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Vocal Function and Upper Airway Thermoregulation in Five Different Environmental Conditions

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Abstract

Purpose—Phonation threshold pressure and perceived phonatory effort were hypothesized to increase and upper airway temperature decrease following exposure to cold and/or dry air. Greater changes were expected with mouth versus nose breathing.

Method—Using a within-participant repeated measures design, 15 consented participants (7 men, 8 women) completed 20-minute duration trials to allow for adequate thermal equilibration for both nose and mouth breathing in five different environments: three temperatures (°C) matched for relative humidity (%RH): cold (15°C/40% RH), thermally neutral (25°C/40% RH), and hot (35°C/40% RH); and two temperatures with variable relative humidity to match vapor pressure for the neutral environment (25°C/40% RH): cold (15°C/74% RH) and hot (35°C; 23% RH). Following each equilibration trial, measures were taken in this order: upper airway temperature (transnasal thermistor probe), phonation threshold pressure, and perceived phonatory effort.

Results—Data were analyzed using repeated measures analysis of variance and no significant differences were established.

Conclusions—The study hypotheses were not supported. Findings suggest that the upper airway is tightly regulated for temperature when challenged by a realistic range of temperature/relative humidity environments. This is the first study of its kind to include measurement of upper airway temperature in conjunction with measures of vocal function.

Introduction

Professionals working in thermally extreme environments are subjected to varying temperature environments during vocally demanding tasks. There is evidence that laborers and professional voice users working in thermally challenging environments have a higher incidence of voice disorder (Herrington-Hall, Lee, Stemple, Niemi, & McHone, 1988;

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Hoffman-Ruddy, Lehman, Crandell, Ingram, & Sapienza, 2001). Well-established beliefs regarding the ideal environment for the performing voice exist, e.g., the performance space should be adequately humidified and the temperature should be comfortable and not too cold (Benninger, Jacobson, & Johnson, 1994). There also exist specific claims regarding the deleterious effects of extreme temperature or humidity on voice function (Lawrence, 1986; Tucker, 1993) that are not evidence supported. Our understanding of how temperature changes may support optimal voice use or contribute to work-related vocal injury is incomplete.

Investigations of environmental factors on voice function have largely been limited to studies of superficial hydration (Sivasankar & Erickson, 2009; Sivasankar & Fisher, 2002, 2003), systemic hydration (Verdolini-Marston, Sandage, & Titze, 1994; Verdolini-Marston, Titze, & Druker, 1990; Verdolini, Titze, & Fennell, 1994), ambient humidity (Sivasankar, Erickson, Schneider, & Hawes, 2008; Sivasankar & Fisher, 2002, 2003; Verdolini-Marston et al., 1994), and mouth versus nose breathing (Sivasankar et al., 2008; Sivasankar & Fisher, 2002, 2003), all factors that have been shown to influence optimal voice function and perception of vocal ease. These investigations have contributed to the current understanding that higher humidity environments and nasal breathing reduce phonation threshold pressure (PTP) and perceived phonatory effort (PPE). Conversely, mouth breathing and drier ambient conditions are believed to drive PTP measures and PPE measures higher. The findings from these investigations have been readily accepted into clinical vocal hygiene guidelines that often advise a minimum of 40% relative humidity in work, performance, and home environments.

Little attention has been paid to the influence of ambient temperature on voice function. Despite the inability to directly measure laryngeal viscosity and skeletal muscle function, investigation of ambient temperature and vocal function requires basic understanding of environmental influences on laryngeal surface fluid maintenance; the influence of laryngeal surface fluid viscosity on voice function; and the influence of tissue temperature change on skeletal muscle physiology to account for physiologic variables that may influence the findings. Figure 1 illustrates the synergistic nature of the relevant physiological variables discussed below.

Environmental Influences on Laryngeal Surface Fluid Maintenance

Pulmonary air conditioning (Cole, 1953a, 1953b, 1953c, 1954; McCutchan & Taylor, 1951; McFadden, Pichurko, & Pichurko, 1985; McFadden, et al., 1985; McFadden, 1983) and respiratory epithelial water transport literature (Widdicombe, 1997; Williams, Rankin, Smith, Galler, & Seakins, 1996) describes an *interdependence* between humidity and temperature with percent saturation of ambient water vapor changing dramatically with small changes in temperature. This basic research supports the importance of considering the influence of environmental temperature, humidity, and water vapor pressure on the physiology of the human upper airway.

Given that study of the laryngeal structures *in vivo* represents a great technical challenge, much of our knowledge of laryngeal surface fluid maintenance comes from animal models. Our initial understanding of laryngeal surface fluid transport was translated from studies of tracheal epithelia using canine and ovine models. Investigation of the effect of temperature, humidity, and mode of breathing in anaesthetized dogs found that bulk water flux across the epithelium was higher with mouth breathing than nasal breathing (Man, Adams, & Proctor, 1979) and the transepithelial osmotic gradient created by evaporative cooling may account for hydration of the tracheal surface (Boucher, Stutts, & Gatzy, 1981). Work by Freed and Davis (1999) postulated that hyperventilation in dry air increased airway surface fluid osmolality in a canine model. Widdicombe (1997) investigated the maintenance of the

airway surface liquid (ASL) as influenced by mechanisms of evaporative water loss and he calculated that the degree of fluid loss would depend on many factors that could include inspired air temperature and humidity.

Fisher and colleagues (Fisher, Telser, Phillips, & Yeates, 2001) found evidence of bidirectional water flux controlled by $\text{Na}^+\text{K}^+\text{ATPase}$ channels for both the excised canine and ovine laryngeal epithelium. In a later study using an excised ovine laryngeal model, investigators identified luminally-directed water flux following luminal exposure to osmotic perturbations (Sivasankar & Fisher, 2007). These studies provide evidence of laryngeal epithelial viscosity maintenance in the ovine and canine larynx. Translation of laryngeal epithelial research conducted with canine and ovine models to human laryngeal epithelial physiology may not be optimal because, unlike humans (Robertshaw, 2006), these panting species employ the respiratory mechanism as a primary means of thermoregulation (Fayez, Marai, Alnaimy, & Habeeb, 1994; Hales & Brown, 1974; Ingram & Legge, 1972; Ray, Ingenito, Streck, Schumacker, & Solway, 1989; Solway et al., 1986). This primary physiologic difference for thermoregulation may signal key differences in respiratory epithelial physiology that are yet to be described in the human larynx. More recently, Sivasankar and colleagues have conducted basic investigations of laryngeal epithelial physiology with the porcine laryngeal epithelium, a species that relies on behavior and not on respiration for thermoregulation (Erickson & Sivasankar, 2010; Sivasankar & Blazer-Yost, 2009) which may be more similar to human physiology. Their findings provide evidence for laryngeal epithelial regulatory mechanisms that are triggered when challenged by gastroesophageal reflux and corticosteroids.

A recent review by Leydon, et al., (2009) summarized recent basic and applied research of superficial liquid homeostasis of the larynx and the role that these mechanisms have in maintaining optimal vibratory characteristics of the vocal fold mucosa. In their discussion of vocal fold epithelial viscosity, temperature of the conditioned air was not discussed as an agent of change despite evidence in the pulmonary literature (Widdicombe, 1997) that describes temperature as an environmental variable of interest for upper airway fluid maintenance. Additionally, much of the applied research cited in the review (Sivasankar & Fisher, 2002, 2003; Tanner, Roy, Merrill, & Elstad, 2007) hypothesized that superficial laryngeal fluid would be reduced with mouth breathing, an assumption not supported in the work of McFadden and others (McFadden, et al, 1985).

Laryngeal surface fluid viscosity and voice function

Phonation threshold pressure (PTP), an aerodynamic assessment of vocal fold function at voice onset, has been used in most investigations of environmental influences on vocal function. PTP is described as the minimum amount of lung pressure required to initiate vocal fold vibration for voicing, a measurement that will vary as a function of vocal fold viscosity, velocity of mucosal wave of the vocal fold cover, pre-voice glottal width, and vocal fold thickness (Titze, 1988). PTP is theoretically proportional to vocal fold tissue viscosity, which may have an inverse relationship with hydration level (Verdolini-Marston et al., 1994). Titze's model does not include temperature of the vocal fold tissue or temperature of the conditioned respiratory air in the laryngeal environment in the model despite a likely role of temperature on superficial laryngeal viscosity and muscle function, both intramuscularly and in response to the environment.

There is ample published evidence supporting the effect that ambient humidity may have on vocal function, with drier air driving PTP measures higher and higher humidity environments facilitating lower PTP (Sivasankar & Erickson, 2009; Sivasankar et al., 2008; Sivasankar & Fisher, 2002, 2003; Verdolini-Marston et al., 1994; Verdolini-Marston et al., 1990; Verdolini, Titze, & Fennell, 1994; Verdolini et al., 1994). In a series of investigations

of airway conditioning on superficial laryngeal viscosity, Sivasankar and colleagues (Sivasankar et al., 2008; Sivasankar & Fisher, 2002, 2003) studied the influence of oral versus nasal breathing on measurements of PTP, with the working hypothesis that oral breathing would thin the sol layer on the surface of the vocal folds with a subsequent increase in laryngeal viscosity and rise in PTP as compared to nasal breathing. Their initial findings suggested increased PTP and PPE following oral breathing, particularly for the higher pitches assessed. More recent research that has targeted humidity manipulations with longer periods of mouth versus nose breathing did not support these findings (Erickson-Levendoski & Sivasankar, 2011; Sundarajan, Erickson-Levendoski, & Sivasankar, 2012).

Temperature and skeletal muscle physiology

Temperature of air in the human upper airway has been shown to vary, with the site of pulmonary air conditioning (warming and humidifying) extending below the level of the vocal folds during exposure to cold air (McFadden & Pichurko, 1985; McFadden, 1983). Given that pulmonary air warming may extend below the level of the vocal folds, passive temperature changes at the level of laryngeal skeletal muscle tissue may signal a change in muscle function (e.g., metabolism, contractile function). Little is known about active warming of laryngeal muscle tissue with voice use. Cooper and Titze (Cooper & Titze, 1985), described the temperature change of muscle tissue in an excised bovine larynx to be between 0.1°C–0.8°C during stimulated phonation. These values were smaller than predicted in a hypothetical model for temperature change with phonation; however, the authors acknowledged the limitations of the excised laryngeal model with regard to exposure to ambient temperature and humidity conditions and inability to simulate blood flow in the vibrating tissues. The excised bovine laryngeal temperature changes described above may not appear to be large; however, temperature changes greater than 0.5°C are considered physiologically significant for skeletal muscle function (Brooks, Fahey, & Baldwin, 2005). Therefore, understanding how temperature changes in the upper airway affect voice function is important for understanding laryngeal muscle physiology *in vivo*.

It is well established that biological systems are sensitive to small incremental changes in temperature and tightly regulated to maintain homeostasis. With regard to muscle physiology, mechanical and energetic parameters have variable responses depending on the degree to which muscle temperature increases (Rall & Woledge, 1990). Tissue temperature changes may occur passively through environmental thermal exposure, actively via muscle contraction, or through a combination of both passive and active factors. Enzyme catalysis is faster with small increases in temperature (Brooks, Hittelman, Faulkner, & Beyer, 1971; Clarke, 2006; Nelson & Cox, 2008; Powers & Howley, 2007). For even small increases in muscle temperature (36°C to 37°C) there is believed to be an acceleration of skeletal muscle metabolic rate (Wirth, Van Lunen, Mistry, Saliba, & McCue, 1998), affecting facilitating enzymes in metabolic pathways such as glycolysis, where six of the 10 steps in the glycolytic pathway are near equilibrium, e.g., aldolase, glucose 6-phosphatase (Nelson & Cox, 2008). Skeletal muscle exercise and heat stress has also been documented to increase intramuscular carbohydrate utilization (Febbraio, 2001; Starkie, Hargreaves, Lambert, Proietto, & Febbraio, 1999), increase the affinity for oxygen binding to hemoglobin and facilitate faster unloading of oxygen from hemoglobin (Brooks et al., 2005), and elevate skeletal muscle ATP turnover with higher (Gray, De Vito, Nimmo, Farina, & Ferguson, 2006).

Temperature changes in the laryngeal tissue that may occur for individuals who work in thermally extreme environments may influence voice function. Our understanding of vocal function will be enhanced through manipulation of ambient temperature and vapor pressure, both of which collectively influence relative humidity. Accounting for vapor pressure is

necessary because the saturation of water vapor in the environment varies widely relative to temperature. Relative humidity (%) is calculated as follows: ratio of the partial pressure of water/ saturated water vapor pressure of water at a specific temperature $\times 100$ (Lawrence, 2005; Miers, 1991). In order to isolate the effect of temperature on voice function both relative humidity and vapor pressure need to be accounted for. If the two are not isolated, then there may be variance in the amount of water vapor in the air that is not accounted for that could affect voice function.

Study of mouth versus nose breathing in temperature variant environments may identify regional pharyngeal temperature changes that can influence clinical measures of laryngeal tissue function. The role of ambient temperature on voice function *in vivo*, an integral environmental parameter that has not been included in current discussions of laryngeal physiology, presents many technical challenges given the difficulty in accessing laryngeal tissue *in vivo*. The purpose of this investigation was to combine thermal environmental manipulation and temperature measurement methods (transnasal supraglottic temperature probe) from the field of kinesiology with established clinical measures of vocal function (phonation threshold pressure [PTP] and perceived phonatory effort [PPE]) in a novel multidisciplinary approach to identify proposed upper airway temperature and voice function changes secondary to passive environmental exposure in five temperature/relative humidity environments. It was hypothesized that PTP and PPE would increase and upper airway temperature decrease following exposure to cold and/or dry air. Greater changes were expected with mouth versus nose breathing.

Methods

Participants

Twenty volunteers (10 female and 10 male) between the ages of 19 and 35 were recruited from the local community following receipt of approval from the Auburn University Institutional Review Board for human subjects. Based on the PTP literature (Roy, Tanner, Gray, Blomgren, & Fisher, 2003; Sivasankar et al., 2008; Verdolini-Marston et al., 1994; Verdolini-Marston et al., 1990), previously published thermal studies (Pascoe, Bellinger, & McCluskey, 1994; Pascoe, Smith, & Molloy, 2004), and power analysis (Stevens, 2002), 10 participants were determined to be adequate for a robust degree of power (0.8) and effect size (0.89). Inclusion criteria were: ability to match pitch on a screening task and no evidence of laryngeal pathology as determined via videostroboscopic screening. Volunteers were excluded from participation for any of the following reasons: health conditions that prohibited exposure to cold/hot environments or interfered with the proper insertion of the transnasal thermal probe; history of laryngeal pathology, diabetes, allergic rhinitis, and/or respiratory disease; active smoker at the time of the investigation; and/or contraindicated medications known to dry the laryngeal mucosa or alter thermal responses. Consented participants also completed a behavioral questionnaire to identify type and amount of daily voice use, history of voice training, amount of caffeine consumed daily, and amount of fluids consumed daily for use in post hoc data analysis.

Experimental Procedures

Prior to inclusion in the study, each volunteer completed a videostroboscopic laryngeal structure and function screening (Digital Videostroboscopy System Model 9295 with a rigid 9106 endoscope, KayPENTAX, Montvale, New Jersey). Images were then reviewed by a board-certified otolaryngologist and determined to be free of laryngeal disease. Modal frequency for use during PTP data collection was determined from repetition of the phrase "The blue spot is on the key again," a phrase that is balanced for both front and back vowels, using Real Time Pitch software (Multi-Speech Model 3700 Version 3.2, KayPENTAX). The

frequency selected for the PTP elicitation task was modeled for the participants using a standard pitch pipe (WM. Kratt Co., Kenilworth, New Jersey) and pitch-matching accuracy was determined using Real Time Pitch software.

Voice Function Assessment

Each participant completed pre-trial familiarization for each of the three data points collected: transnasal placement of the upper airway temperature probe (Exacon thermister probe, T-F1345, Roskilde, Denmark), PTP, and determination of PPE. For determination of upper airway temperature superior to the epiglarynx, the examiner or the participant (participant choice) advanced the flexible thermistor probe (diameter = 1.33 mm) transnasally, approximately 12–15 cm from the tip of the nose to control for a depth that was close to the epiglaryngeal structure (E. R. McFadden, Jr et al., 1985) but not deep enough to cause frequent gagging, throat clearing, coughing, or general discomfort. Depth was determined by centimeter markings on the probe and by visualization of the probe in the participant's pharynx following insertion. Measurement of upper airway temperature (°C) was recorded from a Squirrel Data Logger 2020 Series (Grant Instruments, Hillsborough, New Jersey) at the pre-determined data collection time points.

PTP was measured at modal frequency only to best assess real world conditions for speech tasks and to best capture vocal function changes before any probable temperature change in the upper airway secondary to extended voice use. Because this study protocol involved repeated measures for ten different conditions over a 2–3 day time period, accuracy of pitch-matching ability was reviewed using the Real Time Pitch software prior to the start of any trials. Task elicitation training for phonation threshold pressure was scripted to standardize directions across participants and data were collected using the KayPENTAX Phonatory Aerodynamic System (PAS) Model 6600. Task familiarization included production of a 5 syllable train of the CV combination /pi/ at a rate of 1.5 syllables per second (95 beats per minute), first above threshold, then below threshold, and at threshold (Verdolini-Marston et al., 1994). Consistent rate production for the PTP task was maintained by providing a flashing light from a digital metronome (Korg, TM-40, Japan). Participants were allowed to practice until comfortable with the procedure and it was determined that the data produced met the following criteria: (1) frequency within one semitone of the participant's target modal frequency; (2) productions were perceptually judged to be produced at a level that was as quiet as possible (Verdolini-Marston et al., 1990); (3) airflow measurements were negligible (considered to be less than 20 ml/s) to ensure appropriate lip closure (Solomon & DiMattia, 2000); (4) all of the peaks within the PTP string were judged, via visual inspection, to be of equal height (Milbrath & Solomon, 2003; Verdolini et al., 1994); and (5) voicing was evident during vowel production (Solomon, 2011).

PPE was determined after reading the Grandfather Passage at a comfortable loudness level. Participants were oriented to a 100 mm visual analog scale, with the left anchor labeled "no effort" and the right anchor labeled "maximum effort," on which they were asked to place a mark indicating degree of perceived voicing effort following the reading task.

Environmental Conditions

All trials were conducted within an environmental chamber specifically designed to establish and maintain each of the 5 different temperature and relative humidity conditions chosen for this study. Three temperature (°C) conditions were used at a constant relative humidity (RH,%): cold (15°C/40% RH), thermally neutral (25°C/40% RH), and hot (35°C/40% RH), with 40% selected as the target relative humidity based on the literature review (Fang, Wyon, Clausen, & Fanger, 2004; Richter, Lohle, Maier, Kliemann, & Verdolini, 2000). Two additional temperature (°C) conditions varied relative humidity (RH,%) to

match vapor pressure for the thermally neutral condition (25°C/40% RH) as follows: cold (15°C/74% RH) and hot (35°C; 23% RH). As is evident in Figure 2 the percent water vapor in the environment is not equivalent when matching for 40% relative humidity across the three temperature environments.

The three temperature targets were selected to represent realistic outdoor temperatures in which extensive voice use may be required, i.e., outdoor performance spaces during scheduled music and theatre seasons. Nose versus mouth breathing trials were included for each of the temperature conditions to account for airway conditioning differences that have been suggested to influence superficial laryngeal viscosity and laryngeal function (Sivasankar & Fisher, 2002, 2003).

Environmental parameters were closely monitored with a wet bulb global temperature device (QUEST°34 Thermal Environment Monitor, Quest Technologies), a device commonly used in environmental and thermoregulation research. Participants were scheduled to complete 1–3 different temperature conditions during one laboratory visit with each of the trials scheduled at approximately the same time of day to avoid thermal differences secondary to circadian rhythm (Refinetti, 2010). For all temperature conditions participants were asked to wear similar clothing for the test sessions and to refrain from caffeine, meals, hot/cold beverages, and exercise for at least one hour prior to each trial. All female participants were asked to schedule trials during days 7–12 from the start of menstruation (prior to ovulation) to avoid any effects that hormone levels may have on the data collection (Abitbol, Abitbol, & Abitbol, 1999). Prior to each thermal condition, the participants were asked for a urine sample for specific gravity analysis (Refractometer, ATAGO®, Tokyo, Japan), a routine objective measurement of systemic hydration. A value < 1.020 g/ml was used as a threshold for hydration criterion based on previous thermal studies (Pascoe & Fisher, 2009). Values greater than 1.020 g/ml required consumption of room temperature water until the criterion was established prior to start of any trial.

Data Collection

During each breathing trial, pharyngeal temperature (C°) was recorded 20 minutes after the start of each trial to allow for participant thermal equilibration to the pre-established environmental condition. Twenty minutes was chosen for physiological equilibration to the environment based on recommendations from the International Academy of Clinical Thermography (“Quality Assurance and Patient Safety,” 2003). Immediately after the pharyngeal temperature recording, participants completed the PTP task using the modal frequency that was determined prior to the trial and using the PTP method described above. Finally, participants read the standard passage and indicated their perceived phonatory effort on the 100 mm visual analog scale previously described. Following collection of the above data, the participant changed to the second breathing condition (mouth or nose) with the same measures repeated at the end of the 20 minute exposure. The order of breathing conditions (mouth or nose) within a temperature condition and the order of the 5 different environmental conditions were counterbalanced across participants to account for order effect. Table 1 illustrates the basic methods implementation for this research.

Data Analysis

A within-participant repeated measures analysis of variance (RM-ANOVA) was used in a 2 (mouth vs. nose inhalation) by 5 (5 environmental conditions) design to determine significant differences measured for mouth breathing versus nose breathing for PTP, pharyngeal temperature, and PPE. Significance was set at $\alpha < 0.05$. Pair wise post hoc comparisons were performed where warranted. Normality assessments were made with visual inspection of QQ plots and histograms. All data met normality assumptions and no

transformations of the raw data were required; therefore all of the data analyses reported in this investigation are based on the raw data. The data analysis for this paper was generated using SAS software, Version 9.2 of the SAS System for XP-Pro (2008, SAS Institute Inc., Cary, NC).

Results

Participants

In total, 20 (10 women; 10 men) volunteers met the study inclusion criteria and agreed to participate in the study. Data from 5 participants were excluded from data analysis because two of the participants disclosed a history of asthma after data collection and three of the volunteers experienced intermittent nasal congestion during data collection. The remaining fifteen participants (8 women, 7 men) ranged in age from 20–26 ($M = 21.73$; $SD = 1.53$). Power for this investigation is retained given the previous rationale that indicated a minimum of 10 participants for sufficient power.

Effect of Environment on Phonation Threshold Pressure

All of the PTP data for the 15 participants that met the narrow criteria for inclusion in data analysis for this study as described above were included in the data analysis. To eliminate the possibility of phonation threshold pressure calculation error via manual PTP analysis, all pressure peak trains that met the study criteria were measured and averaged via the automated measurement software for the PAS system. The intensity and frequency at which each PTP trial was produced was carefully examined across trials for each participant to ensure that any increase or decrease in PTP could not be attributed to change in frequency or intensity for any syllable train. As a result of the narrow criteria for valid PTP values, the number of data points in each of the ten groups (2 breathing conditions $\times$ 5 environmental conditions) were not always equal. Within each environmental condition, acceptable PTP trials across subjects ranged from 11–15, with two conditions (15°C/40% RH, mouth breathing & 25°C/40% RH, mouth breathing) having 11, one condition (35°C/40% RH, mouth breathing) with 15, and the remaining seven ranging from 12–14. Inability to achieve minimal airflow during production of /p/ during the task was the primary reason for exclusion of PTP trials. No significant difference was detected for PTP values for nose vs. mouth breathing or between the 5 environmental conditions, $F(9,120)=0.62$, $p=0.779$. Refer to Table 2 for means and standard deviations for each of the ten conditions analyzed.

Effect of Environment on Upper Airway Temperature

Of the 15 volunteers in this study, one was not able to tolerate the transnasal thermistor probe; therefore, data for 14 participants were included in the data analysis. Means and standard deviations for pharyngeal temperature for all 10 conditions analyzed are outlined in Table 2. No significant differences were found between the groups when the full model was applied, $F(9,130)=0.78$, $p=0.634$. The p-value for nose versus mouth breathing, excluding environmental comparisons, was $p=0.69$ ruling out any need for post hoc comparisons. Environmental comparisons with all 10 conditions included, without including breathing condition, fell short of significance at $p=0.19$.

Effect of Environment on Perceived Phonatory Effort

All 15 participants were included in the data analysis for PPE. The means and standard deviations for each of the 10 conditions are included in Table 2. No significant differences were found between the groups when the full model was applied, $F(9, 140) = 0.42$, $p = 0.921$.

Discussion

There are long held beliefs, particularly in the vocal performance arts, that the temperature of the air in which one is vocalizing will dramatically affect voice function, for better or worse. There has been no specific research effort to date to investigate whether there is any merit to these claims. Through complimentary of measures of upper airway thermoregulation from the field of exercise science and well-established vocal function measures, this research effort hypothesized that significant differences in vocal function and upper airway temperature would be observed *in vivo* following equilibration to environments that varied in temperature and relative humidity. It was hypothesized that PTP and PPE would be higher following mouth versus nose breathing and following exposure to cold and/or dry environments. The findings described above do not provide support for these hypotheses in the young participant pool studied who were free of health conditions known to perturb voice function. The absence of significant differences is consistent with John Widdicombe's hypothesis that the upper airway epithelium has a large margin to compensate for water loss secondary to alterations in ambient temperature and humidity (Widdicombe, 1997). These findings suggest that the upper airway is highly efficient at thermoregulatory control and maintenance of laryngeal epithelial viscosity when not challenged by medications, disease, or exercise.

For the realistic environmental conditions used in this study, ambient temperatures ranging from 15–35°C did not influence threshold oscillation pressure for the resting breathing conditions assessed. These findings do not provide support for the earlier assertion that temperature may be an important variable to include in the theoretical construct of phonation threshold pressure; however, this investigation may not have provided enough of a passive or active thermal challenge to the upper airway to realize a difference in PTP. The lack of significant difference in PTP between mouth versus nose breathing in each environmental condition tested departs from early findings (Sivasankar et al., 2008; Sivasankar & Fisher, 2002) of lower PTP values with nose breathing; however, these findings are consistent with more recent data presented by the Sivasankar laboratory (Erickson-Levendoski & Sivasankar, 2011; Sundarajan, et al., 2012). The difference in these findings may be attributed to participant selection differences and protocol scheduling considerations. In this research effort the female volunteers completed the trial protocol during the same phase of the menstrual cycle, between days 7 and 12, to control for proposed changes to laryngeal mucosal viscosity throughout the menstrual cycle (Abitbol, Abitbol, & Abitbol, 1999) and the influence of these viscosity changes on PTP values (Titze, 1988). Research also indicates significant core temperature differences through the primary phases of the menstrual cycle (Coyne, Kesick, Doherty, Kolka, & Stephenson, 2000) which may also influence measurement of upper airway temperature and intrinsic laryngeal skeletal muscle function.

Gastroesophageal reflux, extra-esophageal reflux and any degree of allergy or asthma history were also tightly controlled for in this study to further refine our understanding of this measure in healthy subjects. These conditions and many of the medications taken for these conditions may interrupt the physiology of laryngeal epithelial health as demonstrated in recent basic research (Erickson-Levendoski & Sivasankar, 2011). The potential variability of the measurement of PTP was reduced through careful and repeated training of the task protocol as well as careful maintenance of task elicitation parameters within and between test conditions for each volunteer. Variability inherent in human subject research was also reduced by using a within-volunteer repeated measures design.

While statistical significance was not established for temperature differences between the environmental conditions or the breathing conditions, there were temperature responses identified that exceeded the physiologically meaningful difference of 0.5°C. The location of

the temperature measurement was above the aryepiglottic folds, requiring careful interpretation of probable temperature changes in the larynx. Using the airway thermal mapping research (McFadden, et al., 1985) and the distance reported from the tip of the nares to the glottis (mean=18.2 cm, SE+0.6), it is likely that the thermistor probe to glottis distance in this study ranged from 3–6 cm, a distance sufficient enough to alter the temperature of the air in either direction by the time it passed the vocal fold tissue. Conclusions cannot be made regarding degree of temperature change to the laryngeal tissue itself.

The non-significant findings for PPE and PTP are consistent with the literature referenced earlier that describes these measures as changing in a similar direction in response to environmental or behavioral perturbations (Sivasankar et al., 2008). The task choice for this investigation was use of a standard reading passage, a voice task that does not typically challenge the vocal mechanism. The Grandfather Passage may not have been the best task to discern small changes in vocal function.

Given that both vocal function measure (PTP and PPE) differences pre/post environmental exposure failed to meet significance, we are left with several possible conclusions: 1) maintenance of superficial epithelial viscosity in healthy men and women may be more tightly regulated than previously proposed with mouth versus nose breathing; 2) the vocal function measures used in this study were not sensitive enough to measure changes in the laryngeal physiology that may have occurred; 3) the upper airway, which serves as a primary buffering region between the environment and the human body's core with a constant stream of air moving in both directions, may have unique physiological mechanisms which are yet to be identified and described. Further research will be needed to delineate which of these explanations help to explain the non-significant vocal function measures described in this study.

A strength of this research is that it establishes a foundational environmental design from which to study vocal function *in vivo* to better understand vocal perturbations that are hypothesized to lead to voice disorders, e.g., gastroesophageal reflux, high intensity use of the voice, respiratory disease, and medications. An additional strength was the consistent task elicitation procedures for phonation threshold pressure over several environmental conditions and multi-day data collection time points. The successful use of PTP within the tightly controlled parameters required supports the clinical utility of PTP measures between clinical visits when appropriate training is provided.

There are also weaknesses identified in this project. While participant selection was strong for inclusion of both sexes and regionally-balanced representation of different ethnic groups, the volunteer pool was largely limited to young adults, limiting generalizability to all adults. Additionally, the study design did not introduce any vocal perturbations that are believed to influence voice function. Because of the complexity of the study protocol and the high number of variables that required tight control, it was decided to limit this initial effort to a basic design.

Limiting measurement of PTP to modal frequency only may have limited the ability to discern small changes in vocal function. For this foundational investigation on environmental influences on vocal function following resting breathing, variables that could potentially influence threshold oscillatory pressures and PPE were tightly managed. Given the physiological constraints of the measures being taken before probable laryngeal tissue temperature or superficial laryngeal viscosity changes from repeated voicing attempts, it was decided to measure only one pitch and limit it to three trials to avoid excessive voice use prior to assessment of PPE. Past experience has shown that obtaining PTP for high

frequencies can be difficult and effortful for many volunteers and changes to the upper airway/laryngeal environment secondary to that effort may have influenced the PPE measure that was being measured immediately afterward. Clinically, little attention has been paid to the amount of voice use prior to objective voice assessment and how the physiological ramifications of vocal dose impact objective voice measures. If small changes in laryngeal skeletal muscle temperature and/or viscosity occur with voice use and influence vocal function, the order in which objective voice measures are taken may also matter and warrants further study.

The environmental conditions selected may not have been challenging enough to influence voice function; however, the conditions used reflected realistic environmental conditions for extensive voice use by professional and occupational voice users. Greater upper airway temperature differences would have likely been realized if the arctic temperatures used in the early airway conditioning literature (Cole, 1953a; McFadden, et al., 1985) had been replicated. Additionally, the lack of significant findings may be due to use of a resting breathing condition without voice use; however, this effort focused on passive thermal challenge on voice function with limitation of as many extraneous variables as possible that might change laryngeal skeletal muscle physiology between participants. Subsequent work with this environmental model should include challenges of vocal duration and intensity in realistic environmental conditions that mirror those of professionals believed to be at greater risk of developing voice disorder. Investigation of active vocal loading in temperature challenging environments will contribute to a body of evidence that will address long-held beliefs regarding ambient environment and voice function. Refining our knowledge of environmental influences on voice function may also support workplace policy development for reducing incidence of occupationally-based voice disorder.

This study manipulated environmental temperature to better understand how passive warming or cooling of the upper airway may influence voice function. The findings of this investigation suggest that upper airway and laryngeal physiology is tightly regulated for thermoregulation and superficial laryngeal viscosity in the population included in this study. The findings also indicate that there is much to learn regarding the unique role that upper airway tissue and muscles play with regard to airway conditioning and the persistent exposure to variable external environmental conditions that must then be accommodated for core temperature maintenance. Given the dual role of the larynx for respiration and phonation, temperature of the environment and the influence of that temperature change on muscle function and superficial viscosity maintenance deserve further study. Many long-held beliefs about human behavior and disease are founded in careful observation of those environmental and physiological factors that influence function, with voice being a primary function of interest for all professionals who require effective voice use to be successful in their work. There is a strong rationale for additional investigation into environmental impacts on voice function to provide a scientific basis from which to refine workplace environmental policy and potentially prevent work-related voice impairment.

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Figure 1. Model of relevant physiological variables

Ambient temperature may influence voice function via changes from upper airway conditioning response, laryngeal viscosity and/or laryngeal tissue temperature.

A

B

Figure 2. Partial Vapor Pressure & Relative Humidity by Temperature

A represents the variability in partial vapor pressure when relative humidity is held constant. B represents the relative humidity (RH%) variability when partial vapor pressure is held constant. Water vapor pressure is referred to as partial vapor pressure in an environment that is mixed with other gases. *mbar represents the unit millibar. Values for the graphs were derived by the author from a standard vapor pressure of water table (Diem, 1962).

Table 1

Study Design.

Environment	Experiment	Measures
15°C/40% RH	Nasal route breathing	Hydration level (g/ml) Pharyngeal temperature (°C) Phonation threshold pressure (cm/H ₂ O) Perceived phonation effort (mm/100mm)
15°C/74% RH		
25°C/40% RH		
35°C/23% RH	Oral route breathing	
35°C/40% RH		

RH=relative humidity. The highlighted environmental conditions are those that were matched for partial vapor pressure.

Table 2

Means and Standard Deviations for Pre/Post Voice Measures

Environment		PTP(cm H ₂ O)	Effort (mm)	Temperature (°C)
<i>Nose/Mouth</i>				
15°C/40% RH	nose	4.14 + 0.99	11.73 + 9.24	34.97 + 2.40
15°C/40% RH	mouth	4.41 + 0.69	14.93 + 13.70	34.61 + 1.96
15°C/74% RH	nose	4.14 + 0.85	12.87 + 10.08	34.39 + 1.88
15°C/74% RH	mouth	3.92 + 0.82	16.13 + 15.26	34.48 + 1.56
25°C/40% RH	nose	4.07 + 1.30	9.53 + 10.22	34.17 + 1.99
25°C/40% RH	mouth	3.96 + 1.02	12.33 + 11.25	34.46 + 1.68
35°C/23% RH	nose	3.63 + 0.78	14.33 + 8.24	35.28 + 0.94
35°C/23% RH	mouth	4.07 + 1.17	13.13 + 7.86	35.51 + 1.56
35°C/40% RH	nose	4.05 + 0.82	12.80 + 10.83	34.52 + 2.14
35°C/40% RH	mouth	3.82 + 0.73	15.00 + 14.10	34.86 + 1.29

Values are reported as means +/- standard deviations. The highlighted conditions are those that were matched for vapor pressure. RH=relative humidity