In Vivo Measures of Vocal Function Response and Upper Airway Thermoregulation Following Exposure to Varying Environmental Conditions

by

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Abstract

Occupationally-related voice disorders affect a large number of individuals each year, costing the U.S. healthcare system the equivalent in health care dollars spent for the treatment of pediatric ear infections. To date, the influence of upper airway temperature changes on voice function, either passively through environmental temperature manipulations or actively through physical activity, has not been studied. The influences of skeletal muscle tissue temperature change and muscle physiology have been studied extensively in limb skeletal muscles with evidence of thermal effects on muscle biochemistry, contractile function and bioenergetics, yet it is unclear if this research can be translated to the intrinsic laryngeal skeletal muscles. The primary goals of this research were as follows: 1) Determine vocal function differences in five different temperature/relative humidity environments, and 2) Determine vocal function differences following a short bout of submaximal exercise with a targeted respiratory rate of 20 breaths per minute. For both investigations the following measures were taken: phonation threshold pressure, perceived phonatory effort, and pharyngeal temperature. For Aim 1, no significant differences were found for phonation threshold pressure, perceived phonatory effort, or pharyngeal temperature across environments, indicating that the upper airway is tightly regulated for mouth and nose breathing conditions in healthy young adults who were free of any conditions that are currently attributed to the development of voice disorders. For Aim 2, significant increases in phonation threshold pressure and perceived phonatory effort were found with exercise as well as a significant reduction in pharyngeal temperature (1.9°C). The findings support the well-established belief that voice use with physical activity requires greater physical effort. Future investigations should extend these basic models of ambient temperature/relative humidity environments and increased respiratory rate to include volunteers with conditions that

are believed to contribute to the development of voice disorder, e.g., reflux, asthma, allergies. Findings from this research may contribute to the development of workplace environmental policy that will promote greater vocal health and reduce the incidence of voice disorders.

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List of Abbreviations and Definitions

Laryngeal viscosity – for the surface fluid of the laryngeal epithelium, viscosity describes the fluid's internal resistance to flow and may be thought of as a measure of fluid friction: low viscosity implies ease of movement, high viscosity indicates increased resistance.

Perceived phonatory effort (PPE) –This measure is typically taken following reading of a standard passage (e.g., Grandfather passage or Rainbow passage). The method for determining effort varies throughout the published literature ranging from a 1-10 scale, to a visual analog scale (100 mm), to participant choice of scale with equalization of scales following data collection.

Phonation – a clinical term for voice production.

Phonation threshold pressure (PTP) – reported in cm of H_2O, a measure of the minimum amount of pulmonary air pressure that is required to initiate vocal fold vibration.

Relative humidity (RH) – actual water vapor pressure divided by saturated water vapor pressure at a given temperature.

Urine Specific Gravity – a measure of the density of urine as compared to pure water

Vapor Pressure – the pressure of a vapor in thermodynamic equilibrium – a liquid evaporates at all pressures *below* its vapor pressure and remains stable at pressures *above* vapor pressure. Water vapor pressure in an environment that is mixed with other gases is referred to as *partial vapor pressure*.

I. Introduction

Voice disorders affect approximately 7.5 million Americans (www.nidcd.nih.gov), have a lifetime prevalence of 28%-57%, and have a negative impact on work productivity and quality of life (1). While the causes of vocal pathology and functional voice disorders are not well understood, investigations of environmental factors on voice function have largely been limited to studies of superficial hydration (2-4), systemic hydration (5-7), and relative humidity (2, 3, 5, 8), all factors shown to influence optimal voice function and perception of vocal ease (3, 5, 7, 9). Despite our understanding of hydration and relative humidity, evidence that some professions are at higher risk of developing voice disorders (1, 10, 11), and clinical advice to avoid voice use in extreme temperatures, U.S. guidelines for avoidance of work-related injury do not fully address environmental standards that may prevent work-related voice disorders. Other countries have standards regarding specific temperature, humidity and dust particle compositions that are acceptable for an optimal work environment (12-14). A comprehensive review of the U.S. Department of Labor Occupational Safety and Health Administration work environment standards (15) identified standards for workplace ventilation (i.e., targeted reduction of dusts, fumes, mists, vapors, and gases) with criteria for ambient temperature in the range of 68-76°F and humidity range of 20-60%. The environmental criteria described may be cool or too dry for optimal for vocal health. This mismatch between government environmental standards, published evidence, and recommendations made by healthcare providers treating voice disorders signals a gap in our understanding of environmental influences on voice production. Specifically, we lack knowledge about the potential influences of conditions that may alter laryngeal skeletal muscle temperature and physiology, either passively through

ambient temperature manipulations or actively via increased respiratory rate secondary to physical activity on vocal function.

Professionals working in thermally extreme environments (e.g., coaches, physical education teachers, performers, laborers, military personnel) (1, 10, 16) are subjected to varying temperature environments during vocally demanding tasks, yet we know little of how these temperature changes may support optimal voice use or contribute to work-related vocal injury. A study of laryngeal pathology by occupation reported a higher incidence of vocal polyps and nodules in factory workers who sought care for a voice disorder (16), a population that is exposed to highly variable environmental conditions. A study of laryngeal appearance and function in outdoor performers reported a significantly higher degree of vocal fold edema and decreased vocal fold vibratory behavior for those in the worst environmental conditions (11). Evidence from meteorological (17, 18), pulmonary air conditioning (19-26), and respiratory epithelial water transport (27, 28) literature describes an *interdependence* between humidity and temperature with percent saturation of water vapor changing dramatically with small changes in temperature. The temperature of the 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 and with increased ventilation rate (20, 29). Given that pulmonary air warming may extend below the level of the vocal folds, a temperature change at the level of laryngeal tissue may cause a change in tissue function (e.g., bioenergetics). Our understanding of vocal function will be enhanced through manipulation of ambient temperature and vapor pressure, both of which collectively influence relative humidity. Increased respiratory rate secondary to submaximal exercise may cause upper airway temperature and superficial laryngeal viscosity changes that influence clinical measures of laryngeal tissue function.

The combination of thermal environmental manipulation and temperature measurement methods (transnasal supraglottic temperature probe) from the field of kinesiology with established clinical measures of vocal function (i.e., phonation threshold pressure [PTP] and

perceived phonatory effort [PPE]) used in these investigations represents a novel multidisciplinary approach for the study of relationships between proposed upper airway temperature changes and vocal function. Given the anatomical and physiological challenges of accessing the laryngeal tissues *in vivo*, the combination of these indirect measures of tissue temperature and tissue function were best suited to investigate the role of ambient temperature/humidity changes and vocal function through manipulation of ambient temperature/relative humidity and respiratory rate secondary to submaximal exercise.

II. Review of Literature

Our knowledge of environmental influences on vocal function, the development of voice disorder or pathology, and recovery from voice disorder is limited despite evidence that voice disorders affect approximately 7.5 million Americans (www.nidcd.nih.gov), have a lifetime prevalence of 28%-57%, and have a negative impact on work productivity and quality of life (1). The upper airway, which includes the larynx, serves a unique role as a transitional structure between the environment outside of the body and the body's tightly thermoregulated core, creating the need for specific physiological regulation of temperature and superficial epithelial viscosity that may be unlike any other structures of the human body. This primary role of the upper airway, to condition (warm and humidify) inspired air, may also influence vocal function.

It is well established that higher ambient humidity has a positive effect for vocal function and perception of vocal ease (2, 3, 5, 7, 8); however, the potential influence of upper airway temperature change on vocal function has not been described. Identifying the degree to which temperature changes in the upper airway tissue, including the larynx, is important to better understand epithelial, muscle and tissue physiology (metabolic, biochemical, and mechanical) that may be altered with tissue temperature changes. The pulmonary science literature has established that upper airway temperature may be altered by exposure to ambient temperature changes (19, 21-23) and/or with changes in respiratory rate (20, 25). Professionals who work in thermally extreme environments, e.g., physical education teachers, performers, military personnel and laborers, are subjected to varying temperature environments during vocally demanding tasks. Other professionals require durable voice production while the respiratory rate is elevated above resting, e.g., military personnel calling cadence, fitness instructors, and

teachers. Our present understanding of measures of vocal function, phonation threshold pressure (PTP) and perceived phonatory effort (PPE), typically gathered in a thermally neutral clinical or research environment, may not adequately describe vocal function in realistic environments or during respiratory tasks that fall outside of this neutral range. Investigation of ambient temperature and vocal function requires basic understanding of laryngeal epithelial fluid maintenance; the influence of pulmonary behaviors and ambient air conditions on fluid maintenance; and the relationship of airway temperature to respiratory rate and oral versus nasal breathing route. Finally, knowledge of the strengths and limitations of widely used measures of vocal function is necessary to interpret upper airway responses to varying ambient conditions and respiratory behaviors.

Assessing Vocal Function

Given the anatomical and physiological challenges of accessing the laryngeal tissue, indirect measures, such as phonation threshold pressure (PTP) and perceived phonatory effort (PPE), have been used to evaluate vocal function. Voice researchers have assessed the effect of environment to a limited degree with a primary emphasis on ambient relative humidity manipulation, for voice signal acoustic parameters (9, 30), aerodynamic measures of phonation threshold pressure (PTP) (2, 5, 7), and perceived phonatory effort (PPE) (3, 8). In these studies, acoustic, aerodynamic measures, and PPE were used as indirect measures of voice function in the typical clinical environment or following exposure to environments with different relative humidity levels. These indirect measures of laryngeal function were collected in a range of humidity environments (20%-75% humidity) with two of the studies (7, 9) reporting an ambient temperature of 27°C. A third study reported ambient temperature as a factor when studying the variability of objective voice measures (30), however, temperature values measured and statistical analysis of this variable relative to clinical voice measurement were not detailed in the methods or results sections of the paper.

Assessment of perceived phonatory effort following exposure to environments of varying humidity levels have been described by several investigators (3, 5, 7, 8), all of whom found a reduction in perceived phonatory effort (PPE) following exposure to a high humidity environment or when comparing nasal versus oral breathing. None of these investigations controlled for menstrual cycle in their female participants, a factor that may have influenced the PPE measures taken (31). The method used to determine vocal effort varied between studies as there is currently no standardized method for determining PPE.

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. Phonation threshold pressure (PTP) is described as the minimum amount of lung pressure required to initiate vocal fold vibration for voicing (32). For many years, this measurement has been used as an indirect measurement of intrinsic laryngeal muscle function and condition of the laryngeal mucosa, with the working hypothesis that PTP will vary as a function of vocal fold viscosity, velocity of mucosal wave of the cover of the thyroarytenoid muscle, pre-voice glottal width, and vocal fold thickness. The last three variables listed will vary with the frequency of vocal fold vibration. Titze's original mathematical model is as follows:

$$PTP = (2k/T)(Bc)(w/2)$$

where k is transglottal pressure coefficient, T is vocal fold thickness, B is a damping coefficient that is proportional to vocal fold viscosity, *c* is a constant, and w/2 is pre-phonatory glottal half-width (32). Specific for the present research focus, PTP is theoretically proportional to vocal fold tissue viscosity, which may have an inverse relationship with hydration level (5). This model does not include temperature of the vocal fold tissue or temperature of the conditioned respiratory air in the laryngeal environment in the calculation despite a likely role of temperature on muscle function, both intramuscularly and in response to the environment. The exclusion of temperature from this model may be accounted for in an earlier work co-authored by Titze (33), where the temperature change within an excised bovine larynx was found to be between 0.1°C-

0.8°C during stimulated phonation. These values were smaller than predicted in the 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.

To date, there is ample published evidence that ambient humidity may have a direct effect on vocal function, with drier air having a deleterious effect on vocal function and higher humidity environments facilitating less muscular effort for voice initiation as measured with PTP (2-8, 34). In a series of investigations of airway conditioning on superficial laryngeal viscosity, Sivasankar and colleagues (2, 3, 8) 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 findings suggested increased PTP and PPE following oral breathing, particularly for the higher pitches assessed.

More recently, the thesis that oral breathing leads to a decline of vocal function as evidenced by increased PTP and increased PPE was expanded by adding resting increased respiratory rate during the oral breathing challenge to further induce proposed airway dehydration (4). This is the first published evidence of the effects of increased respiratory rate with oral breathing as measured with PTP. Additionally, controls were compared to smokers, who as a group, are at increased risk of developing voice problems. Ambient temperature was not reported for this study and the study was limited to females. This investigation found that PTP did not differ between smokers and nonsmoking controls; however, both groups realized higher PTP values following the accelerated oral breathing challenge. Of interest, the potential role of temperature or alterations in upper airway thermoregulation from smoking was not discussed in this investigation. The temperature changes of the conditioned air with oral versus nose breathing and with increased ventilation rate, which were described above as a factor in respiratory system conditioning of inspired air, were not addressed.

Vocal Fold Surface Hydration

Our initial understanding of laryngeal epithelial fluid transport was translated from studies of tracheal epithelia using canine and ovine models. An investigation by Man et. al (35) that targeted the effect of temperature, humidity, and mode of breathing on secretions in the upper trachea of anaesthetized dogs, concluded that bulk water flux across the epithelium was higher with mouth breathing than nasal breathing. Boucher et al. (36) confirmed the findings by Man et al. (35) and concluded that the transepithelial osmotic gradient created by evaporative cooling may account for hydration of the tracheal surface. Additional work by Freed and Davis (37) postulated that hyperventilation in dry air increased airway surface fluid osmolality in a canine model, the findings of which were heavily criticized by McFadden and colleagues (38). McFadden and others identified primary flaws in Freed's work as follows: 50% or more of water loss during hyperventilation occurs above the level of the glottis, reliance on unidirectional airflow in Freed's model disregarded the replenishment of epithelial surface fluid through evaporative water during expiration, and similar studies failed to find the same results. These observations may indicate an up-regulation of mechanisms that maintain superficial epithelial viscosity.

Widdicombe investigated the role and maintenance of the airway surface liquid (ASL) as influenced by mechanisms of evaporative water loss (28). In his description of evaporative water loss from the ASL, he calculated that the degree of fluid loss would depend on many factors which could include ventilation rate and inspired air temperature and humidity. He qualified this calculation with the observation that there "seems to be a large margin to compensate for water loss" (p. 11).

A small body of literature has bridged the findings from the canine tracheal epithelium to the canine and ovine laryngeal epithelium. Fisher and colleagues (39) found evidence of bidirectional water flux controlled by $Na^{+}K^{+}$ATPase channels for both the excised canine and ovine laryngeal epithelium. In a later study (40), using the excised ovine laryngeal model,

investigators identified luminally-directed water flux following luminal exposure to osmotic perturbations. These studies provide evidence of laryngeal epithelial viscosity maintenance in the rabbit larynx. Translation of laryngeal epithelial research conducted with canine and ovine models to human laryngeal epithelial physiology may not be optimal because unlike humans (41), these panting species employ the respiratory mechanism (42-46) as a primary means of thermoregulation. This primary physiologic difference for thermoregulation may signal key differences in respiratory epithelial physiology that are yet to be described and considered in translational models. More recently, some investigators have conducted basic investigations of laryngeal epithelial physiology with the porcine laryngeal epithelium, a species that does not rely on respiration for thermoregulation (47, 48).

A recent review of vocal fold surface hydration mechanisms by Leydon et al., (49) provides a comprehensive summary of current knowledge from both basic and applied research of superficial liquid homeostasis of the larynx and the role that these mechanisms play in maintaining optimal vibratory characteristics of the vocal fold mucosa as follows: 1) the vocal fold epithelium plays a role in regulating surface liquid of the vocal folds; 2) superficial viscosity of the vocal folds are challenged by behavioral and environmental challenges; 3) vocal fold epithelium has a sodium-potassium ($Na^{+}K^{+}$-ATPase) pump, sodium-potassium-chloride ($Na^{+}K^{+}2Cl^{-}$) cotransporter, epithelial sodium channel (ENaC), and cystic fibrosis transmembrane regulator (CFTR) chloride channels to support ion transport across the membrane; 4) bidirectional water fluxes across the epithelium have been identified; and 5) airway epithelium can be both secretory and absorptive. 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 that describes temperature as an environmental variable of interest for upper airway fluid maintenance. Additionally, much of the applied research cited in the review (2, 3, 50) hypothesized that superficial laryngeal fluid would be reduced with mouth breathing, an assumption with which McFadden and others take issue.

Upper Airway Conditioning

The pulmonary airway conditioning literature should be considered in light of what we know of laryngeal epithelial viscosity and the mechanisms by which superficial laryngeal viscosity is maintained given the hypothesized influence of laryngeal viscosity for optimal voice function. The coupling of ambient temperature to humidity is emphasized in pulmonary research for the following reasons: 1) the percent saturation of expired air is a direct function of inspired air humidity and an inverse function of inspired air temperature (19), and 2) the existing relationship between evaporative losses and thermally induced secretion and reabsorption in mucociliary transport (20). The partial pressure of water vapor changes dramatically with small changes in temperature, particularly in the human physiological range of 30-40°C (17, 18).

The physiological responses that occur in respiratory tract conditions *in vivo* of inspired air at varying temperatures and at varying respiratory rates have been studied for some time (19, 21-23). An early investigation by McCutchan and Taylor (1951) described the respiratory heat exchange measured at the mouth with varying room air temperature (21° - 93°C) and vapor pressure (4.0-30mmHg) of inspired air. The authors concluded that expired air is not fully saturated, with the percent saturation a direct function of inspired air humidity level and an inverse function of inspired air temperature. Two initial studies by Cole (22, 23) measured nasally inspired air temperature and humidity in the pharynx, concluding the following: increased ventilation rate from 8 to 40 liters/minutes had little effect on the temperature and water content of nasally inspired air at the level of the pharynx; the inspiratory pharyngeal air temperature following nasal inhalation ranged between 31°C and 37°C when participants were exposed to ambient temperatures between “arctic and tropical” (-30°C to 50°C); water loss varied directly and heat loss inversely with the temperature of dry inspired air; and water and heat losses inversely corresponded with the water content of inspired air. A third investigation by Cole (24) described temperature changes of the conditioned air at several points along the respiratory pathway. Temperatures were measured at the nares, oropharynx, hypopharynx,

and trachea with a 3° C temperature increase from ambient air in the lower pharynx and an additional 2°-3° C increase of temperature as the air continued through the larynx into the trachea.

McFadden (20) hypothesized that respiratory heat exchange may challenge mucociliary transport with increased ventilation rate or reduced environmental temperature with the respiratory structures playing an increasing role in heat exchange. In an elegant study of airway temperatures *in vivo*, McFadden et al. (1985) inserted a flexible probe, with six thermistor probes placed 4.3 cm apart, into the tracheobronchial tree to measure air stream temperature at different points from the glottis to the distal bronchi under different inspired air conditions and during different ventilation rates. These investigators found that at resting ventilation, the conditioning process is largely confined to the upper airway, as was described by Cole (22, 23). However, the site of the conditioning process varied with increasing ventilation and changes in temperature and water content of the air breathed. The direct measurement of temperatures along the airway demonstrated the magnitude of airway cooling when participants were asked to increase ventilation in frigid air.

McFadden and colleagues (26) described the direct measurement of airway temperatures in participants during 5 minutes of exercise and 5 minutes of voluntary hyperventilation while inhaling frigid air (-17.8$\pm$1.8°C). They concluded that the airway temperatures measured did not differ significantly in the two groups. They hypothesized that the local thermal need of the human airway to recover heat and water may be independent of moderate increases in cardiac output, a primary difference from canine models of respiratory function.

Temperature and Tissue Function

The role of local temperature changes, increased (51) and reduced (52), in skeletal muscle contractile function and local metabolic activity of active skeletal muscle (53, 54), provide additional scientific rationale to support investigation of the role of ambient temperature and increased respiratory rate relative to proposed upper airway (laryngeal) muscle temperature

changes and voice function. The upper airway temperature changes described in the pulmonary conditioning section may not appear to be large; however, temperature changes greater than 0.5°C are considered physiologically meaningful for skeletal muscle function (55). 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. With regard to muscle physiology, mechanical and energetic parameters have variable responses depending on the degree to which muscle temperature increases (56). Temperature changes may occur passively, with changes in ambient temperature, or actively with exercise.

A key area of study in limb skeletal muscle physiology has been the speed with which biochemical reactions occur in skeletal muscle tissue secondary to temperature change within the tissue and the influence of these biochemical changes on muscle bioenergetics. Enzyme catalysis is faster with small increases in temperature (57-60), a well-described phenomenon called the Q10 effect (55, 58-60). Within the normal range of muscle temperature, the rate of reaction can double (55). Muscle temperature rises proportionally to workload which may be augmented by environmental thermal stress. For a realistic 2°C increase in muscle temperature that arises because of increased muscle workload and/or environmental thermal stress, application of the Q_{10} effect would translate to an approximate enzyme reaction increase of 30 to 40% (61).

For even smaller increases in muscle temperature (36°C to 37°C) there is believed to be an acceleration of metabolic rate (62), 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 (58). A comprehensive review of energy metabolism during exercise and heat stress (61) concluded that for physical activity in the heat that is sufficient to raise the core temperature (> 0.5°C) metabolic differences were characterized by increased intramuscular carbohydrate utilization. Starkie, et al. (63) described greater muscle glycogen

utilization with increased tissue temperature during submaximal exercise. There is additional evidence of increased muscle lactate accumulation during exercise and heat stress (64). The affinity for oxygen binding to hemoglobin is also temperature sensitive where increases in contracting skeletal muscle temperature facilitates unloading of oxygen from hemoglobin as described by the Bohr effect (55). Gray, et al. (51) described elevated skeletal muscle adenosine triphosphate (ATP) turnover with higher muscle temperatures.

There is also evidence that temperature variability has an influence on skeletal muscle contractile function, specifically force/velocity and power/velocity relationships (51, 65) if the increase in local muscle tissue temperature is sufficient (2°C to 3°C), sometimes referred to as the therapeutic temperature level (62). In an early discussion of temperature and muscle, Bennett (66) described that maximal force generation *in vivo* was not influenced by increasing temperature in the muscle tissue; however, time-dependent mechanisms were faster at higher temperatures. A later review of the influence of temperature on muscle contraction mechanics and energetics by Rall and Woledge (56) concluded that some non-time dependent factors are also influenced by temperature increases, e.g., tetanic force production. While a dramatic increase in tetanic forces observed with temperature changes from 0-20 °C have been attributed to greater cross bridge formation, force production in the more realistic physiological range of 30-35°C appears to plateau and then fall off slightly (56). With temperature increases in skeletal muscle, there is also evidence of a reduction of the affinity of Ca^{2+} for the Ca^{2+}-specific sites of troponin (67), an affinity that is required for rapid attachment of the myosin contractile protein to the actin contractile protein within the sarcomere, the smallest contractile unit of the muscle cell. Reduced affinity for Ca^{2+} binding would directly influence the speed with which the primary contractile proteins (actin and myosin) form crossbridges for muscle contraction. In a study of human skeletal muscle *in vivo*, De Ruiter and De Haan (65) manipulated temperature of the adductor pollicis muscle of the hand and found significant increases in peak tetanic force, maximal rate of force production, and relaxation rate as

temperature increased. There is also indication that muscle temperature may have a different effect on force fluctuations with age (52) and muscle fiber conduction velocity (51).

Finally, there is evidence that biomechanical advantages may occur with increases in tissue temperature. Specifically, increases in tissue temperature may increase range of motion (ROM) and facilitate greater compliance in the muscle-tendon unit (68). There is disagreement in the literature regarding the universal benefit of muscle-warming exercise. A recent review of the role of warm-up and stretching on performance concluded that they may be of particular importance for physical activity that requires high intensity stretch-shortening cycles of the muscle-tendon unit (68-71).

The extensive influence of skeletal muscle temperature on muscle tissue bioenergetics, contractile function, and biomechanics supports the importance of understanding the influence of tissue temperature changes in the upper airway on voice function. Thermoregulation of the laryngeal muscle and tendon tissues is a great challenge to study given the primary role of the upper airway in warming and humidifying the air that we breathe, a physiologic role not shared by limb skeletal muscle *in vivo*.

Review of Literature Summary

There is support in the literature for the hypothesis that ambient humidity and increased ventilation rate influence superficial viscosity of the laryngeal epithelium, resulting in reduction of airway surface liquid and directly influencing clinical and research measurement of phonation threshold pressure and perceived phonatory effort; however, this is not generally accepted across disciplines due to a belief that the upper airway is highly responsive to environmental perturbations including water loss. As of yet, there is no direct evidence of reduced sol layer depth in humans *in vivo* and given the disagreement in the pulmonary literature regarding this very issue, it is compelling to consider other physiological changes that may influence laryngeal muscle and tissue function. A relationship between ambient temperature change and vocal function has not yet been investigated despite a close relationship between temperature, partial

vapor pressure of water, and humidity as reported in the respiratory conditioning literature and a well-established understanding that temperature changes affect skeletal muscle function in limb skeletal muscle. Systematic study of the role of temperature changes to the conditioned air of the respiratory system would further our knowledge of vocal function in environments other than the thermally neutral environment of the typical clinical or research setting. This knowledge would provide additional insight to the demands placed on laryngeal tissue for vocal behaviors produced during exposure to cold and warm environments as well as with increased ventilation rates, all conditions described as influencing the temperature of the conditioned air of the respiratory system in which the vocal folds reside. A better understanding of temperature influences on clinical voice assessment may help refine our current instrumental data collection practices as well as expand our ability to better habilitate effective voice use under these realistic conditions.

This scientific investigation of upper airway thermoregulation and vocal function *in vivo* includes the following hypotheses:

1) *PTP and PPE will increase as the ambient temperature is lowered, with greater increase of PTP observed with oral breathing versus nasal breathing.*
2) *PTP and PPE will be higher and upper airway temperature will be lower following a submaximal aerobic challenge versus resting ventilation rate with nasal breathing.*

III. Journal Manuscript

Vocal Function and Upper Airway Thermoregulation in Five Different Environmental Conditions

ABSTRACT

Through a merger of voice science, exercise science, and pulmonary science literature, it was hypothesized that vocal function (phonation threshold pressure and perceived phonatory effort) and upper airway temperature (transnasal thermistor probe), would change following exposure to variable temperature conditions. Specifically, it was hypothesized that phonation threshold pressure and perceived phonatory effort would increase and upper airway temperature would decrease following exposure to cold and/or dry air. Significantly greater changes were expected with mouth versus nose breathing. Using a within-participant repeated measures design, 15 consented participants (10 men, 10 women) completed 20-minute equilibration trials for both nose and mouth breathing in five different environments (10 trials): 3 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 2 temperatures (°C) with variable relative humidity (RH,%) to match vapor pressure for the thermally neutral environment (25°C/40% RH) as follows: cold (15°C/74% RH) and hot (35°C; 23% RH). At the end of each 20 minute equilibration trial, measures were taken in this order: upper airway temperature, phonation threshold pressure, and perceived phonatory effort. Data were analyzed using repeated measures analysis of variance and no statistically significant differences were established. Findings from this investigation suggest that the upper airway is tightly regulated for temperature and superficial laryngeal viscosity and may have unique physiological characteristics when compared to limb skeletal muscle tissue.

INTRODUCTION

Professionals working in thermally extreme environments (e.g., military personnel, physical education teachers, performers, laborers)(1, 10, 16) are subjected to varying temperature environments during vocally demanding tasks, yet we know little of how these temperature changes may support optimal voice use or contribute to work-related vocal injury. A study of laryngeal pathology by occupation reported a higher incidence of vocal polyps and nodules in factory workers who sought care for a voice disorder(16), a population that is exposed to highly variable environmental conditions. An investigation of laryngeal appearance and function in outdoor performers reported significantly higher degree of vocal fold edema and decreased vocal fold vibratory behavior for those in the worst environmental conditions (11).

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 (72). There also exist specific claims regarding the deleterious effects of extreme temperature or humidity on voice function (73, 74); however, we lack adequate evidence to support these claims. Investigations of environmental factors on voice function have largely been limited to studies of superficial hydration (2-4), systemic hydration (5-7), ambient humidity (2, 3, 5, 8), and mouth versus nose breathing (2, 3, 8). All factors have been shown to influence optimal voice function and perception of vocal ease (3, 5, 7, 9). Specifically, these investigations have contributed to our current understanding that higher humidity environments and nasal breathing reduce both 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 practice with general vocal hygiene guidelines including suggestions to have a minimum of 40% relative humidity in work, performance, and home environments; however, little attention has been paid to the role of ambient temperature and voice function.

Despite our understanding of hydration and ambient relative humidity, evidence that some professions are at higher risk of developing voice disorders (1, 10, 11), and clinical advice to avoid voice use in extreme temperatures, particularly cold temperatures, U.S. guidelines for avoidance of work-related injury do not fully address environmental standards that may prevent work-related voice disorders. Other countries have standards regarding specific temperature, humidity and dust particle compositions that are acceptable for an optimal work environment (12-14). A comprehensive review of the U.S. Department of Labor Occupational Safety and Health Administration work environment standards (15) describe criteria for workplace ventilation (i.e., targeted reduction of dusts, fumes, mists, vapors, and gases) with criteria for ambient temperature (68-76° F) and relative humidity (20%-60%).

Our present understanding of two measures of vocal function, phonation threshold pressure (PTP) and perceived phonatory effort (PPE), typically gathered in a thermally neutral clinical or research environment, may not adequately describe vocal function in realistic environments that fall outside of this neutral range. Investigation of ambient temperature and vocal function requires basic understanding of environmental influences on laryngeal epithelial fluid maintenance; the influence of laryngeal epithelial fluid viscosity on voice function; and the influence of skeletal muscle tissue temperature change on muscle physiology.

Environmental Influences on Laryngeal Epithelial Fluid Maintenance

Evidence from meteorological (17, 18), pulmonary air conditioning (19-26), and respiratory epithelial water transport (27, 28) literature describes an *interdependence* between humidity and temperature with percent saturation of water vapor changing dramatically with small changes in temperature (24, 25). This basic research supports the importance of including environmental temperature as a vital factor in the study of the physiology of the human upper airway. Given that study of the laryngeal structures *in vivo* represents a great challenge, much of our knowledge of laryngeal epithelial fluid maintenance comes from animal models.

Our initial understanding of laryngeal epithelial fluid transport was translated from studies of tracheal epithelia using canine and ovine models. Man et. al (35), in an 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. Boucher et al. (36) confirmed the findings by Man et al. (35) and concluded that the transepithelial osmotic gradient created by evaporative cooling may account for hydration of the tracheal surface. Additional work by Freed and Davis (37) postulated that hyperventilation in dry air increased airway surface fluid osmolality in a canine model, the findings of which were heavily criticized by McFadden and colleagues (38). McFadden and others identified primary flaws in Freed's work as follows: 50% or more of the water loss during hyperventilation occurs above the level of the glottis, reliance on unidirectional airflow in Freed's model disregarded the replenishment of epithelial surface fluid through evaporative water during expiration, and similar studies failed to find the same results. John Widdicombe investigated the maintenance of the airway surface liquid (ASL) as influenced by mechanisms of evaporative water loss (28) and he calculated that the degree of fluid loss would depend on many factors that could include inspired air temperature and humidity.

A small body of literature bridges the findings from the canine tracheal epithelium to the canine and ovine laryngeal epithelium. Fisher and colleagues (39) found evidence of bidirectional water flux controlled by Na^+K^+ATPase channels for both the excised canine and ovine laryngeal epithelium. In a later study (40), using an excised ovine laryngeal model, investigators identified luminally-directed water flux following luminal exposure to osmotic perturbations. These studies provide evidence of laryngeal epithelial viscosity maintenance in the rabbit larynx. Translation of laryngeal epithelial research conducted with canine and ovine models to human laryngeal epithelial physiology may not be optimal because, unlike humans (41), these panting species employ the respiratory mechanism (42-46) as a primary means of thermoregulation. 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 (47, 48) which is more similar to human physiology.

A recent review of vocal fold surface hydration mechanisms by Leydon, et al., (49) 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 that describes temperature as an environmental variable of interest for upper airway fluid maintenance. Additionally, much of the applied research cited in the review (2, 3, 50) hypothesized that superficial laryngeal fluid would be reduced with mouth breathing, an assumption with which McFadden and others take issue.

Laryngeal epithelial 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. Phonation threshold pressure (PTP) is described as the minimum amount of lung pressure required to initiate vocal fold vibration for voicing (32). For many years, this measurement has been used as an indirect measurement of the condition of the laryngeal mucosa, with the working hypothesis that PTP will vary as a function of vocal fold viscosity, velocity of mucosal wave of the cover of the thyroarytenoid muscle, pre-voice glottal width, and vocal fold thickness (32). PTP is theoretically proportional to vocal fold tissue viscosity, which may have an inverse relationship with hydration level (5). Titze's original mathematical model is as follows:

$$PTP = (2k/T)(Bc)(w/2)$$

where k is transglottal pressure coefficient, T is vocal fold thickness, B is a damping coefficient that is proportional to vocal fold viscosity, *c* is a constant, and w/2 is pre-phonatory

glottal half-width (32). This model does not include temperature of the vocal fold tissue or temperature of the conditioned respiratory air in the laryngeal environment in the calculation despite a likely role of temperature on 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 having a deleterious effect on vocal function and higher humidity environments facilitating less muscular effort for voice initiation as measured with PTP (2-8, 34). In a series of investigations of airway conditioning on superficial laryngeal viscosity, Sivasankar and colleagues (2, 3, 8) 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 findings suggested increased PTP and PPE following oral breathing, particularly for the higher pitches assessed.

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 (20, 75). 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 (33), 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.

Even small skeletal muscle temperature changes influence a host of physiological functions that directly affect muscle performance. The role of local temperature changes, increased (51) and reduced (52), in skeletal muscle contractile function and local metabolic activity of active skeletal muscle (53, 54), provide a scientific rationale to support investigation of the role of ambient temperature, its relationship to local muscle temperature changes, and laryngeal skeletal muscle function. 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 (55). 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. With regard to muscle physiology, mechanical and energetic parameters have variable responses depending on the degree to which muscle temperature increases (56). Enzyme catalysis is faster with small increases in temperature (57-60) and the change in reaction rate is generally referred to as the Q_{10} effect. Within the normal range of muscle temperature, the rate of reaction can about double (55). Muscle temperature rises proportionally to workload which may be augmented by environmental thermal stress. For a realistic 2°C increase in muscle temperature that arises because of increased muscle workload and/or environmental thermal stress, application of the Q_{10} effect would translate to an approximate enzyme reaction increase of 30 to 40% (61). For even smaller increases in muscle temperature (36°C to 37°C) there is believed to be an acceleration of metabolic rate (62), 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 (58).

A comprehensive review of energy metabolism during exercise and heat stress (61) concluded that for physical activity in the heat that is sufficient to raise the core temperature (> 0.5°C) metabolic differences were characterized by increased intramuscular carbohydrate

utilization. Starkie, et al. (63) described greater muscle glycogen utilization with increased tissue temperature during submaximal exercise. There is additional evidence of increased muscle lactate accumulation during exercise and heat stress (64). The affinity for oxygen binding to hemoglobin is also temperature sensitive where increases in contracting skeletal muscle temperature facilitates unloading of oxygen from hemoglobin as described by the Bohr effect (55). Gray, et al. (51) described elevated skeletal muscle ATP turnover with higher muscle temperatures.

There is also evidence that temperature has an influence on skeletal muscle contractile function, specifically force/velocity and power/velocity relationships (51, 65) if the increase in local muscle tissue temperature is sufficient (2°C to 3°C), sometimes referred to as the therapeutic temperature level (62). In an early discussion of temperature and muscle, Bennett (66) summarized that maximal force generation *in vivo* was not influenced by increasing temperature in the muscle tissue; however, time-dependent mechanisms, e.g., maximal velocity of shortening, were faster at higher temperatures. A later review of the influence of temperature on muscle contraction mechanics and energetics by Rall and Woledge (56) concluded that some non-time dependent factors are also influenced by temperature increases, e.g., tetanic force production. In a study of human skeletal muscle *in vivo*, De Ruiter and De Haan (65) manipulated temperature of the adductor pollicis muscle and found significant increases in peak tetanic force, maximal rate of force production, and relaxation rate as temperature increased. There is also indication that muscle temperature may have a different effect on force fluctuations with age (52). With temperature increases in rabbit skeletal muscle, there is also evidence of a reduction of the affinity of Ca2+ for the Ca2+-specific sites of troponin (67). Finally, there is evidence that higher muscle temperatures achieved by passively increasing muscle tissue temperature to 37-37.5°C may increase muscle fiber conduction velocity (51) .

In short, temperature changes in the laryngeal tissue that may occur for certain 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, and accounting for differences in mouth versus nose breathing, to discover how regional pharyngeal temperature changes may 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 (i.e., 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.

METHODS AND PROCEDURES

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 (IRB) for human subjects. Based on the phonation threshold pressure literature (5, 6, 8, 76), previously published thermal studies (77, 78), and power analysis sample size described by Stevens (79), 10 participants were determined to be adequate for a robust degree of power (0.8) and effect size (0.89). Inclusion criteria included: 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

Pre-trial Voice Screening

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), the images of which 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 Speech software (Multi-Speech Model 3700 Version 3.2, KayPENTAX). The frequency selected for the phonation threshold pressure (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. The modal frequency determined was recorded for use during the study trial.

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 probes, T-F1345, Roskilde, Denmark), phonation threshold pressure (PTP), and determination of perceived phonatory effort (PPE). For determination of upper airway temperature superior to the epilarynx, 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 epilaryngeal structure (25) 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.

Phonation threshold pressure (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 temperature change in the upper airway may have occurred with use of the larynx. Because this study protocol involved repeated measures 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 was 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 (5). 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 criteria for accuracy of pitch target produced within one semitone of the target pitch, consistent peak height (middle 3 peaks all within 1 cm/H_20), low intensity of production, and negligible airflow during production of /p/ (80).

Perceived voicing effort 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. Following each participant trial, the distance from the left end of the perceived effort line was measured in millimeters (mm) and recorded.

Environmental Conditions

All trials were conducted within an environmental chamber specifically designed to establish and maintain each of the 5 different temperature/relative humidity conditions chosen for this study: 3 temperatures (°C) with constant relative humidity (RH,%) maintained as follows: 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 (12, 13), and 2 temperatures (°C) with variable relative humidity (RH,%) to match vapor pressure for the thermally neutral (25°C/40% RH) as follows: cold (15°C/74% RH) and hot (35°C; 23% RH). Because the saturation of water vapor in the environment varies widely relative to temperature (17, 18), vapor pressure was manipulated for the 15°C and 35°C environments to match the water vapor pressure at 25°C/40% RH. As is evident in Figure 1 the percent water vapor 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 (2, 3).

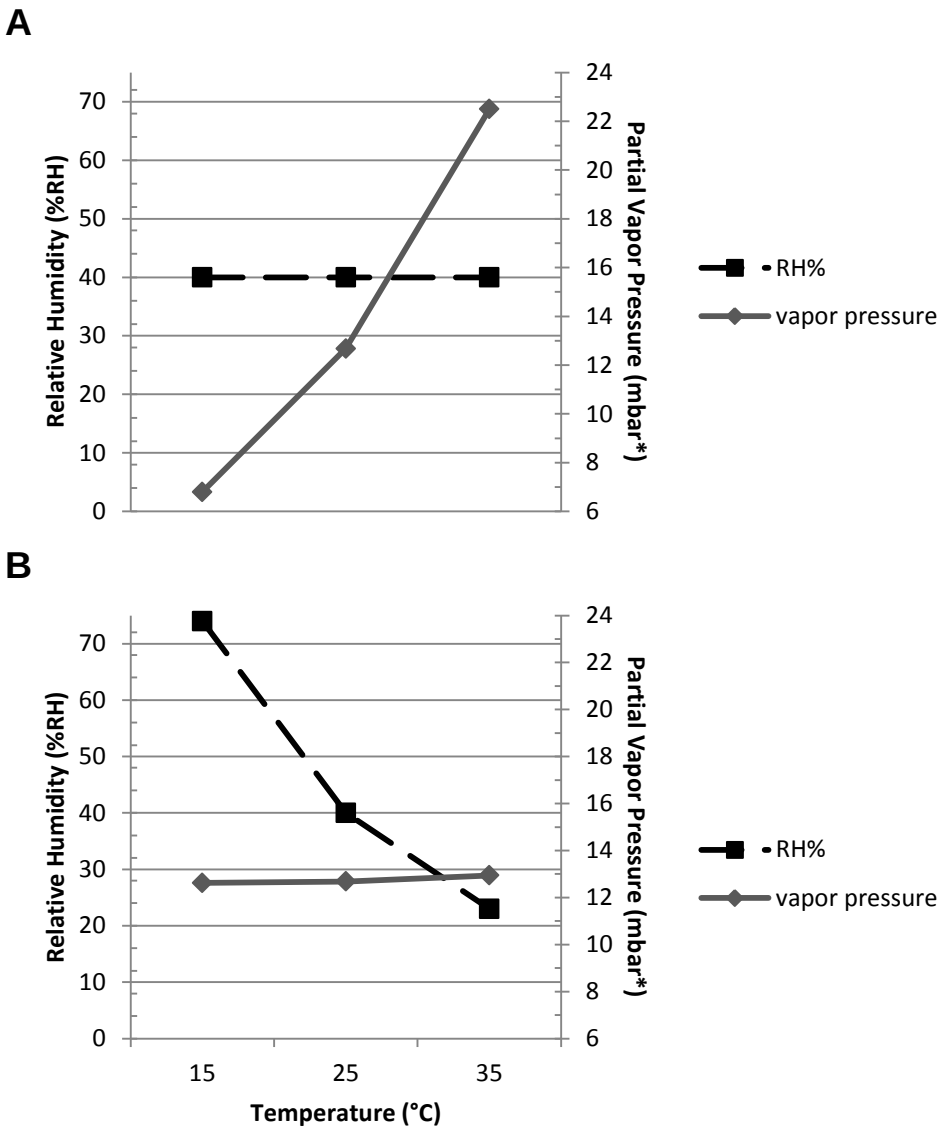

Figure 1. Partial vapor pressure & relative humidity by temperature

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 (81).

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 (82). 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 (83). 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 $\leq$ 1.020 g/ml was used as a threshold for hydration criterion based on previous thermal studies (84). 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 at 20 minutes after start of trial to allow for room equilibration. Immediately after the pharyngeal temperature recording, participants completed the phonation threshold pressure task (PTP) 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 vocal effort on the 100 mm visual analog scale described above. 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.

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_20) Perceived phonation effort (mm/100mm)
15°C/74% RH 25°C/40% RH 35°C/23% RH	Oral route breathing	
35°C/40% RH		

The highlighted environmental conditions are those that were matched for partial vapor pressure.

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 perceived phonatory effort (PPE). Significance was set at $\alpha<0.05$. Pair wise post hoc comparisons were performed where warranted. Information gathered in the voice use questionnaire was used to interpret the data analyses 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 (Copyright © [2002-2008] *SAS* Institute Inc. *SAS* and all other *SAS* Institute Inc. product or service names are registered trademarks or trademarks of *SAS* Institute Inc., Cary, NC, USA).

RESULTS

Participants

In total, 20 (10 women; 10 men) volunteers met the study inclusion criteria and agreed to participate in the study. In order for a participant's data to be included in the study, the PTP data recorded had to meet the following requirements: (1) frequency had to be within one semitone of the participant's target modal frequency; (2) each participant's productions were perceptually judged to be produced at a level that was as quiet as possible (6); (3) airflow measurements had to be negligible (considered to be less than 20 ml/s) to ensure appropriate lip closure (85, 86); (4) all of the peaks within the PTP string were judged, via visual inspection, to be of equal height (7, 87); and (5) voicing was evident during vowel production. 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 for equal height, low intensity within a narrow range across trials, negligible airflow during production of /p/, and pitch production within ½ semitone across trials 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 numbers of data points in each of the ten groups

(2 breathing conditions x 5 environmental conditions) were not always equal. Refer to Table 2 for means and standard deviations for each of the ten conditions analyzed. 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.

Table 2. Means and standard deviations for pre/post voice measures

Environment	Breathing Condition	PTP(cm H_2O)	Effort (mm)	Temperature (°C)
	Nose/Mouth			
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.

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 environment comparisons, was p=0.69 ruling out any need for post hoc comparisons. The p-value for between environment comparisons with all 10 conditions included, without including breathing condition, fell short of significance at p=0.19. Post-hoc comparison of the environmental conditions was completed by analyzing the vapor pressure-matched conditions

as a separate group from the humidity-matched conditions. For the vapor pressure-matched conditions (15°C/74% RH, 25°C/40% RH and 35°C/23% RH), RM-ANOVA analysis using the full model also failed to meet significance at $F(5, 78)=1.54$, $p=0.19$; however, the Tukey's Studentized Range Test indicated that the temperatures for the 25°C/40% RH and 35°C/23% RH environments may be significantly different. Further post-hoc analysis for these two environments further reduced the p-value to 0.09. While significant differences in temperature were not found statistically, there was evidence of temperature differences (ΔT) between some environmental conditions for nose and mouth breathing trials that signal a physiologically meaningful temperature change (>0.5°C) in the upper airway. As shown in Tables 3 and 4, separating the nose and mouth breathing conditions respectively, there were temperature differences that were greater than 1°C when comparing the 35°C/23%RH environment to the 25°C/40%RH for nasal and mouth inhalation conditions.

Table 3. Nasal inhalation temperature differences between environmental conditions.

15°C/ 40%RH	15°C/ 74%RH	25°C/ 40%RH	35°C/ 23%RH	35°C/ 40%RH	
	0.58°C	**0.8°C**	0.31°C	0.45°C	**15°C/ 40%RH**
		0.22°C	**0.89°C**	0.13°C	**15°C/ 74%RH**
			1.11°C	0.35°C	**25°C/ 40%RH**
				0.76°C	**35°C/ 23%RH**
					35°C/ 40%RH

Values reported in the boxes are absolute differences in temperature measured in the upper airway between the condition described in the top horizontal row and the corresponding condition described in the column on the right side of the graph following 20 minutes of breathing in through the nose, e.g., 1.11 °C is the absolute difference between the 35°C/23% RH and 25°C/40% RH environments. The bolded numbers denote differences > 0.5°C which may be physiologically meaningful for skeletal muscle function. Values in the gray boxes represent temperature differences for environmental conditions that were matched for vapor pressure.

Table 4. Mouth inhalation temperature differences between environmental conditions.

	15°C/ 40%RH	15°C/ 74%RH	25°C/ 40%RH	35°C/ 23%RH	35°C/ 40%RH
15°C/ 40%RH					
15°C/ 74%RH	0.13°C				
25°C/ 40%RH	0.15°C	0.02°C			
35°C/ 23%RH	**0.9°C**	**1.03°C**	**1.05°C**		
35°C/ 40%RH	0.25°C	0.38°C	0.4°C	**0.65°C**	

Values reported in the boxes are absolute differences in temperature measured in the upper airway between the condition described in the top horizontal row and the corresponding condition described in the column on the left side of the graph following 20 minutes of breathing in through the mouth, e.g., 1.03 °C is the absolute difference between the 15°C/74% RH and 35°C/23% RH environments. The bolded numbers denote differences > 0.5°C which may be physiologically meaningful for skeletal muscle function. Values in the gray boxes represent temperature differences for environmental conditions that were matched for vapor pressure.

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. With a basic combination 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. Specifically, it was hypothesized that phonation threshold pressure and perceived phonatory effort would increase for mouth versus nose breathing and with exposure to cold and/or dry environments.

The findings described above do not provide support for the hypotheses that nose breathing is superior to mouth breathing or that cold or dry environments are particularly deleterious to voice function following a period of resting breathing in young men and women who were free of health conditions that may perturb voice function. The failure to establish 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 (28). 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.

The lack of significant difference in PTP between mouth versus nose breathing in each environmental condition tested departs from early findings (3, 8) of lower PTP values with nose breathing. 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. Given the proposed changes to laryngeal mucosal viscosity throughout the menstrual cycle (31) and the theory that viscosity influences PTP values (32), it was felt that this initial research effort into vocal function and environment (temperature and humidity) should control for menstrual cycle differences between subjects. Research also indicates significant core temperature differences through the primary phases of the menstrual cycle (88) which may also influence 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 by Erickson-Levendoski and Sivasankar (89). 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 subjects 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 differences 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 intrinsic laryngeal muscles. Using the airway thermal mapping research conducted by McFadden et al., (25) and the distance reported from the tip of the nares to the glottis (mean=18.2 cm, SE$\pm$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.

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) the laryngeal tissue temperature did not change sufficiently to influence intrinsic laryngeal skeletal muscle function as may be expected particularly with increases in tissue temperature; 2) the intrinsic laryngeal skeletal muscles of the human larynx do not share the same physiological response to thermal changes as has been well-established in limb skeletal muscle; 3) maintenance of superficial epithelial viscosity in healthy men and women may be more tightly regulated than previously proposed with mouth versus nose breathing; 4) the vocal function measures used in this study were not sensitive enough to measure changes in the laryngeal physiology that may have occurred ; 5) 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 passed in both directions, may have unique physiological mechanisms which are yet to be identified and described.

A strength of this research is that it establishes an environmental foundation from which to build additional research efforts to better understand vocal perturbations that are hypothesized to lead to voice disorders, e.g., gastroesophageal reflux, high intensity use of the voice. Understanding upper airway responses to changes in temperature and vapor pressure required investigation free of the factors believed to lead to the development of voice disorder to better understand how disease, medication, and voice use pattern influence voice function. An additional strength was the consistent task elicitation procedures for phonation threshold pressure over several environmental conditions and multi-day lab appointments. 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 several weaknesses identified in this project. While participant selection was strong for inclusion of both men and women, 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 controlling, it was decided to limit this initial effort to a basic design.

Limiting measurement of PTP to modal frequency only may be seen as a limitation given that the literature indicates that use of high pitch may discern small changes in laryngeal function (5, 85). It may be that if high frequency was also used, that significant differences in PTP would have been discovered. Given the time constraints of the measures being taken before upper airway temp changed from repeated voicing attempts, it was decided to do only one pitch and limit it to three trials to avoid excessive voice use prior to the standardized reading

passage. Past experience has shown that obtaining PTP for high frequencies can be very difficult for many volunteers and the time it may have taken to obtain that measure may have influenced the perceived phonatory effort measure that was being compared to upper airway temperature.

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 (22, 25) 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 the foundational study of 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.

This is the first known study to specifically manipulate environmental temperature to better understand how passive warming of the upper airway may influence voice function. The findings of this investigation of environmental temperature and voice function 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.

REFERENCES

1. Herrington-Hall BL, Lee L, Stemple JC, Niemi KR, McHone MM. Description of Laryngeal Pathologies by Age, Sex, and Occupation in a Treatment-Seeking Sample. J Speech Hear Disord. 1988;53(1):57 -64.

2. Roy N, Merrill RM, Thibeault S, Parsa RA, Gray SD, Smith EM. Prevalence of Voice Disorders in Teachers and the General Population. Journal of Speech, Language & Hearing Research. 2004;47(2):281 -93.

3. Titze IR, Lemke J, Montequin D. Populations in the U.S. workforce who rely on voice as a primary tool of trade: a preliminary report. Journal of Voice. 1997;11(3):254 -9.

4. Hoffman-Ruddy B, Lehman J, Crandell C, Ingram D, Sapienza C. Laryngostroboscopic, Acoustic, and Environmental Characteristics of High-Risk Vocal Performers. Journal of Voice. 2001;15(4):543 -52.

5. Benninger M, Jacobson BH, Johnson AF. Vocal arts medicine : the care and prevention of professional voice disorders. Stuttgart ; New York: Thieme Medical Publishers; 1994. ix, 374 p.

6. Tucker HM. The larynx. 2nd ed. New York: Thieme Medical Publishers : Georg Thieme Verlag, Stuttgart; 1993. x, 326 p. p.

7. Lawrence V. Sermon on Hydration (The Evils of Dry). The NATS Journal. 1986;42(4):22 -3.

8. Sivasankar M, Fisher KV. Oral breathing challenge in participants with vocal attrition. J Speech Lang Hear Res. 2003;46(6):1416 -27.

9. Sivasankar M, Fisher KV. Oral breathing increases Pth and vocal effort by superficial drying of vocal fold mucosa. J Voice. 2002;16(2):172 -81.

10. Sivasankar M, Erickson E. Short-Duration Accelerated Breathing Challenges Affect Phonation. Laryngoscope. 2009;119(8):1658 -63.

11. Verdolini-Marston K, Sandage M, Titze IR. Effect of hydration treatments on laryngeal nodules and polyps and related voice measures. Journal of Voice. 1994;8(1):30 -47.

12. Verdolini-Marston K, Titze IR, Druker DG. Changes in phonation threshold pressure with induced conditions of hydration. Journal of Voice. 1990;4(2):142 -51.

13. Verdolini K, Titze IR, Fennell A. Dependence of phonatory effort on hydration level. J Speech Hear Res. 1994;37(5):1001 -7. Epub 1994/10/01. PubMed PMID: 7823546.

14. Sivasankar M, Erickson E, Schneider S, Hawes A. Phonatory Effects of Airway Dehydration: Preliminary Evidence for Impaired Compensation to Oral Breathing in Individuals With a History of Vocal Fatigue. J Speech Lang Hear Res. 2008;51(6):1494 -506.

15. Hemler RJB, Wieneke GH, Dejonckere PH. The effect of relative humidity of inhaled air onacoustic parameters of voice in normal subjects. Journal of Voice. 1997;11(3):295 -300.

16. Fang L, Wyon D, Clausen G, Fanger P. Impact of indoor air temperature and humidity in an office on perceived air quality, SBS symptoms and performance. Indoor Air. 2004;14(s7):74 -81.

17. Richter B, Lohle E, Maier W, Kliemann B, Verdolini K. Working conditions on stage: climatic considerations. Logoped Phoniatr Vocol. 2000;25(2):80 -6.

18. Fang L, Clausen G, Fanger P. Impact of Temperature and Humidity on the Perception of Indoor Air Quality. Indoor Air. 1998;8(2):80 -90.

19. Fairfax R. OSHA Policy on Indoor Air Quality: Office Temperature/Humidity and Environmental Tobacco Smoke. In: Administration OSH, editor. www.osha.gov2003.

20. Lawrence MG. The Relationship between Relative Humidity and the Dewpoint Temperature in Moist Air: A Simple Conversion and Applications. Bulletin of the American Meteorological Society. 2005;86(2):225 -33.

21. Miers BT. On the interrelation of temperature, pressure, air density, and humidity. In: Laboratory UALCAS, editor. White Sands Missile Range1991. p. 23.

22. McCutchan J, Taylor CL. Respiratory heart exchange with varying temperature and humidity of inspired air. Journal of Applied Physiology. 1951;4(2):121 -35.

23. McFadden ER. Respiratory heat and water exchange: physiological and clinical implications. Journal of Applied Physiology: Respiratory, Environmental & Exercise Physiology. 1983;54(2):331 -6.

24. Cole P. Temperature and humidity of respiratory air. Journal of Physiology. 1953;122(Suppl):51P.

25. Cole P. Further observations on the conditioning of respiratory air. Journal of Laryngology & Otology. 1953;67(11):669 -81.

26. Cole P. Some aspects of temperature, moisture and heat relationships in the upper respiratory tract. Journal of Laryngology & Otology. 1953;67(8):449 -56.

27. Cole P. Recordings of respiratory air temperature. Journal of Laryngology & Otology. 1954;68(5):295 -307.

28. McFadden ER, Pichurko BM, Bowman HF, Ingenito E, Burns S, Dowling N, et al. Thermal mapping of the airways in humans. J Appl Physiol. 1985;58(2):564 -70.

29. Mcfadden ER, Pichurko BM. Intra-Airway Thermal Profiles during Exercise and Hyperventilation in Normal Man. Journal of Clinical Investigation. 1985;76(3):1007 -10.

30. Williams R, Rankin N, Smith T, Galler D, Seakins P. Relationship between the humidity and temperature of inspired gas and the function of the airway mucosa. Crit Care Med. 1996;24(11):1920 -9.

31. Widdicombe JG. Airway Surface Liquid: Concepts and Measurements. In: Rogers DF, Lethem MI, editors. Airway Mucus; basic mechanisms and clinical perspectives. Basel: Birkhauser Verlag; 1997. p. 1-17.

32. Man SF, Adams GK, 3rd, Proctor DF. Effects of temperature, relative humidity, and mode of breathing on canine airway secretions. J Appl Physiol. 1979;46(2):205 -10.

33. Boucher RC, Stutts MJ, Gatzy JT. Regional differences in bioelectric properties and ion flow in excised canine airways. J Appl Physiol. 1981;51(3):706 -14.

34. Freed AN, Davis MS. Hyperventilation with dry air increases airway surface fluid osmolality in canine peripheral airways. Am J Respir Crit Care Med. 1999;159(4 Pt 1):1101 -7.

35. Freed AN, Anderson SD, Daviskas E, McFadden J, E.R., Nelson JA, Skowronski ME, et al. THERMALLY INDUCED ASTHMA AND AIRWAY DRYING. Am J Respir Crit Care Med. 2000;161(6):2112 -3.

36. Fisher KV, Telser A, Phillips JE, Yeates DB. Regulation of vocal fold transepithelial water fluxes. J Appl Physiol. 2001;91(3):1401 -11.

37. Sivasankar M, Fisher KV. Vocal fold epithelial response to luminal osmotic perturbation. J Speech Lang Hear Res. 2007;50(4):886 -98.

38. Robertshaw D. Mechanisms for the control of respiratory evaporative heat loss in panting animals. J Appl Physiol. 2006;101(2):664 -8.

39. Hales JRS, Brown GD. Net energetic and thermoregulatory efficiency during panting in the sheep. Comparative Biochemistry and Physiology Part A: Physiology. 1974;49(3):4 13-22.

40. Ingram DL, Legge KF. The influence of deep body temperatures and skin temperatures on respiratory frequency in the pig. J Physiol. 1972;220(2):283 -96.

41. Ray DW, Ingenito EP, Strek M, Schumacker PT, Solway J. Longitudinal distribution of canine respiratory heat and water exchanges. Journal of Applied Physiology. 1989;66(6):2788 -98.

42. Solway J, Leff AR, Dreshaj I, Munoz NM, Ingenito EP, Michaels D, et al. Circulatory heat sources for canine respiratory heat exchange. J Clin Invest. 1986;78(4):1 015-9.

43. Fayez I, Marai M, Alnaimy A, Habeeb M. Thermoregulation in rabbits. Cahiers Options Mediterraneennes. 1994;8:33-41.

44. Sivasankar M, Blazer-Yost B. Effects of Long-Acting Beta Adrenergic Agonists on Vocal Fold Ion Transport. Laryngoscope. 2009;119(3):602 -7.

45. Erickson E, Sivasankar M. Simulated reflux decreases vocal fold epithelial barrier resistance. The Laryngoscope. 2010;120(8):1569 -75.

46. Leydon C, Sivasankar M, Falciglia DL, Atkins C, Fisher KV. Vocal Fold Surface Hydration: A Review. Journal of Voice. 2009;23(6):658 -65.

47. Tanner K, Roy N, Merrill RM, Elstad M. The effects of three nebulized osmotic agents in the dry larynx. J Speech Lang Hear Res. 2007;50(3):635 -46.

48. Titze IR. The physics of small-amplitude oscillation of the vocal folds. J Acoust Soc Am. 1988;83(4):1536 -52.

49. Verdolini K, Titze IR, Fennell A. Dependence of Phonatory Effort on Hydration Level. J Speech Hear Res. 1994;37(5):1001 -7.

50. McFadden, Pichurko BM. Intraairway thermal profiles during exercise and hyperventilation in normal man. J Clin Invest. 1985;76(3):1007 -10. Epub 1985/09/01. doi: 10.1172/JCI112052. PubMed PMID: 4044825; PubMed Central PMCID: PMC423970.

51. Cooper DS, Titze IR. Generation and Dissipation of Heat in Vocal Fold Tissue. J Speech Hear Res. 1985;28(2):207 -15.

52. Gray SR, De Vito G, Nimmo MA, Farina D, Ferguson RA. Skeletal muscle ATP turnover and muscle fiber conduction velocity are elevated at higher muscle temperatures during

maximal power output development in humans. American Journal of Physiology-Regulatory Integrative and Comparative Physiology. 2006;290(2):R376 -R82.

53. Dewhurst S, Graven-Nielsen T, De Vito G, Farina D. Muscle temperature has a different effect on force fluctuations in young and older women. Clinical Neurophysiology. 2007;118(4):762 -9.

54. Febbraio MA, Carey MF, Snow RJ, Stathis CG, Hargreaves M. Influence of elevated muscle temperature on metabolism during intense, dynamic exercise. American Journal of Physiology-Regulatory Integrative and Comparative Physiology. 1996;40(5):R1251 -R5.

55. Morton JP, Kayani AC, McArdle A, Drust B. The exercise-induced stress response of skeletal muscle, with specific emphasis on humans. Sports Med. 2009;39(8):643 -62.

56. Brooks GA, Fahey TD, Baldwin KM. Exercise physiology : human bioenergetics and its applications. 4th ed. Boston: McGraw-Hill; 2005. xxi, 876, 7, 22 p. p.

57. Rall JA, Woledge RC. Influence of temperature on mechanics and energetics of muscle contraction. American Journal of Physiology - Regulatory, Integrative and Comparative Physiology. 1990;259(2):R197 -R203.

58. Brooks G, Hittelman K, Faulkner J, Beyer R. Temperature, skeletal muscle mitochondrial functions, and oxygen debt. American Journal of Physiology -- Legacy Content. 1971;220(4):1053 -9.

59. Nelson DL, Cox MM. Lehninger Principles of Biochemistry. Fifth Edition ed. New York, NY: W.H. Freeman and Company; 2008. 1158 p.

60. Powers SK, Howley ET. Exercise physiology : theory and application to fitness and performance. 6th ed. Boston: McGraw-Hill; 2007.

61. Clarke A. Temperature and the metabolic theory of ecology. Wiley-Blackwell; 2006. p. 405-12.

62. Febbraio MA. Alterations in energy metabolism during exercise and heat stress. Sports Med. 2001;31(1):47 -59.

63. Wirth VJ, Van Lunen BL, Mistry D, Saliba E, McCue FC. Temperature changes in deep muscles of humans during upper and lower extremity exercise. Journal of athletic training. 1998;33(3):211 -5.

64. Starkie RL, Hargreaves M, Lambert DL, Proietto J, Febbraio MA. Effect of temperature on muscle metabolism during submaximal exercise in humans. Experimental physiology. 1999;84(4):775 -84.

65. Febbraio MA, Snow RJ, Stathis CG, Hargreaves M, Carey MF. Effect of heat stress on muscle energy metabolism during exercise. J Appl Physiol. 1994;77(6):2827 -31.

66. De Ruiter CJ, De Haan A. Temperature effect on the force/velocity relationship of the fresh and fatigued human adductor pollicis muscle. Pflügers Archiv European Journal of Physiology. 2000;440(1):163 -70.

67. Bennett AF. Temperature and muscle. The Journal of experimental biology. 1985;115:333-44.

68. Potter JD, Hsu FJ, Pownall HJ. Thermodynamics of Ca2+ binding to troponin-C. J Biol Chem. 1977;252(7):2452 -4.

69. Roy N, Tanner K, Gray SD, Blomgren M, Fisher KV. An evaluation of the effects of three laryngeal lubricants on phonation threshold pressure (PTP). J Voice. 2003;17(3):331 -42.

70. Pascoe DD, Bellingar TA, McCluskey BS. Clothing and exercise. II. Influence of clothing during exercise/work in environmental extremes. Sports Med. 1994;18(2):94 -108.

71. Pascoe DD, Smith JW, Molloy JM. Regional surface temperature responses during a high intensity heat acclimation protocol. Medicine and Science in Sports and Exercise. 2004;36(5):0573.

72. Stevens J. Applied multivariate statistics for the social sciences. 4th ed. Mahwah, N.J.: Lawrence Erlbaum Associates; 2002. xiv, 699 p. p.

73. Solomon NP. Assessment of laryngeal airway resistance and phonation threshold presure: Glottal Enterprises. In: Ma EPM, Yiu EML, editors. Handbook of Voice Assessments. San Diego, CA: Plural Publishing; 2011.

74. Refinetti R. The circadian rhythm of body temperature. Front Biosci. 2010;15:564-94.

75. Abitbol J, Abitbol P, Abitbol B. Sex hormones and the female voice. Journal of Voice. 1999;13(3):424 -46.

76. Pascoe DF, G. Comparison of measuring sites for the assessment of body temperature. Thermology International. 2009;19(1):35 -42.

77. Solomon NP, DiMattia MS. Effects of a vocally fatiguing task and systemic hydration on phonation threshold pressure. J Voice. 2000;14(3):341 -62.

78. Morgan MD, Triana MA, Milroy TJ. The effect of auditory feedback on phonation threshold pressure measurement. J Voice. 2004;18(1):46 -55.

79. Milbrath RL, Solomon NP. Do Vocal Warm-Up Exercises Alleviate Vocal Fatigue? Journal of Speech, Language & Hearing Research. 2003;46(2):422.

80. Abitbol J, Abitbol P, Abitbol B. Sex hormones and the female voice. J Voice. 1999;13(3):424 -46.

81. Coyne MD, Kesick CM, Doherty TJ, Kolka MA, Stephenson LA. Circadian rhythm changes in core temperature over the menstrual cycle: method for noninvasive monitoring. American Journal of Physiology - Regulatory, Integrative and Comparative Physiology. 2000;279(4):R1316 -R20.

82. Erickson-Levendoski E, Sivasankar M. The Laryngeal Epithelium in Reflux. Perspectives on Voice and Voice Disorders. 2011;21(3):112 -7.

83. Smith E, Kirchner HL, Taylor M, Hoffman H, Lemke JH. Voice problems among teachers: Differences by gender and teaching characteristics. Journal of Voice. 1998;12(3):328 -34.

84. Wolfe V, Long J, Youngblood HC, Williford H, Olson MS. Vocal Parameters of Aerobic Instructors with and without Voice Problems. Journal of Voice. 2002;16(1):52 -60.

85. Mcfadden ER, Pichurko BM, Bowman HF, Ingenito E, Burns S, Dowling N, et al. Thermal Mapping of the Airways in Humans. Journal of Applied Physiology. 1985;58(2):564 -70.

86. Katch VL, McArdle WD, Katch FI. Essentials of exercise physiology. 4th ed.

Philadelphia: Wolters Kluwer/Lippincott Williams & Wilkins Health; 2011. p. p.

IV. Journal Manuscript

Voice Function Differences Following Resting Nasal Breathing vs. Submaximal Exercise

ABSTRACT

The purpose of this investigation was to determine if vocal function and upper airway temperature were significantly different as measured following a resting breathing condition versus a bout of submaximal exercise. Specifically, it was hypothesized that phonation threshold pressure and perceived phonatory effort would increase, and pharyngeal temperature would decrease following an exercise bout. Using a within-participant repeated measures design, 18 consented participants (10 men, 10 women) completed a 20-minute equilibration task immediately followed by 8 minutes of submaximal exercise on a stationary bike in a thermally neutral environment (25°C/40% RH). At the end of the equilibration trial and the exercise trial measures were taken: pharyngeal temperature, phonation threshold pressure, and perceived phonatory effort were taken. Data were analyzed using paired t-tests with significance set at $\alpha<0.05$. Significantly increased phonation threshold pressure and perceived phonatory effort and significantly decreased pharyngeal temperature (*1.9°C)* were found, supporting the initial hypotheses. Findings from this investigation support the widely held belief that voice use associated with physical activity requires additional laryngeal effort and closure forces, both of which may contribute to the development of voice disorder.

INTRODUCTION

Physical education teachers, military personnel, performers, and fitness instructors, represent a handful of the many individuals who are in occupations that require reliable, effective voice use during moderate to high intensity physical activity. While there is a general understanding that these professionals are at higher risk for development of occupationally-based voice disorders (90, 91), there is little known about the effect of moderate intensity exercise on voice production. Physiologically, study of vocal function following moderate intensity exercise is complex, the challenges of which are, in part, resolved with use of well-established exercise science methodology.

Understanding voice function immediately following submaximal exercise requires knowledge of superficial vocal fold viscosity mechanisms and skeletal muscle tissue properties (biochemical, contractile, & bioenergetic) that may be altered with changes in tissue temperature secondary to increased ventilation rate. A recent review of vocal fold surface hydration mechanisms (49) described behavioral and environmental challenges as important variables for maintenance of superficial laryngeal viscosity. Maintenance of superficial vocal fold viscosity, or airway surface liquid (ASL), may be influenced by mechanisms of evaporative water loss, the degree of fluid loss depending on many factors which could include ventilation rate and inspired air temperature and humidity (28).

The pulmonary literature describes variations in the site of the conditioning process (warming and humidification) with increasing ventilation (resting and secondary to exercise), driving warming and conditioning of the air below the level of the glottis (20-23, 92). The shift in airway conditioning toward and below the level of the vocal folds may influence the temperature of the vibrating vocal fold surfaces and the deeper intrinsic laryngeal muscles. Even small changes (> 0.5°C) in skeletal muscle tissue temperatures have been shown to influence skeletal muscle physiology via changes in metabolic activity and contractile function (51-54). In addition to changes in upper airway tissue temperature that may be proposed with increased respiratory

rate, core temperature increases that occur with moderate intensity exercise (59) may also affect laryngeal tissue temperature, potentially counteracting any lowering of tissue temperature that may occur with increased respiratory rate. Further, a change in upper airway tissue temperature may influence the thermoregulatory mechanisms of convective, conductive, and evaporative cooling. The latter of these mechanisms, evaporation, provides an important defense against overheating with heat loss attributed to both sweat and water vapor from the respiratory passages (93). How this water vapor loss through respiration during exercise influences laryngeal function is unknown. To date, little is known about laryngeal skeletal muscle temperature regulation and if those thermoregulation mechanisms mirror those of limb skeletal muscle.

Environmental investigations of voice function using phonation threshold pressure (PTP) and perceived phonatory effort (PPE) measures have largely focused on hydration and ambient humidity manipulations (2, 3, 5, 6, 8, 34) during sitting resting breathing. More recently, Sivasankar and Erickson (4) investigated the influence of resting accelerated breathing on voice function in smokers and nonsmokers, concluding that even short durations of accelerated breathing significantly increased PTP in both groups studied. While providing insight for certain environmental or behavioral conditions that may influence voice function, current evidence may not adequately describe vocal function that is required for individuals who use the voice professionally while the respiratory rate is elevated during physical activity. The purpose of this study was to determine if increased ventilation rate with submaximal exercise influenced upper airway tissue temperature, phonation threshold pressure, and perceived phonatory effort to better understand voice use following in the realistic scenario of physical activity, a previously unstudied condition. Specifically, it was hypothesized that phonation threshold pressure and perceived phonatory effort would increase, and pharyngeal temperature would decrease following a bout of submaximal exercise.

METHODS AND PROCEDURES

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 (IRB) for human subjects. Based on the phonation threshold pressure literature (5, 6, 8, 76), previously published thermal studies (77, 78), and power analysis sample size described by Stevens (79), 10 participants were determined to be adequate for a robust degree of power (0.8) and effect size (0.89). Inclusion criteria included: 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 or hot environments or interfered with the proper placement of the transnasal thermal probe, history of laryngeal pathology, diabetes, allergic rhinitis, respiratory disease, active smoker at the time of the investigation, and/or contraindicated medications known to dry the laryngeal mucosa or alter thermal responses.

Preliminary Procedures and Assessments

Voice Screening

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), the images of which 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 Speech software (Multi-Speech Model 3700 Version 3.2, KayPENTAX). The frequency selected for the phonation threshold pressure elicitation task was modeled for the participants using a standard pitch pipe (WM. Kratt Co.) and pitch-matching ability was determined using Real Time Pitch software. The modal frequency determined was recorded for use during the study trial.

Work Rate Determination for Targeted Respiratory Rate

Pre-trial determination of submaximal cycle ergometer work rate for each participant was completed using a standardized cycle ergometer (Quinton Instrument Company, Howell, New Jersey) protocol beginning at 50 watts (W) pedal resistance, from which a work rate was determined that averaged a respiratory rate of 20 breaths per minute for 8 minutes. Participants were asked to select and maintain the pedaling rate per minute (RPM), and both work rate (W) and pedaling rate (RPM) were recorded for replication during the actual trial. Respiratory rate was determined objectively using standard exercise physiology gas analysis software (ParvoMedics, Provo, Utah) that counts respiratory rate per minute from the expired air sample that is collected from a mouthpiece designed for this purpose. A submaximal exercise target of 20 breaths per minute was chosen so that a wide range of individuals could participate, thus not limiting this research to only fit individuals. The 8-minute length of the aerobic cycling exercise was chosen because that would allow time for all participants to reach submaximal steady state with consistent breathing rate and stable heart rate. Target submaximal work rate (W) and pedaling RPM varied between participants given variable levels of individual fitness as detailed in Table 1.

Table 1. Participant cycle work rate.

Work Rate (W)	Peddling Rate (RPM)	Age
Women		
0	55	20
5	30	23
25	60	21
40	48	23
45	65	21
50	45	21
55	70	24
70	45	20*
75	75	21
140	40	21
Men		
50	40	21
65	60	21*
70	30	20
75	90	22
85	80	23
100	90	23
105	80	21
175	90	21
180	50	21
180	80	24

*Denotes consented participants whose data were not included in data analysis. Large differences in both work rate (W) and RPM required to maintain an average of 20 breaths per minute are due to widely varying fitness levels of participants, e.g., sedentary individuals and college athletes.

Experimental Procedures

Environmental Conditions

All trials were conducted within an environmental chamber in which a thermally neutral temperature/relative humidity condition (25°C/40% RH) was maintained. Environmental parameters were closely monitored with a wet bulb global temperature device (QUEST°34 Thermal Environment Monitor, Quest Technologies, Oconomowoc, Wisconsin), a device commonly used in environmental and thermoregulation science.

Participants were scheduled to complete each of the trials at approximately the same time of day to avoid thermal differences secondary to circadian rhythm (82). Participants were asked to refrain from caffeine, meals, hot/cold beverages, and exercise for at least one hour prior to the trial. All female participants were asked to schedule the trial 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 (83).

Voice Function Assessment

Pre-trial familiarization training was completed for transnasal placement of the upper airway temperature probe (Exacon thermistor probes, T-F1345, Roskilde, Denmark), phonation threshold pressure (PTP) collection procedures, and perceived voicing effort procedures. The investigator advanced the flexible pharyngeal probe (diameter = 1.33 mm) approximately 12-15 cm from the tip of the nose to control for a depth that was close to the epilaryngeal structure (25), 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 pharynx following insertion. Measurement of upper airway temperature (°C) was then recorded from a Squirrel Data Logger 2020 Series (Grant Instruments, Hillsborough, New Jersey).

Task elicitation training for phonation threshold pressure was scripted to standardize directions across participants and data was collected using the Phonatory Aerodynamic System (PAS) Model 6600 (KayPENTAX. Task familiarization included training of a 5 syllable train of the consonant-vowel combination /pi/ at a rate of 1.5 syllables per second (95 beats per minute), first above threshold, then below threshold, and at threshold (5). 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 criteria for accuracy of pitch

target produced, consistent peak height (middle 3 peaks all within 1 cm/H_20), low intensity of production, absence of airflow during initial production of /p/.

Perceived voicing effort (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 PPE following the reading task. Following each participant trial, the distance from the left end of the perceived effort line was measured in millimeters (mm) and recorded.

Systemic hydration

Prior to the start of the experiment, each participant provided a urine sample for objective assessment of hydration level using a refractometer (ATAGO®,Tokyo, Japan) with hydration level set at less than or equal to 1.02 g/ml (78). Participants were allowed to drink additional room temperature fluids to reach the hydration target as needed.

Data Collection

Once hydration criteria were achieved, the transnasal pharyngeal temperature probe was positioned and secured in place by placing the distal end of the probe over the ipsilateral ear and securing the probe to the cheek with skin tape. The participant then entered the thermal chamber to acclimate to the 25°C/40% RH environment for 20 minutes, using nasal breathing only. After the 20 minute acclimation period, temperature, baseline PTP data, and PPE were collected in that order. Participants then moved from a seated position to the cycle ergometer for the increased respiratory rate trial. Participants cycled for 8 minutes at the pre-determined submaximal aerobic work rate to elicit an average of 20 breaths per minute with oral breathing (nose clip in place) in the same thermally-neutral environment of 25°C/50% RH. Immediately after completion of the 8 minute cycle, measures of pharyngeal temperature, PTP, and perceived phonatory effort were repeated. Please refer to Table 2 for a complete list of measurements made before and after the submaximal exercise trial.

Table 2. Trial design time points.

Time Points	Measures
Pre-Trial	Submaximal work rate (Watts, breaths per minute) Hydration level (g/ml)
Resting nasal route (20 minutes)	Pharyngeal temperature (°C) Phonation threshold pressure (cm/H_20) Perceived phonation effort (mm/100)
8 minutes of submaximal cycling exercise	

Data Analysis

A within-participant repeated measures design was used. Paired t-tests were used to determine significance of differences measured during rest breathing versus increased ventilation secondary to submaximal aerobic exercise for PTP, pharyngeal temperature, and PPE. Significance was set at $\alpha<0.05$. All data analyzed met normality assumptions based on visual inspection of the respective QQ plots and histograms. Information gathered in the voice use questionnaire was used to interpret the data analyses. The data analysis for this paper was generated using SAS software, Version 9.2 of the SAS System for XP-Pro (Copyright © [2002-2008] *SAS* Institute Inc. *SAS* and all other *SAS* Institute Inc. product or service names are registered trademarks or trademarks of *SAS* Institute Inc., Cary, NC, USA).

RESULTS

Participants

In total, 20 (10 women; 10 men) volunteers met the study inclusion criteria and agreed to participate in the study. Data from 2 participants *(1 male and 1 female)* were excluded from data analysis due to post-data collection disclosure of history of asthma. The remaining 18 participants (9 women, 9 men) ranged in age from 20-24 (M = 21.72; SD = 1.27). No significant differences were statistically discerned between the men and women in this study, therefore for all of the statistical analysis below, all participant data were analyzed together. Given that 10

volunteers was deemed sufficient for achieving appropriate power for this investigation as discussed earlier, loss of data from 2 participants should not affect the power of the data analysis for the remaining 18 participants.

Effect of Submaximal Activity on Phonation Threshold Pressure

Of the 18 participants who met the criteria for inclusion in the data analysis in general, only 14 produced PTP targets that met the following narrow criteria for inclusion in data analysis: (1) frequency within one semitone of the participant's target low, modal, or high frequency; (2) productions were perceptually judged to be produced at a level that was as quiet as possible(6); (3) airflow measurements was negligible (considered to be less than 20 ml/s) to ensure appropriate lip closure (85, 86); (4) all of the peaks within the PTP string were judged, via visual inspection, to be of equal height (7, 87); and voicing was evident during vowel production. *A paired-samples t-test was conducted to compare PTP before and after* the exercise trial. There was a significant difference in the PTP values measured before exercise (M = 3.97 cm H_2O, SD = 1.36) and after 8 minutes of submaximal exercise (M = 4.77 cm H_2O, SD = 1.13) conditions; $t(13) = -2.66$, $p = 0.019$. Phonation threshold pressure data is presented in Figure 1.

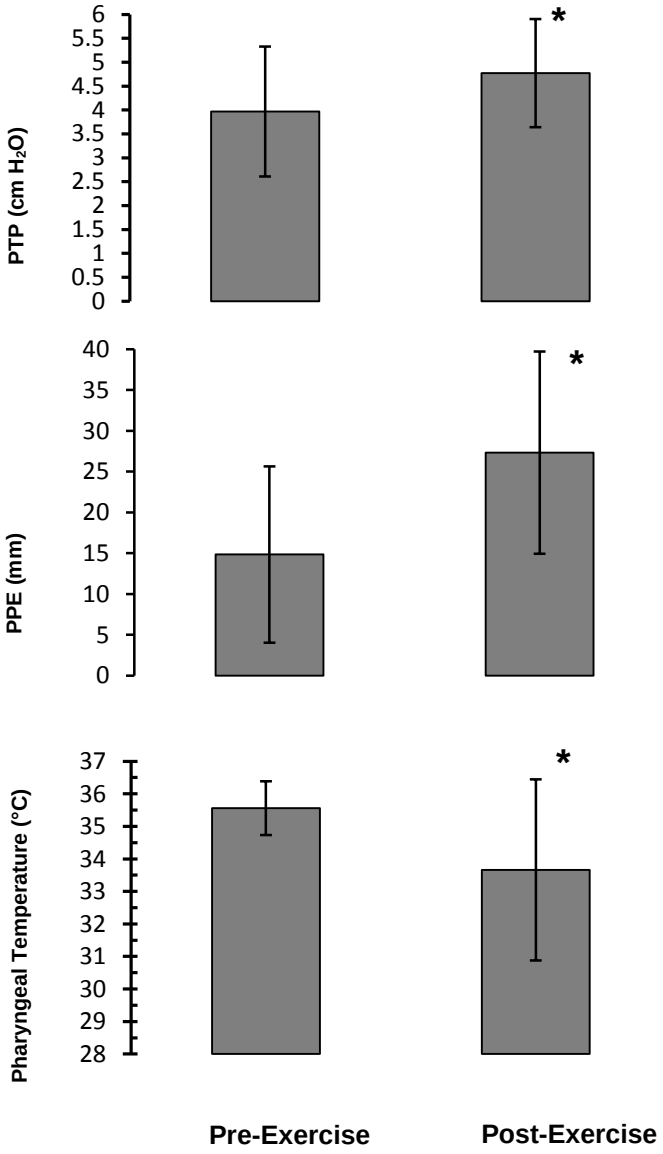

Figure 1. Submaximal exercise effects on study variables. Significant differences are denoted by the * ($p < 0.05$).

Intraexaminer Reliability of Data Measurement

To eliminate the possibility of phonation threshold pressure calculation error via manual PTP analysis, all pressure peak trains that met the study criteria for equal height, low intensity, negligible airflow during production of /p/, and pitch production within ½ semitone were measured and averaged via the automated measurement software for the PAS system.

Effect of Submaximal Activity on Perceived Phonatory Effort

Perceived phonatory effort (PPE) values were determined by measuring from the left anchor of the 100 mm visual analog scale to the mark made by the participant. A paired-samples t-test was conducted to compare PPE before and after the exercise trial. There was a significant difference in the effort values measured for the before exercise (M = 14.8 mm, SD = 10.8) and after 8 minutes of submaximal exercise (M = 27.3 mm, SD = 12.4) conditions; $t(17) = -3.81$, $p = 0.001$. These results indicate that with only moderate increases in activity, perception of effort for voicing tasks increases.

Effect of Submaximal Activity on Pharyngeal Temperature

One participant could not tolerate the transnasal temperature probe but completed the other measurements, therefore upper airway temperature data were analyzed for 17 participants. A paired-samples t-test was conducted to compare pharyngeal temperature before and after the exercise trial. There was a significant difference in the temperature values measured for the before exercise (M = 35.6 *°C*, SD = 0.83) and after 8 minutes of submaximal exercise (M = 33.7 *°C*, SD = 2.78) conditions; $t(16) = 3.38$, $p = 0.004$. These results demonstrate an average decrease of *1.9°C* in upper airway temperature with a moderate increase in ventilation rate in young, healthy volunteers, a finding that is consistent with the upper airway conditioning literature. Temperature changes greater than 0.5°C are considered physiologically significant for muscle physiology (55-57, 59).

DISCUSSION

The primary goal of this investigation was to determine if measureable changes in vocal function and pharyngeal temperature could be identified. It was hypothesized that phonation threshold pressure (PTP) and perceived phonatory effort (PPE) would increase and pharyngeal temperature would decrease following a bout of submaximal exercise. The results of this investigation offer support for significant changes in all three of the study measures.

We hypothesized that PTP would be higher after exercise because participants produced the target train of syllables immediately after cessation of cycling when the respiratory drive was increased from the resting condition. These findings are consistent with the resting elevated respiratory rate research conducted by Sivasankar and Erickson (4); however, this method of actively increasing respiratory rate through physical activity may be a more realistic representation of respiratory challenge and vocal function. Respiratory rate typically remains elevated for a period of time following cessation of exercise in the body's effort to return to homeostasis (59). The standard deviations measured for the pre-exercise and post-exercise values were similar, indicating that fitness differences between participants did not result in highly variable post-exercise threshold pressures as might be hypothesized. It could be that the submaximal nature of the exercise trial was not of sufficient intensity to drive PTP values higher. During the course of data collection, it was also learned that some of the participants typically talked to an exercise partner while working out, suggesting that some individuals may have learned to overcome the increase in pulmonary drive during exercise to engage the larynx for speech. This may be hypothesized given the rule of specificity in exercise science – muscles become more efficient when trained for a specific task. If some of the volunteers already had muscle experience, entraining the intrinsic laryngeal muscles for phonation while the respiratory drive was elevated, PTP values may be lower.

The differences measured for PPE, while statistically significant, remained on the lower half of the 100 mm visual analog scale used for this study. If a mark in the middle of the scale (50

mm) is associated with perception of moderate voicing effort, then it appears that all of the participants' perception of effort could be considered to remain in the mild-moderate range. This suggests that perception of voicing effort with submaximal exercise may not be a great physical stressor in young healthy individuals who are free of those health conditions that may promote either less than optimal laryngeal tissue condition or use, e.g., pulmonary disease, reflux, allergies, or anticholinergic medications.

The average reduction in pharyngeal temperature (*1.9°C)* measured in this investigation is potentially physiologically significant if the muscles of the upper airway, including the larynx, have metabolic and contractile features similar to those in the limb skeletal muscles. With changes of >0.5°C the ability of muscle tissue to access stored fuel substrate and offload oxygen from the circulating blood supply is enhanced (55, 63). Contractile function, both for speed and force production, is also enhanced with small increases in temperature (51, 65). Therefore, with a potential reduction in upper airway tissue temperature, which may include intrinsic laryngeal skeletal muscle, muscle inefficiencies may be hypothesized. To date, there is a large gap in the literature regarding bioenergetics, thermoregulation, and contractile function in vivo in the human larynx. What is apparent is that any increases in core temperature during the submaximal exercise bout did not completely offset pharyngeal temperature reductions that were measured.

An additional aspect of this investigation, that cannot be directly assessed, is the state of superficial laryngeal viscosity post submaximal exercise. The pre-exercise superficial laryngeal viscosity was expected to be adequate secondary to the participant selection constraints as well as pre-trial constraints on exercise, diet, time of day scheduling, menstrual cycle limitations to days 7-12, and objective assurance of hydration status. It has been hypothesized that mouth breathing for a period of time may desiccate the superficial fluid layer of the laryngeal epithelium, with degree of desiccation increased with increased respiratory rate (4, 35, 37). There are those investigators who believe, however, that the upper airway is remarkably well

suited to adequately maintain respiratory epithelial surface liquid despite pulmonary challenge (20, 28). Changes in viscosity may have influenced measures of PTP and PPE; however, reductions in tissue temperature and increased pulmonary drive could also account for increases in these measures.

A primary strength of this investigation was the use of well-established procedures used in exercise science to objectively discern the target work rate to match all volunteers for 20 breaths per minute. As can be seen from the values in Table 1, some participants achieved the target respiratory rate working with little to no resistance set on the cycle ergometer (watts) at a low RPM while other volunteers matched the same respiratory rate while pedaling at high resistance and high RPMs. This investigation also introduced the novel application of pharyngeal temperature, measured with transnasal thermistor probes, typically used in exercise science to measure core esophageal temperature. To date, temperature has not been considered in understanding laryngeal physiology. Investigations of temperature influences on laryngeal skeletal muscle tissue physiology and superficial laryngeal viscosity are needed.

An obvious limitation of this study is the youthful age range of the participants recruited, rendering generalization of the findings to older adults difficult. In this initial effort to understand the vocal function perturbation of increased respiratory rate, the volunteer pool was limited to only those individuals who were free of several of the known medical conditions and medications that affect vocal function. The volunteer criteria were also designed to limit the age range to those individuals 35 and younger to exclude women who were nearing or past menopause. These limitations excluded many of the potential older volunteers from participation. An additional limitation of this study was exclusion of acoustic measures of voice function from the study protocol. The addition of acoustic measures would have strengthened this study in many ways; however, the study was conducted in an exercise science laboratory in order to have access to the gas analysis equipment, the cycle ergometer, and the

environmental chamber and the ambient noise level in the laboratory made accurate collection of acoustic measures impossible.

With regard to the exercise challenge in this study, cycling was selected because it was the safest way to maintain the transnasal temperature probe for volunteer comfort while increasing respiratory rate. Cycling is not considered full body exercise, as is walking where the large muscle groups of the arms and legs are engaged. Use of full body exercise in this study would have better represented the type of physical activity that typically accompanies voice use for fitness trainers, military personnel, performers, and other individuals who require a durable voice while engaged in physical activity.

Conclusions

This investigation represents a novel effort to combine well-established physiological assessment tools used in exercise science with well-established vocal function measures used in speech language pathology to better understand vocal function immediately following submaximal exercise. As hypothesized, measures of PTP, PPE, and pharyngeal temperature all increased following the bout of submaximal exercise on the cycle ergometer in healthy young men and women. The increased vocal effort measured, both perceptually and objectively, in this study supports the hypothesis that individuals in occupations that require voice use during physical activity may be at greater risk of developing voice disorder. From the foundational work established in this investigation, additional research could discern vocal function differences following submaximal exercise for individuals with specific health conditions that are believed to contribute to the development of voice disorders, e.g., allergies and reflux. Identification of potential risk factors for the development of voice disorders using realistic environmental and behavioral conditions will provide a more thorough understanding for the prevention of work-related voice disorders and aide in the development of voice habilitation and rehabilitation programs.

REFERENCES

1. Smith E, Kirchner HL, Taylor M, Hoffman H, Lemke JH. Voice problems among teachers: Differences by gender and teaching characteristics. Journal of Voice. 1998;12(3):328 -34.

2. Wolfe V, Long J, Youngblood HC, Williford H, Olson MS. Vocal Parameters of Aerobic Instructors with and without Voice Problems. Journal of Voice. 2002;16(1):52 -60.

3. Leydon C, Sivasankar M, Falciglia DL, Atkins C, Fisher KV. Vocal Fold Surface Hydration: A Review. Journal of Voice. 2009;23(6):658 -65.

4. Widdicombe JG. Airway Surface Liquid: Concepts and Measurements. In: Rogers DF, Lethem MI, editors. Airway Mucus; basic mechanisms and clinical perspectives. Basel: Birkhauser Verlag; 1997. p. 1-17.

5. Cole P. Some aspects of temperature, moisture and heat relationships in the upper respiratory tract. Journal of Laryngology & Otology. 1953;67(8):449 -56.

6. Cole P. Further observations on the conditioning of respiratory air. Journal of Laryngology & Otology. 1953;67(11):669 -81.

7. Cole P. Temperature and humidity of respiratory air. Journal of Physiology. 1953;122(Suppl):51P.

8. Mcfadden ER, Pichurko BM, Bowman HF, Ingenito E, Burns S, Dowling N, et al. Thermal Mapping of the Airways in Humans. Journal of Applied Physiology. 1985;58(2):564 -70.

9. McFadden ER. Respiratory heat and water exchange: physiological and clinical implications. Journal of Applied Physiology: Respiratory, Environmental & Exercise Physiology. 1983;54(2):331 -6.

10. Febbraio MA, Carey MF, Snow RJ, Stathis CG, Hargreaves M. Influence of elevated muscle temperature on metabolism during intense, dynamic exercise. American Journal of Physiology-Regulatory Integrative and Comparative Physiology. 1996;40(5):R1251 -R5.

11. Morton JP, Kayani AC, McArdle A, Drust B. The exercise-induced stress response of skeletal muscle, with specific emphasis on humans. Sports Med. 2009;39(8):643 -62.

12. Dewhurst S, Graven-Nielsen T, De Vito G, Farina D. Muscle temperature has a different effect on force fluctuations in young and older women. Clinical Neurophysiology. 2007;118(4):762 -9.

13. Gray SR, De Vito G, Nimmo MA, Farina D, Ferguson RA. Skeletal muscle ATP turnover and muscle fiber conduction velocity are elevated at higher muscle temperatures during maximal power output development in humans. American Journal of Physiology-Regulatory Integrative and Comparative Physiology. 2006;290(2):R376 -R82.

14. Powers SK, Howley ET. Exercise physiology : theory and application to fitness and performance. 6th ed. Boston: McGraw-Hill; 2007.

15. Katch VL, McArdle WD, Katch FI. Essentials of exercise physiology. 4th ed. Philadelphia: Wolters Kluwer/Lippincott Williams & Wilkins Health; 2011. p. p.

16. Sivasankar M, Erickson E, Schneider S, Hawes A. Phonatory Effects of Airway Dehydration: Preliminary Evidence for Impaired Compensation to Oral Breathing in Individuals With a History of Vocal Fatigue. J Speech Lang Hear Res. 2008;51(6):1494 -506.

17. Verdolini-Marston K, Sandage M, Titze IR. Effect of hydration treatments on laryngeal nodules and polyps and related voice measures. Journal of Voice. 1994;8(1):30 -47.

18. Verdolini-Marston K, Titze IR, Druker DG. Changes in phonation threshold pressure with induced conditions of hydration. Journal of Voice. 1990;4(2):142 -51.

19. Verdolini K, Titze IR, Fennell A. Dependence of Phonatory Effort on Hydration Level. J Speech Hear Res. 1994;37(5):1001 -7.

20. Sivasankar M, Fisher KV. Oral breathing increases Pth and vocal effort by superficial drying of vocal fold mucosa. J Voice. 2002;16(2):172 -81.

21. Sivasankar M, Fisher KV. Oral breathing challenge in participants with vocal attrition. J Speech Lang Hear Res. 2003;46(6):1416 -27.

22. Sivasankar M, Erickson E. Short-Duration Accelerated Breathing Challenges Affect Phonation. Laryngoscope. 2009;119(8):1658 -63.

23. Roy N, Tanner K, Gray SD, Blomgren M, Fisher KV. An evaluation of the effects of three laryngeal lubricants on phonation threshold pressure (PTP). J Voice. 2003;17(3):331 -42.

24. Pascoe DD, Bellingar TA, McCluskey BS. Clothing and exercise. II. Influence of clothing during exercise/work in environmental extremes. Sports Med. 1994;18(2):94 -108.

25. Pascoe DD, Smith JW, Molloy JM. Regional surface temperature responses during a high intensity heat acclimation protocol. Medicine and Science in Sports and Exercise. 2004;36(5):0573.

26. Stevens J. Applied multivariate statistics for the social sciences. 4th ed. Mahwah, N.J.: Lawrence Erlbaum Associates; 2002. xiv, 699 p. p.

27. Refinetti R. The circadian rhythm of body temperature. Front Biosci. 2010;15:564-94.

28. Abitbol J, Abitbol P, Abitbol B. Sex hormones and the female voice. Journal of Voice. 1999;13(3):424 -46.

29. McFadden ER, Pichurko BM, Bowman HF, Ingenito E, Burns S, Dowling N, et al. Thermal mapping of the airways in humans. J Appl Physiol. 1985;58(2):564 -70.

30. Solomon NP, DiMattia MS. Effects of a vocally fatiguing task and systemic hydration on phonation threshold pressure. J Voice. 2000;14(3):341 -62.

31. Morgan MD, Triana MA, Milroy TJ. The effect of auditory feedback on phonation threshold pressure measurement. J Voice. 2004;18(1):46 -55.

32. Milbrath RL, Solomon NP. Do Vocal Warm-Up Exercises Alleviate Vocal Fatigue? Journal of Speech, Language & Hearing Research. 2003;46(2):422.

33. Verdolini K, Titze IR, Fennell A. Dependence of phonatory effort on hydration level. J Speech Hear Res. 1994;37(5):1001 -7. Epub 1994/10/01.

34. Rall JA, Woledge RC. Influence of temperature on mechanics and energetics of muscle contraction. American Journal of Physiology - Regulatory, Integrative and Comparative Physiology. 1990;259(2):R197 -R203.

35. Brooks GA, Fahey TD, Baldwin KM. Exercise physiology : human bioenergetics and its applications. 4th ed. Boston: McGraw-Hill; 2005. xxi, 876, 7, 22 p. p.

36. Brooks G, Hittelman K, Faulkner J, Beyer R. Temperature, skeletal muscle mitochondrial functions, and oxygen debt. American Journal of Physiology -- Legacy Content. 1971;220(4):1053 -9.

37. Starkie RL, Hargreaves M, Lambert DL, Proietto J, Febbraio MA. Effect of temperature on muscle metabolism during submaximal exercise in humans. Experimental physiology. 1999;84(4):775 -84.

38. De Ruiter CJ, De Haan A. Temperature effect on the force/velocity relationship of the fresh and fatigued human adductor pollicis muscle. Pflügers Archiv European Journal of Physiology. 2000;440(1):163 -70.

39. Man SF, Adams GK, 3rd, Proctor DF. Effects of temperature, relative humidity, and mode of breathing on canine airway secretions. J Appl Physiol. 1979;46(2):205 -10.

40. Freed AN, Davis MS. Hyperventilation with dry air increases airway surface fluid osmolality in canine peripheral airways. Am J Respir Crit Care Med. 1999;159(4 Pt 1):1101 -7.

Cumulative References

1. Roy N, Merrill RM, Thibeault S, Parsa RA, Gray SD, Smith EM. Prevalence of Voice Disorders in Teachers and the General Population. Journal of Speech, Language & Hearing Research. 2004;47(2):281 -93.

2. Sivasankar M, Fisher KV. Oral breathing challenge in participants with vocal attrition. J Speech Lang Hear Res. 2003;46(6):1416 -27.

3. Sivasankar M, Fisher KV. Oral breathing increases Pth and vocal effort by superficial drying of vocal fold mucosa. J Voice. 2002;16(2):172 -81.

4. Sivasankar M, Erickson E. Short-Duration Accelerated Breathing Challenges Affect Phonation. Laryngoscope. 2009;119(8):1658 -63.

5. Verdolini-Marston K, Sandage M, Titze IR. Effect of hydration treatments on laryngeal nodules and polyps and related voice measures. Journal of Voice. 1994;8(1):30 -47.

6. Verdolini-Marston K, Titze IR, Druker DG. Changes in phonation threshold pressure with induced conditions of hydration. Journal of Voice. 1990;4(2):142 -51.

7. Verdolini K, Titze IR, Fennell A. Dependence of phonatory effort on hydration level. J Speech Hear Res. 1994;37(5):1001 -7. Epub 1994/10/01. PubMed PMID: 7823546.

8. Sivasankar M, Erickson E, Schneider S, Hawes A. Phonatory Effects of Airway Dehydration: Preliminary Evidence for Impaired Compensation to Oral Breathing in Individuals With a History of Vocal Fatigue. J Speech Lang Hear Res. 2008;51(6):1494 -506.

9. Hemler RJB, Wieneke GH, Dejonckere PH. The effect of relative humidity of inhaled air onacoustic parameters of voice in normal subjects. Journal of Voice. 1997;11(3):295 -300.

10. Titze IR, Lemke J, Montequin D. Populations in the U.S. workforce who rely on voice as a primary tool of trade: a preliminary report. Journal of Voice. 1997;11(3):254 -9.

11. Hoffman-Ruddy B, Lehman J, Crandell C, Ingram D, Sapienza C. Laryngostroboscopic, Acoustic, and Environmental Characteristics of High-Risk Vocal Performers. Journal of Voice. 2001;15(4):543 -52.

12. Fang L, Wyon D, Clausen G, Fanger P. Impact of indoor air temperature and humidity in an office on perceived air quality, SBS symptoms and performance. Indoor Air. 2004;14(s7):74 -81.

13. Richter B, Lohle E, Maier W, Kliemann B, Verdolini K. Working conditions on stage: climatic considerations. Logoped Phoniatr Vocol. 2000;25(2):80 -6.

14. Fang L, Clausen G, Fanger P. Impact of Temperature and Humidity on the Perception of Indoor Air Quality. Indoor Air. 1998;8(2):80 -90.

15. Fairfax R. OSHA Policy on Indoor Air Quality: Office Temperature/Humidity and Environmental Tobacco Smoke. In: Administration OSH, editor. www.osha.gov2003.

16. Herrington-Hall BL, Lee L, Stemple JC, Niemi KR, McHone MM. Description of Laryngeal Pathologies by Age, Sex, and Occupation in a Treatment-Seeking Sample. J Speech Hear Disord. 1988;53(1):57 -64.

17. Lawrence MG. The Relationship between Relative Humidity and the Dewpoint Temperature in Moist Air: A Simple Conversion and Applications. Bulletin of the American Meteorological Society. 2005;86(2):225 -33.

18. Miers BT. On the interrelation of temperature, pressure, air density, and humidity. In: Laboratory UALCAS, editor. White Sands Missile Range1991. p. 23.

19. McCutchan J, Taylor CL. Respiratory heart exchange with varying temperature and humidity of inspired air. Journal of Applied Physiology. 1951;4(2):121 -35.

20. McFadden ER. Respiratory heat and water exchange: physiological and clinical implications. Journal of Applied Physiology: Respiratory, Environmental & Exercise Physiology. 1983;54(2):331 -6.

21. Cole P. Temperature and humidity of respiratory air. Journal of Physiology. 1953;122(Suppl):51P.

22. Cole P. Further observations on the conditioning of respiratory air. Journal of Laryngology & Otology. 1953;67(11):669 -81.

23. Cole P. Some aspects of temperature, moisture and heat relationships in the upper respiratory tract. Journal of Laryngology & Otology. 1953;67(8):449 -56.

24. Cole P. Recordings of respiratory air temperature. Journal of Laryngology & Otology. 1954;68(5):295 -307.

25. McFadden ER, Pichurko BM, Bowman HF, Ingenito E, Burns S, Dowling N, et al. Thermal mapping of the airways in humans. J Appl Physiol. 1985;58(2):564 -70.

26. Mcfadden ER, Pichurko BM. Intra-Airway Thermal Profiles during Exercise and Hyperventilation in Normal Man. Journal of Clinical Investigation. 1985;76(3):1007 -10.

27. Williams R, Rankin N, Smith T, Galler D, Seakins P. Relationship between the humidity and temperature of inspired gas and the function of the airway mucosa. Crit Care Med. 1996;24(11):1920 -9.

28. Widdicombe JG. Airway Surface Liquid: Concepts and Measurements. In: Rogers DF, Lethem MI, editors. Airway Mucus; basic mechanisms and clinical perspectives. Basel: Birkhauser Verlag; 1997. p. 1-17.

29. Mcfadden ER, Pichurko BM, Pichurko BM. Intra-Airway Thermal Profiles during Exercise and Hyperventilation in Normal Man. Journal of Clinical Investigation. 1985;76(3):1007 -10.

30. Bough JID, Heuer RJ, Sataloff RT, Hills JR, Cater JR. Intrasubject variability of objective voice measures. Journal of Voice. 1996;10(2):166 -74.

31. Abitbol J, Abitbol P, Abitbol B. Sex hormones and the female voice. J Voice. 1999;13(3):424 -46.

32. Titze IR. The physics of small-amplitude oscillation of the vocal folds. J Acoust Soc Am. 1988;83(4):1536 -52.

33. Cooper DS, Titze IR. Generation and Dissipation of Heat in Vocal Fold Tissue. J Speech Hear Res. 1985;28(2):207 -15.

34. Verdolini K, Titze IR, Fennell A. Dependence of Phonatory Effort on Hydration Level. J Speech Hear Res. 1994;37(5):1001 -7.

35. Man SF, Adams GK, 3rd, Proctor DF. Effects of temperature, relative humidity, and mode of breathing on canine airway secretions. J Appl Physiol. 1979;46(2):205 -10.

36. Boucher RC, Stutts MJ, Gatzy JT. Regional differences in bioelectric properties and ion flow in excised canine airways. J Appl Physiol. 1981;51(3):706 -14.

37. Freed AN, Davis MS. Hyperventilation with dry air increases airway surface fluid osmolality in canine peripheral airways. Am J Respir Crit Care Med. 1999;159(4 Pt 1):1101 -7.

38. Freed AN, Anderson SD, Daviskas E, McFadden J, E.R., Nelson JA, Skowronski ME, et al. THERMALLY INDUCED ASTHMA AND AIRWAY DRYING. Am J Respir Crit Care Med. 2000;161(6):2112 -3.

39. Fisher KV, Telser A, Phillips JE, Yeates DB. Regulation of vocal fold transepithelial water fluxes. J Appl Physiol. 2001;91(3):1401 -11.

40. Sivasankar M, Fisher KV. Vocal fold epithelial response to luminal osmotic perturbation. J Speech Lang Hear Res. 2007;50(4):886 -98.

41. Robertshaw D. Mechanisms for the control of respiratory evaporative heat loss in panting animals. J Appl Physiol. 2006;101(2):664 -8.

42. Hales JRS, Brown GD. Net energetic and thermoregulatory efficiency during panting in the sheep. Comparative Biochemistry and Physiology Part A: Physiology. 1974;49(3):413 -22.

43. Ingram DL, Legge KF. The influence of deep body temperatures and skin temperatures on respiratory frequency in the pig. J Physiol. 1972;220(2):283 -96.

44. Ray DW, Ingenito EP, Strek M, Schumacker PT, Solway J. Longitudinal distribution of canine respiratory heat and water exchanges. Journal of Applied Physiology. 1989;66(6):2788 -98.

45. Solway J, Leff AR, Dreshaj I, Munoz NM, Ingenito EP, Michaels D, et al. Circulatory heat sources for canine respiratory heat exchange. J Clin Invest. 1986;78(4):1015 -9.

46. Fayez I, Marai M, Alnaimy A, Habeeb M. Thermoregulation in rabbits. Cahiers Options Mediterraneennes. 1994;8:33-41.

47. Sivasankar M, Blazer-Yost B. Effects of Long-Acting Beta Adrenergic Agonists on Vocal Fold Ion Transport. Laryngoscope. 2009;119(3):602 -7.

48. Erickson E, Sivasankar M. Simulated reflux decreases vocal fold epithelial barrier resistance. The Laryngoscope. 2010;120(8):1569 -75.

49. Leydon C, Sivasankar M, Falciglia DL, Atkins C, Fisher KV. Vocal Fold Surface Hydration: A Review. Journal of Voice. 2009;23(6):658 -65. doi: DOI 10.1016/j.jvoice.2008.03.010. PubMed PMID: ISI:000271777500003.

50. Tanner K, Roy N, Merrill RM, Elstad M. The effects of three nebulized osmotic agents in the dry larynx. J Speech Lang Hear Res. 2007;50(3):635 -46.

51. Gray SR, De Vito G, Nimmo MA, Farina D, Ferguson RA. Skeletal muscle ATP turnover and muscle fiber conduction velocity are elevated at higher muscle temperatures during maximal power output development in humans. American Journal of Physiology-Regulatory Integrative and Comparative Physiology. 2006;290(2):R376 -R82.

52. Dewhurst S, Graven-Nielsen T, De Vito G, Farina D. Muscle temperature has a different effect on force fluctuations in young and older women. Clinical Neurophysiology. 2007;118(4):762 -9.

53. Febbraio MA, Carey MF, Snow RJ, Stathis CG, Hargreaves M. Influence of elevated muscle temperature on metabolism during intense, dynamic exercise. American Journal of Physiology-Regulatory Integrative and Comparative Physiology. 1996;40(5):R1251 -R5.

54. Morton JP, Kayani AC, McArdle A, Drust B. The exercise-induced stress response of skeletal muscle, with specific emphasis on humans. Sports Med. 2009;39(8):643 -62.

55. Brooks GA, Fahey TD, Baldwin KM. Exercise physiology : human bioenergetics and its applications. 4th ed. Boston: McGraw-Hill; 2005. xxi, 876, 7, 22 p. p.

56. Rall JA, Woledge RC. Influence of temperature on mechanics and energetics of muscle contraction. American Journal of Physiology - Regulatory, Integrative and Comparative Physiology. 1990;259(2):R197 -R203.

57. Brooks G, Hittelman K, Faulkner J, Beyer R. Temperature, skeletal muscle mitochondrial functions, and oxygen debt. American Journal of Physiology -- Legacy Content. 1971;220(4):1053 -9.

58. Nelson DL, Cox MM. Lehninger Principles of Biochemistry. Fifth Edition ed. New York, NY: W.H. Freeman and Company; 2008. 1158 p.

59. Powers SK, Howley ET. Exercise physiology : theory and application to fitness and performance. 6th ed. Boston: McGraw-Hill; 2007.

60. Clarke A. Temperature and the metabolic theory of ecology. Wiley-Blackwell; 2006. p. 405-12.

61. Febbraio MA. Alterations in energy metabolism during exercise and heat stress. Sports Med. 2001;31(1):47 -59.

62. Wirth VJ, Van Lunen BL, Mistry D, Saliba E, McCue FC. Temperature changes in deep muscles of humans during upper and lower extremity exercise. Journal of athletic training. 1998;33(3):211 -5.

63. Starkie RL, Hargreaves M, Lambert DL, Proietto J, Febbraio MA. Effect of temperature on muscle metabolism during submaximal exercise in humans. Experimental physiology. 1999;84(4):775 -84.

64. Febbraio MA, Snow RJ, Stathis CG, Hargreaves M, Carey MF. Effect of heat stress on muscle energy metabolism during exercise. J Appl Physiol. 1994;77(6):2827 -31.

65. De Ruiter CJ, De Haan A. Temperature effect on the force/velocity relationship of the fresh and fatigued human adductor pollicis muscle. Pflügers Archiv European Journal of Physiology. 2000;440(1):163 -70.

66. Bennett AF. Temperature and muscle. The Journal of experimental biology. 1985;115:333-44.

67. Potter JD, Hsu FJ, Pownall HJ. Thermodynamics of Ca2+ binding to troponin-C. J Biol Chem. 1977;252(7):2452 -4.

68. Witvrouw E, Mahieu N, Danneels L, McNair P. Stretching and Injury Prevention: An Obscure Relationship. Sports Medicine. 2004;34(7):443 -9.

69. Behm D, Chaouachi A. A review of the acute effects of static and dynamic stretching on performance. European Journal of Applied Physiology. 2011:1-19.

70. Herbert RD, Gabriel M. Effects of stretching before and after exercising on muscle soreness and risk of injury: systematic review. BMJ. 2002;325(7362):468.

71. Thacker SB, Gilchrist J, Stroup DF, Kimsey CD, Jr. The impact of stretching on sports injury risk: a systematic review of the literature. Med Sci Sports Exerc. 2004;36(3):371 -8.

72. Benninger M, Jacobson BH, Johnson AF. Vocal arts medicine : the care and prevention of professional voice disorders. Stuttgart ; New York: Thieme Medical Publishers; 1994. ix, 374 p.

73. Tucker HM. The larynx. 2nd ed. New York: Thieme Medical Publishers : Georg Thieme Verlag, Stuttgart; 1993. x, 326 p. p.

74. Lawrence V. Sermon on Hydration (The Evils of Dry). The NATS Journal. 1986;42(4):22 -3.

75. McFadden, Pichurko BM. Intraairway thermal profiles during exercise and hyperventilation in normal man. J Clin Invest. 1985;76(3):1007 -10. Epub 1985/09/01. doi: 10.1172/JCI112052. PubMed PMID: 4044825; PubMed Central PMCID: PMC423970.

76. Roy N, Tanner K, Gray SD, Blomgren M, Fisher KV. An evaluation of the effects of three laryngeal lubricants on phonation threshold pressure (PTP). J Voice. 2003;17(3):331 -42.

77. Pascoe DD, Bellingar TA, McCluskey BS. Clothing and exercise. II. Influence of clothing during exercise/work in environmental extremes. Sports Med. 1994;18(2):94 -108.

78. Pascoe DD, Smith JW, Molloy JM. Regional surface temperature responses during a high intensity heat acclimation protocol. Medicine and Science in Sports and Exercise. 2004;36(5):0573.

79. Stevens J. Applied multivariate statistics for the social sciences. 4th ed. Mahwah, N.J.: Lawrence Erlbaum Associates; 2002. xiv, 699 p. p.

80. Solomon NP. Assessment of laryngeal airway resistance and phonation threshold presure: Glottal Enterprises. In: Ma EPM, Yiu EML, editors. Handbook of Voice Assessments. San Diego, CA: Plural Publishing; 2011.

81. Diem K. Vapor Pressure and Boiling Point of Water. New York: Geigy Pharmaceuticals; 1962.

82. Refinetti R. The circadian rhythm of body temperature. Front Biosci. 2010;15:564-94.

83. Abitbol J, Abitbol P, Abitbol B. Sex hormones and the female voice. Journal of Voice. 1999;13(3):424 -46.

84. Pascoe DF, G. Comparison of measuring sites for the assessment of body temperature. Thermology International. 2009;19(1):35 -42.

85. Solomon NP, DiMattia MS. Effects of a vocally fatiguing task and systemic hydration on phonation threshold pressure. J Voice. 2000;14(3):3 41-62.

86. Morgan MD, Triana MA, Milroy TJ. The effect of auditory feedback on phonation threshold pressure measurement. J Voice. 2004;18(1):46 -55.

87. Milbrath RL, Solomon NP. Do Vocal Warm-Up Exercises Alleviate Vocal Fatigue? Journal of Speech, Language & Hearing Research. 2003;46(2):422.

88. Coyne MD, Kesick CM, Doherty TJ, Kolka MA, Stephenson LA. Circadian rhythm changes in core temperature over the menstrual cycle: method for noninvasive monitoring. American Journal of Physiology - Regulatory, Integrative and Comparative Physiology. 2000;279(4):R1316 -R20.

89. Erickson-Levendoski E, Sivasankar M. The Laryngeal Epithelium in Reflux. Perspectives on Voice and Voice Disorders. 2011;21(3):112 -7.

90. Smith E, Kirchner HL, Taylor M, Hoffman H, Lemke JH. Voice problems among teachers: Differences by gender and teaching characteristics. Journal of Voice. 1998;12(3):328 -34.

91. Wolfe V, Long J, Youngblood HC, Williford H, Olson MS. Vocal Parameters of Aerobic Instructors with and without Voice Problems. Journal of Voice. 2002;16(1):52 -60.

92. Mcfadden ER, Pichurko BM, Bowman HF, Ingenito E, Burns S, Dowling N, et al. Thermal Mapping of the Airways in Humans. Journal of Applied Physiology. 1985;58(2):564 -70.

93. Katch VL, McArdle WD, Katch FI. Essentials of exercise physiology. 4th ed.

Philadelphia: Wolters Kluwer/Lippincott Williams & Wilkins Health; 2011. p. p.

Appendices

Appendix A:

Informed Consent Aim 1

Auburn University
Auburn University, Alabama 36849-5323

Department of Kinesiology
2050 Memorial Coliseum
Thermal Lab (Room 2118)

Telephone: (334) 844-4483
Fax: (334) 844-1467
Thermal Lab: (334) 844-1479

Informed Consent for a research Study Entitled
"In Vivo Measures of Vocal Function Response to Environmental Conditions -
Aim 1: Voice Measurement Following Exposure to Six Environmental Conditions"

Project Overview: You are invited to participate in a research study that investigates the influence of environmental temperature and humidity on voice function. There are many professionals who require use of the voice in varying environmental conditions such as hot & humid air or cold & dry air. The effect of breathing warm or cool air on voice function is currently unknown. This study will be conducted by researchers in the Department of Kinesiology (Principle Investigator: Mary J. Sandage, MA, CCC-SLP, Doctoral Candidate).

Purpose: Our research objective is to compare temperature measures of the throat and upper airway (infrared thermography and pharyngeal [throat] temperature) to voice function measures (phonation threshold pressure and perceived voice effort) as influenced by short term exposure to environmental conditions that vary for temperature and humidity level and nose versus mouth breathing.

Participation Requirements: To be eligible, you must:

1. be at least 19 and no older than 35 years of age;
2. be a non-smoker;
3. have no history of voice problems;
4. be able to match a pitch/tone;
5. be free of any medical conditions that would make exposure to cool or warm, humid air problematic;
6. and be free of nasal obstruction or allergies.

If you meet these participation requirements, you will be asked to read and sign this informed consent, complete a medical questionnaire, pass a voice screening, and demonstrate the ability to match specific tones with your voice. The voice screening will be scheduled at the Auburn University Voice Laboratory in the Department of Communication Disorders, Haley Center. The pitch/tone matching screening and all of the study experiments will be completed in the Thermal Laboratory, 2118 Memorial Coliseum. Your involvement will take 4 appointments, the first lasting 1 hour and the next 3 lasting about 3 hours each, for a total of about 10 hours participation.

Participant's initials _____

"In Vivo Measures of Vocal Function Response to Environmental Conditions - Aim 1: Voice Measurement Following Exposure to Six Environmental Conditions"

Testing Protocol:

After you have agreed to participate and have passed the medical screening questionnaire, your vocal folds will be studied to make sure that your voice box is healthy. This voice screening is a standard procedure in most clinics that perform voice evaluations and the investigator who will be doing this procedure is a licensed speech language pathologist who has over 17 years of experience doing this procedure (Mary Sandage- Adjunct Clinical Faculty, Dept. of Communicative Disorders, Auburn University).

During the voice screening you will be asked to hold out the sound "ee" while the investigator gently holds your tongue with gauze and places a small-diameter endoscope toward the back of your mouth. This endoscope allows for digital filming of your vocal folds (muscles that make your voice sounds) while they are vibrating. You will get to see the film of your vocal folds and the film will be explained to you at the time of the screening. The non-identifiable stroboscopic image of the vocal folds will be reviewed by a licensed otolaryngologist (Stites Whatley, MD) a medical doctor who specializes in the health of the ears, nose, and throat. Following review by the licensed otolaryngologist, you will receive a letter indicating if you would benefit from additional medical assessment of your voice with a potential site referral for further medical assessment as appropriate. This letter will be given directly to you following the physician review. The presence or absence of laryngeal (vocal fold) disease may influence temperature measures, and may need to be accounted for in the data analysis. After you have completed the voice screening, you will be asked to match a few pitches or tones with your voice. If you are able to match the tones accurately (within one key on the keyboard), then you will be scheduled for the experimental trial.

The first appointment will involve pre-training to familiarize you with the measures that will be taken. During this visit the pitch that you will use for data collection will be determined and you will be familiarized with the other data collection procedures: placement of the pharyngeal temperature probe, phonation threshold pressures (PTP), placement of the laryngeal mirror for the infared thermographic image of the voice box, and estimation of perceived voice effort. The pitch that will be used for the PTP measures will be determined by having you say the sentence, "The blue spot is on the key again", several times into a microphone. The pharyngeal temperature probe is a small diameter (1.3 mm) probe that is advanced through your nose approximately 17 cm until it is near your voice box but not so far that it will cause coughing, gagging or discomfort. PTP, which measures the amount of air pressure that you need to start your voice, will be measured through a mask that is placed over your mouth and nose that will collect the pressure of air that comes out of your mouth. This mask allows you to breathe normally and this is a standard procedure that is done in voice clinics around the world. You will then be trained to do the specific voicing task until you feel comfortable with it. Following PTP training, you will be shown how the laryngeal mirror will be placed at the back of your mouth, with a chance to practice the procedure until you are comfortable.

Following the familiarization visit, three additional visits will be scheduled, of approximately 3 hours each, at the same time of day, with 2 environmental conditions completed for each visit. All participants will be asked to complete all 6 environmental trials.

 Participant's initials _____

"In Vivo Measures of Vocal Function Response to Environmental Conditions - Aim 1: Voice Measurement Following Exposure to Six Environmental Conditions"

The temperatures being used are 15°C/59°F, 25°C/77°F, and 35°C/95°F with 2 different humidity settings for each temperature. Women participants will schedule these three visits during a point in the menstrual cycle that avoids ovulation, 7-12 days after the first day of menstruation. Participants will be asked to wear the same type of clothing for each of the conditions. For the temperature conditions procedures, you will be asked to avoid eating, drinking, exercising or excessive voice use for one hour prior to reporting to the Thermal Lab. When you first arrive at the Thermal Lab, you will be asked to provide a urine sample to make sure that you are appropriately hydrated. If you are not hydrated well enough, you will be asked to drink water until you are above dehydration level. Once this is determined, the pharyngeal (throat) temperature probe will be placed. Once the temperature probe is placed, you will be asked to sit in the thermal chamber for 20 minutes, breathing either through your mouth or through your nose. Nose clips will be provided for the mouth breathing condition. After you have been in the thermal chamber for 20 minutes the following measures will be taken: pharyngeal temperature, phonation threshold pressure measures, and infrared imaging from the laryngeal mirror. You will then be asked to read a short passage and rate your voice effort. Following these measures, you will remain in the thermal chamber and change to the other mode of breathing (mouth or nose) for 20 minutes, followed by collection of the same measures. After you finish one environmental condition you will be asked to rest and drink 500 ml of room temperature water while the climate room is prepared for the next temperature/humidity condition. The procedure described above will be repeated for the 2nd condition of the scheduled visit. The procedures described above will be repeated for each of thermal conditions.

***Testing Variables**:*
The following measurements will be taken during this investigation: hydration status (urine sample), throat temperature measures via the pharyngeal temperature probe and infrared thermal imaging, and voice function measures via phonation threshold pressure and perceived voice effort. The throat temperature measures and the voice function measures will be taken twice for each environmental condition: 1) 20 minutes after you have adjusted to room temperature using only nose breathing and, 2) 20 minutes after you have adjusted to room temperature using only mouth breathing.

Potential Risks:
There is a possible risk of loss of confidentiality of data. There is also a risk of infection from the endoscopic voice screening and the thermal probe and laryngeal mirror imaging procedures. While inserting the pharyngeal probe, there is a remote possibility that the nasal lining may be scratched with insertion. The gag reflex may be initiated with insertion of the pharyngeal probe, the rigid endoscope for the voice screening, and the laryngeal mirror used for the infrared thermal imaging. These procedures may have associated mild discomfort. In the unlikely event that you may need medical attention, you will be responsible for all expenses.

Participant's initials _____

Precautions:

You have the right to stop any trial at any time. Your participation or lack of participation will not affect your relationship with the researchers, the Department of Kinesiology, or the Department of Communication Disorders. Your data is being recorded on an Excel sheet that is only identifiable by a participant number. The Master code list is maintained by Dr Pascoe, Mary Sandage's academic advisor, in a locked office and locked filing cabinet.

The chance of infection is minimal due to the use of standard endoscope disinfection procedures. Laryngeal mirror and throat temperature probe cleaning procedures will follow methods approved by the Centers for Disease Control. The chance of scratching your nasal lining when placing the pharyngeal probe is unlikely as the investigators have extensive training in this procedure and the diameter of the probe is small (1.3mm). The chance of gagging with placement of the rigid oral endoscope for the voice screening or with placement of the laryngeal mirror for the infrared imaging is reduced because the investigator performing both of these procedures has over 17 years of experience conducting these procedures and knows to avoid making contact with those parts of the throat that trigger the gag reflex, i.e., base of tongue and tonsillar pillars.

Benefits:

Free voice screening with imaging equipment ($75 value for students/ $150 for non-students at the Auburn University Speech & Hearing Clinic) will provide information about laryngeal (voice box) health. Should any referral for additional voice assessment be recommended by the screening otolaryngologist physician, treatment or care related to this medical referral is not part of the investigation and must be paid by you.

Confidentiality*:* Any information obtained in connection with this research study that can be identified with you will remain confidential. Your identity link to the data will consist of a master list of participant and number. This link will be destroyed upon your completion of the trials. Your data recorded during the trial will be recorded by participant number. Non-identifiable data will be used in possible publications if the results warrant a reporting of the data.

If you have any questions, we invite you to ask them now. If you have questions later, you can contact Mary Sandage at (334) 844-1479 or email sandamj@auburn.edu. You will be provided with a copy of this document to keep. For more information regarding your rights as a research participant, you may contact the Auburn University Office of Human Subjects Research or the Institutional Review Board phone (334) 844-5966 or email at hsubjec@auburn.edu or IRBChair@auburn.edu.

HAVING READ THE INFORMATION PROVIDED, YOU MUST DECIDE WHETHER OR NOT YOU WISH TO PARTICIPATE IN THIS RESEARCH STUDY. YOUR SIGNATURE INDCATES YOUR WILLINGNESS TO PARTICIPATE.

_____	_____
Participant's Signature Date	Investigator's Signature Date
_____	_____
Print Name	Print Name
_____	_____
Witness Date	Print Name

Appendix B:

Informed Consent Aim 2

Auburn University
Auburn University, Alabama 36849-5323

Department of Kinesiology
2050 Memorial Coliseum
Thermal Lab (Room 2118)

Telephone: (334) 844-4483
Fax: (334) 844-1467
Thermal Lab: (334) 844-1479

Informed Consent for a research Study Entitled
"In Vivo Measures of Vocal Function Response to Environmental Conditions - Aim 2: Voice Measurement Following Short Term Submaximal Exercise"

Project Overview: You are invited to participate in a research study that investigates the influence of temperature and humidity changes on voice function following short term submaximal cycling exercise. There are many professionals who require use of the voice during or following submaximal aerobic activity, e.g., factory workers, aerobics instructors, physical education teachers, and performers. The effect of increased breathing rate (ventilation) on voice function is currently unknown. This study will be conducted by researchers in the Department of Kinesiology (Principle Investigator: Mary J. Sandage, MA, CCC-SLP, Doctoral Candidate).

Purpose: Our research objective is to compare temperature measures of the throat and upper airway (infrared thermography and pharyngeal [throat] temperature) to voice function measures (phonation threshold pressure and perceived voice effort) as influenced by short term (8-minute) submaximal aerobic cycling.

Participation Requirements: To be eligible, you must:

1. be at least 19 and no older than 35 years of age;
2. be a non-smoker
3. have no history of voice problems;
4. be able to match a pitch (make a pitch with your voice that matches a keyboard pitch);
5. be able to perform submaximal cycling exercise for 8 minutes;
6. and be free of nasal obstruction or allergies.

If you meet these participation requirements, you will be asked to read and sign this informed consent, complete a medical questionnaire, pass a voice screening, and demonstrate the ability to match specific tones with your voice. The voice screening will be scheduled at the Auburn University Voice Laboratory in the Department of Communication Disorders, Haley Center. The pitch/tone matching screening and all of the study experiments will be completed in the Thermal Laboratory, 2118 Memorial Coliseum. Your involvement will require 2 appointments and should take about 2.5 total hours.

Participant's initials _____

"In Vivo Measures of Vocal Function Response to Environmental Conditions - Aim 2: Voice Measurement Following Short Term Submaximal Exercise"

Testing Protocol:

After you have agreed to participate and have passed the medical screening questionnaire, your vocal folds will be studied to make sure that your voice box is healthy. This voice screening is a standard procedure in most clinics that perform voice evaluations and the investigator who will be doing this procedure is a licensed speech language pathologist who has over 17 years of experience doing this procedure (Mary Sandage- Adjunct Clinical Faculty, Dept. of Communicative Disorders, Auburn University). During this screening you will be asked to hold out the sound "ee" while the investigator gently holds your tongue with gauze and places a small-diameter endoscope toward the back of your mouth. This endoscope allows for digital filming of your vocal folds (muscles that make your voice sounds) while they are vibrating.

You will get to see the film of your vocal folds and the film will be explained to you at the time of the screening. The non-identifiable stroboscopic image of the vocal folds will be reviewed by a licensed otolaryngologist (Stites Whatley, MD) a medical doctor who specializes in the health of the ears, nose, and throat. Following review by the licensed otolaryngologist, you will receive a letter indicating if you would benefit from additional medical assessment of your voice with a potential site referral for further medical assessment as appropriate. This letter will be given directly to you following the physician screening. The presence or absence of laryngeal (vocal fold) disease may influence temperature measures, and may need to be accounted for in the data analysis. After you have completed the voice screening, you will be asked to match a few pitches or tones with your voice. If you are able to match the tones accurately (within one key on the keyboard), then you will be scheduled for the experimental trial.

Prior to the experimental trial, you will be asked to refrain from eating, drinking, exercising, and excessive voice use for one hour. When you arrive at the Thermal Lab, you will be asked to provide a urine sample to make sure that you are appropriately hydrated. If you are not hydrated well enough, you will be asked to drink water until you are above dehydration level. Once this is determined, the cycling work rate at which you reach 20 breaths per minute, a mild exercise level, will be determined. This will require that you cycle, on a stationary bike specially designed for this work, at three different work rates, 50, 100, and 150 watts during which time your breathing rate will be monitored. The work rate that is determined will be the rate at which you cycle during the experimental condition. Prior to entering the climate chamber the pitch that you will use for data collection will be determined and you will be familiarized with the other data collection procedures: phonation threshold pressures (PTP), placement of the laryngeal mirror for the infared thermographic image of the voice box, and estimation of perceived voice effort. The pitch that will be used for the PTP measures will be determined by having you say the sentence, "The blue spot is on the key again", several times into a microphone. PTP, which measures the amount of air pressure that you need to start your voice, will be measured through a mask that is placed over your mouth and nose that will collect the pressure of air that comes out of your mouth. This mask allows you to breathe normally and this is a standard procedure that is done in voice clinics around the world. You will then be trained to do the specific voicing task until you feel comfortable with it. Following PTP training, you will be shown how the laryngeal mirror will be placed at the back of your mouth, with a chance to practice the procedure until you are comfortable.

Participant's initials _____

"In Vivo Measures of Vocal Function Response to Environmental Conditions - Aim 2: Voice Measurement Following Short Term Submaximal Exercise"

After your work rate is established and pre-trail familiarization is completed, the pharyngeal (throat) temperature probe will be placed through your nose and into your throat about 17 cm, stopping before you feel uncomfortable. Once the temperature probe is placed, you will be asked to sit in the thermal chamber for 20 minutes, breathing through your nose. The thermal chamber will be set at 25°C/40% relative humidity. After you have been in the thermal chamber for 20 minutes, the following measures will be taken: pharyngeal temperature, phonation threshold pressure measures, and infrared imaging from the laryngeal mirror. You will then be asked to read a short passage and rate your voice effort. Following these measures, you will move to the stationary bicycle and begin cycling at the designated work rate for 8 continuous minutes. The work rate chosen for this study will likely be below a moderate level of exercise for you. During this time you will be asked to breath only through your mouth and a nose clip will be used to ensure that you only mouth breathe. Following the 8 minutes of cycling exercise, the same measures listed above will be repeated.

***Testing Variables**:*
The following measurements will be taken during this investigation: hydration status (urine sample), throat temperature measures via the pharyngeal temperature probe and infrared thermal imaging, and voice function measures via phonation threshold pressure and perceived voice effort. The throat temperature measures and the voice function measures will be taken twice: 1) 20 minutes after you have adjusted to room temperature using only nose breathing and, 2) following the 8 minute submaximal cycling exercise bout.

Potential Risks:
There is a possible risk of loss of confidentiality of data. There is also a risk of infection from the endoscopic voice screening and the thermal probe and laryngeal mirror imaging procedures. While inserting the pharyngeal probe, there is a remote possibility that the nasal lining may be scratched with insertion. The gag reflex may be initiated with insertion of the pharyngeal probe, the rigid endoscope for the voice screening, and the laryngeal mirror used for the infrared thermal imaging. These procedures may have associated mild discomfort. In the unlikely event that you need medical attention, you will be responsible for all expenses.

Precautions:
You have the right to stop any trial at any time. Your participation or lack of participation will not affect your relationship with the researchers, the Department of Kinesiology, or the Department of Communication Disorders. Your data is being recorded on an Excel sheet that is only identifiable by a participant number. The Master code list is maintained by Dr Pascoe, Mary Sandage's academic advisor, in a locked office and locked filing cabinet.

 Participant's initials _____

"In Vivo Measures of Vocal Function Response to Environmental Conditions - Aim 2: Voice Measurement Following Short Term Submaximal Exercise"

The chance of infection is minimal due to the use of standard endoscope disinfection procedures. Laryngeal mirror and throat temperature probe cleaning procedures will follow methods approved by the Centers for Disease Control. The chance of scratching your nasal lining when placing the pharyngeal probe is unlikely as the investigators have extensive training in this procedure and the diameter of the probe is small (1.3mm). The chance of gagging with placement of the rigid oral endoscope for the voice screening or with placement of the laryngeal mirror for the infrared imaging is reduced because the investigator performing both of these procedures has over 17 years of experience conducting these procedures and knows to avoid making contact with those parts of the throat that trigger the gag reflex, i.e., base of tongue and tonsillar pillars.

Benefits: Free voice screening with imaging equipment ($75 value for students/ $150 for non-students at the Auburn University Speech & Hearing Clinic) will provide information about laryngeal (voice box) health. Should any referral for additional voice assessment be recommended by the screening otolaryngologist physician, treatment or care related to this medical referral is not part of the investigation and must be paid by you.

Confidentiality*:* Any information obtained in connection with this research study that can be identified with you will remain confidential. Your identity link to the data will consist of a master list of participant and number. This link will be destroyed upon your completion of the trials. Your data recorded during the trial will be recorded by participant number. Non-identifiable data will be used in possible publications if the results warrant a reporting of the data.

If you have any questions, we invite you to ask them now. If you have questions later, you can contact Mary Sandage, MA, CCC-SLP at (334) 844-1479 or email sandamj@auburn.edu. You will be provided with a copy of this document to keep. For more information regarding your rights as a research participant, you may contact the Auburn University Office of Human Subjects Research or the Institutional Review Board phone (334) 844-5966 or email at hsubjec@auburn.edu or IRBChair@auburn.edu.

HAVING READ THE INFORMATION PROVIDED, YOU MUST DECIDE WHETHER OR NOT YOU WISH TO PARTICIPATE IN THIS RESEARCH STUDY. YOUR SIGNATURE INDCATES YOUR WILLINGNESS TO PARTICIPATE.

_____	_____
Participant's Signature Date	Investigator's Signature Date
_____	_____
Print Name	Print Name
_____	_____
Witness Date	Print Name

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Appendix C:

Medical Screening Instrument

Physical Readiness Activity Questionnaire (Par Q) *

Please read each question carefully and answer honestly. If you do not understand the question, please ask the investigator for clarification. Check the appropriate answer.

1. Are you under the age 19 or over the age of 35?

2. Do you have any history of voice problems?

3. Do you presently smoke or have been a regular smoker?

4. Has your doctor ever said you have heart trouble?

5. Do you have a family history of early cardiovascular death before the age of 50?

6. Have you ever had a heart murmur, rheumatic fever or respiratory problems?

7. Have you ever been told that you have a fast resting heart rate?

8. Have you ever been told by your doctor or nurse that your blood pressure is too high?

9. Have you ever been told that your cholesterol is too high?

10. Have you been told that you have a kidney disorder?

11. Have you been told that you have diabetes or that your blood sugar is too high?

12. Have you been told that your electrocardiogram (EKG), 12 lead EKG or stress test is not normal?

13. Do you have any nose or throat medical issues (e.g. deviated septum, allergies, etc) that would interfere with placement of a 1.3 mm temperature probe through your nose and into the back of your throat?

14. Have you been hospitalized in the past year?

15. Do you have any health conditions that would make it hard to tolerate cold or hot temperatures?

16. Are you taking prescription medicine? If so, what?

17. Do you have any reason to believe that your participation in this investigative effort may put your health or well-being at risk? If so, please state reason.

Signature of subject _____ Date _____

*Adapted from British Columbia Department of Health and Michigan Heart Association

Appendix D:

VOICING HISTORY QUESTIONNAIRE

Subject #:
Date:

These questions refer to behaviors that may influence the interpretation of data collected during this study. *In the following section, if you do NOT want to answer any particular question, leave it blank.*

Voice Use History:

1. Do you sing professionally or in an organized singing group, e.g., choir? Yes ___ No ___
2. Are you taking singing or acting voice lessons at this time? Yes ___ No ___
3. Have you taken singing lessons in the past? Yes ___ No ___
4. Do you use your voice a lot for work or school? Yes ___ No ___
5. Were you ever or are you currently a cheerleader? Yes ___ No ___
6. Do you use your voice loudly or for long periods of time for any hobbies? Yes ___ No ___

Behavioral History

1. How many glasses of water or juice do you drink on an average day? _____
2. Please list all prescribed medications and over the counter supplements that you take. Many medications and supplements can have an effect on voice function:
   _____
3. Do you drink caffeine? Yes ___ No ___
   a. If yes, how many caffeinated drinks to you typically have per day:
4. Do you drink alcohol? Yes ___ No ___
   a. If yes, how many alcoholic beverages do you typically have per day:
5. Do you have a history of reflux/stomach acid problems? Yes ___ No ___
6. When you wake up in the morning is your voice usually rough or hoarse? Yes ___ No ___
7. Do you cough or clear your throat frequently? Yes ___ No ___
8. Have you ever had any throat or neck injuries or surgery? yes ___ No ___

Appendix E:

DATA SUMMARY SHEET AIM 1

Participant #: **Hydration Level:**

(g/ml)

PTP f_0:

Date: **Condition: °C/ %RH**

Breathing Mode	**IR Pharyngeal Temperature (°C)**	**IR Laryngeal Temperature (°C)**	**Phonation Threshold Pressure (PTP)**		
			1	**2**	**3**
Nose					
Mouth					

Perceived Voicing Effort _____

No Effort **Maximum Effort**

Date: **Condition: °C/ %RH**

Breathing Mode	**IR Pharyngeal Temperature (°C)**	**IR Laryngeal Temperature (°C)**	**Phonation Threshold Pressure (PTP)**		
			1	**2**	**3**
Nose					
Mouth					

Perceived Voicing Effort _____

No Effort **Maximum Effort**

Date: **Condition: °C/ %RH**

Breathing Mode	**IR Pharyngeal Temperature (°C)**	**IR Laryngeal Temperature (°C)**	**Phonation Threshold Pressure (PTP)**		
			1	**2**	**3**
Nose					
Mouth					

Perceived Voice Effort _____

No Effort **Maximum Effort**

Date: **Condition: °C/ %RH**

Breathing Mode	**IR Pharyngeal Temperature (°C)**	**IR Laryngeal Temperature (°C)**	**Phonation Threshold Pressure (PTP)**		
			1	**2**	**3**
Mouth					
Nose					

Perceived Voicing Effort ____________________

No Effort **Maximum Effort**

Date: **Condition: °C/ %RH**

Breathing Mode	**IR Pharyngeal Temperature (°C)**	**IR Laryngeal Temperature (°C)**	**Phonation Threshold Pressure (PTP)**		
			1	**2**	**3**
Mouth					
Nose					

Perceived Voicing Effort ____________________

No Effort **Maximum Effort**

Date: **Condition: °C/ %RH**

Breathing Mode	**IR Pharyngeal Temperature (°C)**	**IR Laryngeal Temperature (°C)**	**Phonation Threshold Pressure (PTP)**		
			1	**2**	**3**
Mouth					
Nose					

Perceived Voicing Effort ____________________

No Effort **Maximum Effort**

Appendix F:

DATA SUMMARY SHEET AIM 2

Date:
Participant #
PTP f_0: **f_0**
Work Rate: _____(Watts) **RPM: _____** **VO_2: ____ ml/kg/min**
HR: _____
Hydration Level: **(g/ml)**

Condition: 25°C/40% RH

Breathing Mode	Pharyngeal Temperature (°C)	Laryngeal Temperature (°C)	Phonation Threshold Pressure (cm/H_20)			Breaths Per Minute
			1	2	3	
Nose						
Mouth						

Nose Breathing - Perceived Voicing Effort

No Physical Effort **Maximum Physical Effort**

Mouth Breathing - Perceived Voicing Effort

No Physical Effort **Maximum Physical Effort**