V. (2008). How do I maintain the longevity of my voice? *Journal of Singing, 64*(4), 467-472.

Hirano, S., Minamiguchi, S., Yamashita, M., Ohno, T., Kanemaru, S., Kitamura, M. (2009). Histologic characterization of human scarred vocal folds. *Journal of Voice, 23*(4), 399-407. https://doi.org/10.1016/j.jvoice.2007.12.002.

Hunter, E. J., & Titze, I. R. (2009). Quantifying vocal fatigue recovery: Dynamic vocal recovery trajectories after a vocal loading exercise. *Annals of Otology, Rhinology & Laryngology, 118*(6), 449–460. https://doi.org/10.1177/000348940911800608

Kahrilas, P. (2003). GERD pathogenesis, pathophysiology, and clinical manifestations. *Cleveland Clinic Journal of Medicine, 70*(5), S4-S19.

Karkos, P. D., Yates, P. D., Carding, P. N., & Wilson, J. A. (2007). Is Laryngopharyngeal Reflux Related to Functional Dysphonia? *The Annals of Otology, Rhinology & Laryngology, 116*(1), 24-9. http://ezproxy.fiu.edu/login?url=https://www-proquest-com.ezproxy.fiu.edu/scholarly-journals/is-laryngopharyngeal-reflux-related-functional/docview/217917910/se-2?accountid=10901

Karsdal, M. (2016). Type I Collagen. In *Biochemistry of Collagens, Laminins and Elastin: Structure, Function and Biomarkers* (pp. 1–11). Academic Press.

Koufman, J. A. (2002). Laryngopharyngeal reflux is different from classic gastroesophageal reflux disease. *Ear, Nose & Throat Journal, 81*(9), 7-9. http://ezproxy.fiu.edu/login?url=https://www.proquest.com/scholarly-journals/laryngopharyngeal-reflux-is-different-classic/docview/209382378/se-2?accountid=10901

Koufman, J. A., Aviv, J. E., Casiano, R. R., & Shaw, G. Y. (2002). Laryngopharyngeal reflux: Position statement of the committee on speech, voice, and swallowing disorders of the American Academy of Otolaryngology-Head and Neck Surgery. *Otolaryngology-Head and Neck Surgery: Official Journal of American Academy of Otolaryngology-Head and Neck Surgery, 127*(1), 32–35. https://doi.org/10.1067/mhn.2002.125760

Kuo, C.-Y., Chu, Y.-H., & Wang, C.-H. (2020). Interarytenoid cobblestoning pachydermia in a patient with laryngopharyngeal reflux. *Ear, Nose & Throat Journal.* https://doi.org/10.1177/0145561320940901

Landman, S. M. (2018). Qualitative analysis of alcohol's acute effect on vocal range. *Alpenglow: Binghamton University Undergraduate Journal of Research and*

Creative Activity, 4(1). Retrieved from https://orb.binghamton.edu/alpenglowjournal/vol4/iss1/8

Lechien, J.R., Finck, C., Costa de Araujo, P., Huet, K., Delvaux, V., Piccaluga, M., Harmegnies, B., & Suassez, S. (2017). Voice outcomes of laryngopharyngeal reflux treatment: A systematic review of 1483 patients. *Eur Arch Otorhinolaryngol 274*, 1–23. https://doi.org/10.1007/s00405-016-3984-7

Lechien, J. R., Huet, K., Khalife, M., De Marrez, L. G., Finck, C., Harmegnies, B., & Saussez, S. (2019). Alkaline, protein, low-fat and low-acid diet in laryngopharyngeal reflux disease: Our experience on 65 patients. *Clinical Otolaryngology: Official Journal of ENT-UK; Official Journal of Netherlands Society for Oto-Rhino-Laryngology & Cervico-Facial Surgery*, *44*(3), 379–384. https://doi.org/10.1111/coa.13269

Lechien, J. R., Huet, K., Khalife, M., Fourneau, A. F., Delvaux, V., Piccaluga, M., Harmegnies, B., & Saussez, S. (2016). Impact of laryngopharyngeal reflux on subjective and objective voice assessments: a prospective study. *Journal of Otolaryngology - Head & Neck Surgery, 45*(1), 59. https://doi.org/10.1186/s40463-016-0171-1

Lechien, J. R., Saussez, S., Harmegnies, B., Finck, C., & Burns, J. A. (2017). Laryngopharyngeal reflux and voice disorders: A multifactorial model of etiology and pathophysiology. *Journal of Voice, 31*(6), 733-752.

Lechien, J. R., Saussez, S., Nacci, A., Barillari, M. R., Rodriguez, A., Le Bon, S. D., Crevier-Buchman, L., Harmegnies, B., Finck, C., & Akst, L. M. (2019). Association between laryngopharyngeal reflux and benign vocal folds lesions: A systematic review. *The Laryngoscope, 129*(9), E329–E341. https://doi.org/10.1002/lary.27932

Levendoski, E. E., Leydon, C., & Thibeault, S. L. (2014). Vocal fold epithelial barrier in health and injury: A research review. *Journal of speech, language, and hearing research: JSLHR*, *57*(5), 1679–1691. https://doi.org/10.1044/2014_JSLHR-S-13-0283

Leydon, C., Sivasankar, M., Falciglia, D. L., Atkins, C., & Fisher, K. V. (2009). Vocal fold surface hydration: A review. *Journal of Voice: Official Journal of The Voice Foundation, 23*(6), 658–665. https://doi.org/10.1016/j.jvoice.2008.03.010

Li, X., Huang, Z., Wu, T., Wang, L., & Wu, J. (2014). Role of laryngopharyngeal reflux on the pathogenesis of vocal cord leukoplakia and early glottic cancer. *Chinese Journal of Otorhinolaryngology Head and Neck Surgery*, *49*(5), 362–367.

Lieberman, P. (2012). Vocal tract anatomy and the neural bases of talking. *Journal of Phonetics, 40*(4), 608-622. http://dx.doi.org.ezproxy.fiu.edu/10.1016/j.wocn.2012.04.001

Luakkanen, A. M., Ilomaki, I., Leppanen, K., & Vilkman, E. (2008). Acoustic measures and self-reports of vocal fatigue by female teachers. *Journal of Voice, 22*(3), 283-289. https://doi.org/10.1016/j.jvoice.2006.10.001

Malfertheiner, P. & Hallerbäck, B. (2005). Clinical manifestations and complications of gastroesophageal reflux disease (GERD). *The International Journal of Clinical Practice, 59*(3), 346-355. https://doi.org/10.1111/j.1742-1241.2005.00370.x

Martinucci, I., de Bortoli, N., Savarino, E., Nacci, A., Romeo, S. O., Bellini, M., Savarino, V., Fattori, B., & Marchi, S. (2013). Optimal treatment of laryngopharyngeal reflux disease. *Therapeutic Advances in Chronic Disease, 4*(6), 287–301. https://doi.org/10.1177/2040622313503485

Mattei, A., Magalon, J., Bertrand, B., Philandrianos, C., Veran, J., & Giovanni, A. (2017). Cell therapy and vocal fold scarring. *European annals of otorhinolaryngology, head and neck diseases*, *134*(5), 339–345. https://doi.org/10.1016/j.anorl.2017.06.006

Mendell, D. A., & Logemann, J. A. (2002). A Retrospective Analysis of the Pharyngeal Swallow in Patients with a Clinical Diagnosis of GERD Compared with Normal Controls: A Pilot Study. *Dysphagia, 17*(3), 220-6. http://dx.doi.org.ezproxy.fiu.edu/10.1007/s00455-002-0056-5

Minich, D. & Bland, J. (2007). Acid-alkaline balance: Role in chronic disease and detoxification. *Alternative Therapies, 13*(4), 62-65.

Mosli, M., Alkhathlan, B., Abumohssin, A., Merdad, M., Alherabi, A., Marglani, O., Jawa, H., Alkhatib, T., & Marzouki, H. Z. (2018). Prevalence and clinical predictors of LPR among patients diagnosed with GERD according to the reflux symptom index questionnaire. Saudi journal of gastroenterology: *Official Journal of The Saudi Gastroenterology Association, 24*(4), 236–241. https://doi.org/10.4103/sjg.SJG_518_17

Muñoz, J., Mendoza, E., Fresneda, M. D., Carballo, G., & López, P. (2003). Acoustic and perceptual indicators of normal and pathological voice. *Folia Phoniatrica Et Logopaedica:International Journal of Phoniatrics, Speech Therapy and Communication Pathology, 55*(2), 102-114. doi:http://dx.doi.org.ezproxy.fiu.edu/10.1159/000070092

Naufel de Felippe, A. C., Grillo, M. H., & Grechi, T. H. (2006). Standardization of acoustic measures for normal voice patterns. *Brazilian Journal of Otorhinolaryngology, 72*(5), 659–664. https://doi.org/10.1016/s1808-8694(15)31023-5

Novaleski, C., Carter, B., Sivasankar, M., Ridner, S., Dietrich, M., & Rousseau, B. (2017). Apoptosis and vocal fold disease: Clinically relevant implications of epithelial cell death. *Journal of Speech, Language, and Hearing Research, 60*(5), 1-9. DOI: 10.1044/2016_JSLHR-S-16-0326

Pizolato, R., Rehder, M., de Castro Meneghim, M., Ambrosano, G., Mialhe, F., Pereira, A. (2013). Impact on quality of life in teachers after educational actions for prevention of voice disorders: A longitudinal study. *Health Qual Life Outcomes 11*(28), 1-9. https://doi.org/10.1186/1477-7525-11-28

Richter, J. E., & Rubenstein, J. H. (2018). Presentation and epidemiology of gastroesophageal reflux disease. *Gastroenterology, 154*(2), 267–276. https://doi.org/10.1053/j.gastro.2017.07.045

Rubin, J. S., Sataloff, R. T., & Korovin, G. S. (2014). Laryngopharyngeal Reflux and Voice Disorders. In *Diagnosis and Treatment of Voice Disorders* (pp. 457–469). Plural Publishing.

Sataloff, R., Hawkshaw, M., & Gupta R. (2010). Laryngopharyngeal reflux and voice disorders: An overview on disease mechanisms, treatments, and research advances. *Discovery Medicine, 10*(52), 213-224.

Schwalfenberg, G. (2012). The alkaline diet: Is there evidence that an alkaline pH diet benefits health? *Journal of Environmental and Public Health, 2012*, 1-7. doi:10.1155/2012/727630

Sessions, M. (2020). An analysis of dairy and its effects on vocal production. *Undergraduate Research Journal, 24*(10), 103-109

Simpson, A. P. (2009). Phonetic differences between male and female speech. *Language and Linguistics Compass, 3*(2), 621-640. doi:http://dx.doi.org.ezproxy.fiu.edu/10.1111/j.1749-818X.2009.00125.x

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

Solomon, N. P., & DiMattia, M. S. (2000). Effects of a vocally fatiguing task and systemic hydration on phonation threshold pressure. *Journal of Voice: Official Journal of the Voice Foundation*, *14*(3), 341–362. https://doi.org/10.1016/s0892-1997(00)80080-6

Tierney, W. S., Gabbard, S. L., Milstein, C. F., Benninger, M. S., & Bryson, P. C. (2017). Treatment of laryngopharyngeal reflux using a sleep positioning device: A prospective cohort study. *American Journal of Otolaryngology, 38*(5), 603-607. doi:http://dx.doi.org.ezproxy.fiu.edu/10.1016/j.amjoto.2017.06.012

Teller, S., Farran, A., Xiao, L., Jiao, T., Duncan, R., Clifton, R., & Jia, X. (2012). High-frequency viscoelastic shear properties of vocal fold tissues: Implications for vocal fold tissue engineering, *Tissue engineering. Part A, 18*(19-20), 2008–2019. https://doi.org/10.1089/ten.TEA.2012.0023

Titze, I. (1989). Physiologic and acoustic differences between male and female voices. *The Journal of the Acoustical Society of America, 85*(4). https://doi.org/10.1121/1.397959

Titze, I., Svec, J., & Popolo, P. (2003). Vocal dose measures: Quantifying accumulated vibration exposure in vocal fold tissues. *Journal of Speech, Language, and Hearing Research, 46(*4), 919-932.

Van Houtte, E., Van Lierde, K., & Claeys, S. (2011). Pathophysiology and treatment of of muscle tension dysphonia: A review of the current knowledge. *Journal of Voice, 25*(2), 202-207. https://doi.org/10.1016/j.jvoice.2009.10.009

Vilkman, E. (2000). Voice problems at work: A challenge for occupational safety and health arrangement. *Folia Phoniatrica Et Logopaedica:International Journal of Phoniatrics, Speech Therapy and Communication Pathology, 52*(1-3), 120-125. doi:http://dx.doi.org.ezproxy.fiu.edu/10.1159/000021519

Walimbe, T., Panitch, A., & Sivasankar, P. M. (2017). A review of hyaluronic acid and hyaluronic acid-based hydrogels for vocal fold tissue engineering. *Journal of Voice, 31*(4), 416-423.

Whitling, S., Rydell, R., & Åhlander, V. L. (2015). Design of a clinical vocal loading test with long-time measurement of voice. *Journal of Voice, 29*(2), e13-e27. doi:http://dx.doi.org.ezproxy.fiu.edu/10.1016/j.jvoice.2014.07.012

Zhang, Z. (2016). Mechanics of human voice production and control. *The Journal of Acoustical Society of America, 140*(4), 2614-2635. https://doi.org/10.1121/1.4964509

Zoom Video Communications, Inc. (2020). *ZOOM meetings* (Version 5.4.7) [Computer program]. https://zoom.us

Xue, Q., Zheng, X., Bielamowicz, S., & Mittal, R. (2011). Sensitivity of vocal fold vibratory modes to their three-layer structure: implications for computational modeling of phonation. The Journal of the Acoustical Society of America, 130(2), 965–976. https://doi.org/10.1121/1.3605529

Appendix

Participant Intake Form

Participant number:

Date:

Occupation:

History of any of the following:

	Y	N
Recent voice issues		
Allergies		
Asthma		
Frequent Chronic colds		
Issues with breathing		
Cigarette smoking		
Singing experience		

If yes for any of the above, please provide details:

Please estimate the number of times of the following:

Cups of water per day	
Cups of caffeinated beverages per day	
Any yelling/screaming per day	
Speak above noise	
Alcohol consumption per week	

Are you on any medications currently? If yes, please list.