EFFECTS OF HYPER- AND HYPOCAPNIA ON PHONATORY LARYNGEAL RESISTANCE

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The larynx has a challenging dual role in the simultaneous regulation of gas flow into and out of the lungs while also establishing resistance required for vocal fold vibration in voiced communication. Particular challenges may arise when the larynx is required to alter upper airway resistance to meet respiratory demands in a way that conflicts with requirements for voice production. Little if anything is known about reciprocal relations between these functions, particularly under conditions of respiratory abnormality that affect large sectors of the population- an estimated 25% of the US population who experience respiratory abnormalities and also relies on the larynx for voiced communication.

In order to address this gap, the current study investigated two specific aims in a single within-subjects experiment: **Specific Aim 1 (SA1)** assessed spontaneous fluctuations in phonatory laryngeal resistance during states of (**a**) induced hypocapnia (low arterial carbon dioxide) and (**b**) induced hypercapnia (high arterial carbon dioxide), in comparison to a eupneic

control condition and **Specific Aim 2 (SA2)** investigated the reciprocal effects of laryngeal resistance modulations on respiratory homeostasis.

Results of the first aim demonstrated that phonatory laryngeal resistance remained stable and did not significantly change despite manipulations of inspired gas concentrations causing significant increases and decreases in carbon dioxide (CO_2) levels. For the second aim, results showed that phonation significantly increased levels of end-tidal carbon dioxide ($P_{et}CO_2$) in all experimental conditions, compared to $P_{et}CO_2$ levels during rest breathing. Findings provide support for a theory of voice motor control suggesting that phonatory laryngeal resistance may be an essential, relatively immutable control parameter in phonation (except perhaps under extreme conditions not tested herein), and provides data on the influence of phonation on respiration. The current work sets the foundation for future studies of laryngeal function during phonation in individuals with lower airway disease.

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PREFACE

"Up up up up up up raises the stakes of the game, each day sinks its boot print into her clay, and she's not the same. She looks out on the ledge, and begins to build a home, and it's enough just to look around, and know she's not alone" – Ani DiFranco

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1.0 INTRODUCTION

Examination of the literature reveals an unfortunate divide between research in respiratory physiology versus phonatory physiology. Specifically, literature in both domains largely ignores the complex relationship between the lower and upper airways for simultaneous goals of maintaining blood-gas homeostasis and producing voice. One fairly trivial example is observed in the diverse methodology used to measure laryngeal resistance across domains. In respiratory physiology, laryngeal resistance is often evaluated *during breathing* by measures of glottal area (Bartlett, 1979; England & Bartlett, 1982; England, Bartlett, & Daubenspeck, 1982; England, Bartlett, & Knuth, 1982). In voice science, laryngeal resistance is quantitatively assessed *during phonation* as the ratio of estimated sub-glottal pressure (P_{sub}) to trans-laryngeal airflow (Smitheran & Hixon, 1981). It is clear that lower and upper airway functions are critical for both ventilation and communication. Relevant for the present study, clinical observations suggest that dyspnea (difficulty breathing) and voice problems are common co-morbidities (Hočevar-Boltežar, Janko, & Žargi, 1998; Hoit, Lansing, & Perona, 2007; Koufman & Blalock, 1988; Nguyen, Kenny, Tran, & Livesey, 2009). Regrettably, little information is available about potential mechanisms by which abnormal respiration may affect the larynx's phonatory functions and conversely, how laryngeal mechanics during voice production may in turn affect ventilation. The current investigation is a second step in a long-range plan investigating the intricate balance between respiratory and laryngeal functioning in individuals who both breathe and speak, who clearly constitute the vast majority of the population.

The long-range research plan involves four stages: **(1)** identify aerodynamic profiles of individuals with Primary Muscle Dysphonia (MTD-1), which is thought to involve phonatory-respiratory dysregulation; **(2)** evaluate reciprocal functions of larynx and lower airways with respect to blood-gas homeostasis during phonation; **(3)** identify potential risk factors for voice problems such as MTD-1 in individuals with respiratory abnormalities, and identify bio-behavioral markers connecting them, based on findings from the preceding series; and (**4)** develop a multi-disciplinary evaluation and treatment approach for individuals with co-morbid respiratory and voice abnormalities.

The first of the foregoing issues was addressed by a published, retrospective review of aerodynamic data for 90 female patients diagnosed with MTD-1, at their first clinical presentation. Results identified several distinct patterns of laryngeal resistance, derived from aerodynamic measures discussed shortly. Results also illuminated heterogeneity in the aerodynamic features of the disorder and further suggested a possible interaction between respiratory and phonatory functions at its center. Finally, results also led to the theoretical underpinnings for proposed research phase (2) above, which is the focus of the current dissertation. Research phases 3 and 4 are planned for post-doctoral work.

Specific Aims of the current proposal were (SA1): Assess changes in phonatory laryngeal resistance during **(a)** induced hypocapnia (low arterial carbon dioxide) and **(b)** induced hypercapnia (high arterial carbon dioxide), and **(SA2):** investigate the effects of such changes on respiratory homeostasis. Both SAs were addressed in a single, within-subjects experiment, which involved manipulation of blood-gas concentrations of CO_2 to establish respective states of hypo- and hypercapnia in healthy women, during phonation and rest. For SA1 (a) and (b), changes in

phonatory laryngeal resistance were assessed as a function of respiratory condition (hypocapnia, hypercapnia, and normal “eupneic” breathing). For SA2, changes in end-tidal PCO_2 were evaluated as a function of laryngeal resistance during rest and phonation in hypocapnic, hypercapnic, and eupneic conditions. This project represents a novel convergence of respiratory and voice science that expands basic physiologic research, ultimately with direct application for individuals with co-morbid breathing and voice problems and individuals at risk for them.

2.0 VOICE PROBLEMS

Voice disorders are estimated to affect 3-9% of the population at any given moment in time (Verdolini & Ramig, 2001). Consequences include communication breakdown, lost income, and psychological distress (Verdolini & Ramig, 2001). Voice problems are often divided into two broad categories based on suspected etiology: those due to obvious physical pathology, and those without a clear organic cause. Unfortunately, problems diagnosed as "non-organic" often involve unidentified physical factors. One of the most common putatively "non-organic" conditions is primary muscle tension dysphonia (MTD-1). This condition is often diagnosed based on reported symptoms in the absence of clear clinical findings (Roy, 2010). Patients with MTD-1 report subjective somatic complaints of odynophonia (Koufman & Blalock, 1988; Sapienza, Walton, & Murry, 2000), vocal fatigue (Garrett & Cohen, 2008; Nguyen & Kenny, 2009; Nguyen, et al., 2009; Sapienza, et al., 2000), shortness of breath with speaking, (Nguyen, et al., 2009), cough, and throat clearing (Garrett & Cohen, 2008).

A number of mechanisms are likely responsible for the development of this condition. Relevant for the proposed study, clinicians and researchers have noted changes not only in laryngeal function with MTD-1, but have also made non-specific assertions about respiratory dyscoordination as a foundational element in the development and maintenance of this disorder. We now turn to a fuller discussion of MTD-1 and its possible respiratory involvement.

2.1 PRIMARY MUSCLE TENSION DYSPHONIA

Primary MTD (MTD-1) accounts for up to 40-70% of voice clinic caseloads (Angsuwarangsee & Morrison, 2002; Roy, 2003), and is most often diagnosed in individuals with substantial professional voice demands (Willinger, Volkl-Kernstock, & Aschauer, 2005). The condition has been called by many names, including *hyper/hypofunctional voice disorder, spastic dysphonia, functional dysphonia, psychogenic dysphonia* and *muscle misuse voice disorder* (Aronson, Brown, Litin, & Pearson, 1968; Froeschels, 1952; Hillman, Holmberg, Perkell, Walsh, & Vaughan, 1989a; Morrison & Rammage, 1993; Roy, 2003; Roy et al., 1997). The wide array of diagnostic terminology reflects the challenge in identifying one term for a disorder that presents with a considerable variety of patient-reported symptoms as well as subjective and objective clinical representations. MTD-1 was once thought to be a vocal manifestation of a psychopathology, or a conversion disorder (Aronson, et al., 1968; Kinzl, Biebl, & Rauchegger, 1988; Millar, Deary, Wilson, & MacKenzie, 1999). Today, it is also attributed to "poor speaking or singing technique" (Andrade et al., 2000; Angsuwarangsee & Morrison, 2002; Awan & Roy, 2009; Koufman & Blalock, 1988; Roy, 2010; Roy & Hendarto, 2005; Sapienza, et al., 2000; Van Houtte, Van Lierde, & Claeys, 2010; Vertigan et al., 2006), which may involve some sort of generally poorly defined breathing abnormality (Hixon & Putnam, 1983; Rubin, Macdonald, & Blake, 2010), for example: "surges of uncontrolled expiratory air" (Morrison & Rammage, 1993, p. 431), increased abdominal-thoracic muscle activation (Hočevar-Boltežar, et al., 1998), and "inappropriate phonatory habits" (Iwarsson, Thomasson, & Sundberg, 1998, p. 424).

2.1.1 Respiratory characteristics of muscle tension dysphonia

Consistent with the designation of MTD-1 as a laryngeal muscle tension response, intrinsic and extrinsic laryngeal muscle tension patterns have been described for this condition for decades (Morrison, Rammage, Belisle, Pullan, & Nichol, 1983). However, germane to the present study, suggestions have also been made that MTD-1 may be associated with abnormal coordinative patterns not only at the laryngeal level, but also across respiratory and laryngeal subsystems of phonation. Specifically, according to Morrison and Rammage (1993), individuals with MTD-1 demonstrate "poor coordination among respiratory, phonatory, resonatory and articulatory gestures" (p. 429), "inappropriate muscle tone, disturbed feedback, poor coordination of the voluntary muscle system" (p. 430) and "incoordinate breathing such that the larynx functions more like a valve, controlling the rate of expiratory airflow" (p. 431). In addition, characteristics such as "a lot of respiratory effort in the upper chest" causing a "breath control problem," (Morrison, 1997, p. 110) and "undesirable speech breathing habits" (Hixon & Hoit, 2005, p. 113) have been attributed to individuals with MTD-1. Despite such claims, the literature lacks clear physiologic descriptions of presumed respiratory/phonatory disruptions in MTD-1, as well as a lack of theoretical discourse on *why* or *how* these disruptions may develop. Moreover, numerous studies of MTD-1 ignore its potential respiratory component, focusing instead on laryngeal factors alone (Hočevar-Boltežar, et al., 1998; Morrison, Nichol, & Rammage, 1986; Roy, Bless, Heisey, & Ford, 1997; Sama, Carding, Price, Kelly, & Wilson, 2001; Sapienza, et al., 2000; Stager, Bielamowicz, Regnell, Gupta, & Barkmeier, 2000).

The specific patterns of laryngeal muscle activity in people with MTD-1 have been studied with inconclusive results. In general, studies of laryngeal muscle activity have focused

solely on the muscle activation patterns of the extrinsic laryngeal muscles, overlooking the role of subglottic and respiratory functions in phonation. In such studies, the assumption seems to be that this voice disorder occurs due to "tension" at the level of the glottis or supraglottis (Hočevar-Boltežar, et al., 1998). However, studies of the chest wall (abdominal and thoracic) musculature have found increased or abnormal activity in these muscles as well as in the larynx, indicating the potential contribution of respiratory factors in the condition (Hočevar-Boltežar, et al., 1998; Rubin, et al., 2010). Respiratory-laryngeal coordination has been investigated by other authors as well (for example, Hixon & Putnam, 1983; Iwarsson, 2001; Morrison & Rammage, 1993; Stone, 1993). Those studies have revealed a trend indicating individuals with MTD-1 tend to phonate in lower lung volume ranges (45-10% of vital capacity) than typical (60-40% of vital capacity). In addition, reports have indicated that in MTD-1, muscular forces in the rib cage/thoracic cavity work in opposition to the abdominal musculature, creating co-contraction and a tightly held thoracic-abdominal wall. In these instances, the respiratory-laryngeal interaction could be described as inefficient, contributing to MTD-1 (Hixon & Putnam, 1983). However, it can be argued that in some instances, abdominal-thoracic co-contraction can be beneficial or even normal, as for example, for core stabilization and to provide expiratory checking to prevent rapid loss of inspired air at high lung volumes, or to assist in expiration in obstructive pulmonary diseases such as asthma and chronic obstructive pulmonary disease (Braman, 2007) .

Another approach to address issues of respiratory-phonatory coordination in phonation was described in a seminal study by Hillman and colleagues over 20 years ago (Hillman, et al., 1989a). These authors described a conceptual continuum to distinguish "adducted" versus "non-adducted hyperfunction." The proposal was that *adducted* vocal hyperfunction involves vocal fold "tension," increased subglottal pressure, and increased vocal fold impact stress, raising the

risk of phonotraumatic vocal fold injury. In contrast, in *non-adducted* hyperfunction, achieved with tensed but non-adducted vocal folds, the potential for mucosal trauma would be reduced. However, phonatory subglottal pressures would be increased to initiate and maintain phonation, and increased (turbulent) airflow might occur as a result of the "valve leak." Laryngeal resistance data based on 15 patients with vocal fold lesions and "functional dysphonia" (essentially MTD-1) were generally consistent with the proposed framework.

As an attempt to replicate and expand upon Hillman and colleagues' work, a pre-dissertation research study that was a precursor to the present dissertation compared the aerodynamic profiles of 90 adult females diagnosed with MTD-1 to normative aerodynamic data published elsewhere (Holmberg, Hillman, & Perkell, 1988). In concordance with Hillman's results, on average the patient group demonstrated significantly higher average estimated subglottal pressure (P_{sub}) and phonatory airflow rates than the control means (Gillespie, Gartner-Schmidt, Rubinstein, & Verdolini Abbott, 2012). The data were then further examined in terms of all possible permutations of aerodynamic profiles, specifically, in terms of patterns of subglottal pressure (cmH_20) and phonatory airflow (L/sec) relations. Results are shown in

Table 2.1. Nine data patterns were theoretically possible. However, only five distinct patterns were identified in the data set. The clusters were created based on commonalities in member data. For each cluster, members had less variability around the airflow data than the

pressure data. Therefore, cluster formation was more dependent on airflow than P_{sub}. In other words, more subjects' data fell into a cluster based on similarities in airflow than in P_{sub}.

Table 2.1. Permutations of Aerodynamic Profiles of Females with MTD-1

Rank of the cluster by % of total sample size.	Pattern	Percent of total sample
1	Airflow: normal P_{sub}: normal	32.3% (n=29)
2	Airflow: high P_{sub}: high	20.0% (n=18)
3	Airflow: low P_{sub}: normal	18.9% (n=17)
4	Airflow: normal P_{sub}: high	17.8% (n= 16)
5	Airflow: high P_{sub}: normal	11.1% (n=10)
6	Airflow: normal P_{sub}: low	0% (n=0)
7	Airflow: low P_{sub}: low	0% (n=0)
8	Airflow: low P_{sub}: high	0% (n=0)
9	Airflow: high P_{sub}: low	0% (n=0)

These data expand on data reported by Hillman et al (Hillman, et al., 1989a). The prior authors identified three of the five patterns that we found: pattern 2 (high flow/high P_{sub}), pattern 4 (normal flow/high Psub), and pattern 5 (high flow/normal Psub). The variety of aerodynamic profiles of individuals with MTD-1 indicates that at least conceptually, multiple mechanisms of

respiratory and laryngeal dysfunction may be involved in the condition (Gillespie, et al., 2012). *Why* and *how* the dysfunction or dyscoordination begins is still unknown, and is the focus of the current study. Also unknown are mechanisms of interactions across respiratory and phonatory systems. A review of respiratory physiology, in particular its relation to voice production, follows next.

3.0 RESPIRATORY PHYSIOLOGY

At the most basic level, respiratory functions can be described in terms of interactive processes across cardiovascular and pulmonary systems. In the heart, the right ventricle pumps metabolically produced, carbon dioxide (CO_2)-rich blood into the lungs, which is then exhaled back into the atmosphere (Hlastala & Berger, 2001). Each inspiration brings oxygen (O_2) rich air into the lungs, replacing the expired CO_2. This highly oxygenated blood leaves the lungs via the left pulmonary veins to the left atrium, and is distributed to body tissues by way of the bloodstream (Levitzky, 1995).

3.1 GAS EXCHANGE

The primary role of the human respiratory system is to deliver O_2 to the tissues of the body to meet metabolic tissue demands, using the least amount of energy expended. A second major role is to remove CO_2 by-products of cellular metabolism (Hlastala & Berger, 2001). The cardiovascular and respiratory systems work together to modulate respiratory frequency to meet the body's metabolic needs (Hlastala & Berger, 2001). Increasing CO_2 levels produced by the tissues cause an increase in H^+ (hydrogen ion) concentration and an increase in pH level in arterial blood gas concentrations. One way that homeostatic acid-base balance and pH regulation

is achieved is via removal of CO_2 from the body in ventilation. If blood becomes too acidic (pH decreases), respiratory rate increases to rid the body of more CO_2, freeing fewer hydrogen ions, resulting in an increase in pH to normal levels.

The earth's atmosphere is 78.08% nitrogen, 20.95% oxygen, .04% carbon dioxide, and .93% argon (Hlastala & Berger, 2001). Each inspiration brings in approximately 350mL of air containing approximately 21% O_2, and each expiration releases 350mL of air containing approximately 5-6% CO_2. Typically, 250mL of CO_2/minute diffuses from the blood supply into the alveoli, and 300mL of O_2 diffuses from the alveoli into the blood supply (pulmonary capillaries) per minute (Levitzky, 1995). The partial pressure of a gas equals its "fractional concentration times the total pressure of all the gases in the mixture" (Levitzky, 1995). The prefix "P" refers to the partial pressure of a gas (e.g. PO_2 indicates the partial pressure of oxygen). Measuring the amount of CO_2 or O_2 at the end of a tidal exhalation (end-tidal) is equivalent to measuring arterial CO_2 or O_2. The partial pressure of CO_2 expired at the end of a normal tidal expiration is referred to as end-tidal CO_2 or $P_{et}CO_2$ (Levitzky, 1995). Alveolar PO_2 increases 2-4 mmHg per normal inspiration, and slowly decreases until the next inspiration. Alveolar PCO_2 falls 2-4 mmHg/inspiration and *increases* slowly until the next inspiration when it falls again (Levitzky, 1995). The levels of a gas in arterial circulation are determined by any processes affecting alveolar ventilation, the pH of blood, the body's need for O_2 consumption in the tissues, and CO_2 production as a by-product of cellular metabolism (Levitzky, 1995). As alveolar ventilation increases, alveolar PCO_2 decreases.

The normal partial pressure of arterial CO_2 ranges between 35-45 mmHg. CO_2 levels below 35 mmHg indicate hypocapnia; above 45 mmHg denote hypercapnia (Zvolensky & Eifert, 2001). In healthy humans, imbalances of O_2 and CO_2 are responded to with changes in

ventilatory depth and frequency. The respiratory response to hypercapnia is an increase in breathing frequency and depth in order to rid the body of the excess CO_2 to prevent a decrease in blood pH below 7.3, or *acidosis* (Wilmore, Costill, & Kennedy, 2008; Zvolensky & Eifert, 2001). However, if the increase in respiratory frequency leads to unchecked hypocapnia, *alkalosis* may occur, and with it, a pH elevation above the normal range of 7.35-7.45 (Sikter, Faludi, & Rihmer, 2009; Zvolensky & Eifert, 2001). The typical response to hypocapnia is therefore a decrease in respiratory rate and depth. Changing CO_2 levels exert the greatest influence over the nervous system's control of ventilation. Even a small increase in arterial CO_2 can cause a marked increase in ventilation, as mediated by central chemoreceptors (Rassovsky, Abrams, & Kushner, 2006).

3.2 NEURAL CONTROL OF BREATHING

Respiration is regulated by a dual control system - both voluntary (for speech, singing, swimming, etc.) and involuntary (life sustaining). In both voluntary and involuntary ventilation, every breath must be initiated by the brain. The respiratory control center resides in the medulla of the brainstem. Both peripheral and central chemoreceptors sense changes in the local chemical environment and send afferent information to the medulla to trigger a ventilatory response to alter the body's balance of CO_2, O_2, and pH (Levitzky, 1995).

3.2.1 Central chemoreceptors.

Central chemoreceptors are located on the anterolateral surface of the medulla, near the 4th ventricle (Hixon & Hoit, 2005). These central receptors do not come into contact with the blood supply, but sense chemical changes in cerebrospinal fluid (CSF) (Levitzky, 1995). Receptors respond to fluctuations in Hydrogen (H^+) and CO_2, but not to changing levels of O_2 (Levitzky, 1995; Pain, Biddle, & Tiller, 1988; Smith, Rodman, Chenuel, Henderson, & Dempsey, 2006; Zvolensky & Eifert, 2001). Hydrogen changes, which reflect PCO_2 changes, are sensed more rapidly in the pH of the CSF than in the arterial blood supply (Smith, et al., 2006). Arterial hypercapnia (increased PCO_2 in the blood supply) causes a greater change in CSF H^+ ion concentration than the H^+ ions in the arterial blood. This change in H^+ is then sensed by the central chemoreceptors. A decrease in arterial PCO_2 (hypocapnia), causes an increase in the pH of cerebral spinal fluid, and a decrease in central chemoreceptor stimulation (Levitzky, 1995).

3.2.2 Peripheral chemoreceptors.

Peripheral chemoreceptors are nerve endings in the carotid bodies (in the bilateral common carotid arteries) and in the aortic bodies (in the arch of the aorta) (Hlastala & Berger, 2001). Carotid body receptors are considered more efficient than aortic bodies in sensing blood-gas changes. The carotid bodies are primarily responsible for responses to hypoxia (decrease in O_2), and play a smaller role in sensing and responding to increases in arterial CO_2 and decreases in arterial pH (Atwood, 2010; Hlastala & Berger, 2001; Levitzky, 1995; Smith, et al., 2006; Zvolensky & Eifert, 2001). Hering's nerve, a branch of the glossopharyngeal nerves, and the

vagus nerves, carry afferent information from the carotid and aortic bodies respectively to the central nervous system. Relevant to the current investigation, the only laryngeal abductor, the posterior cricoarytenoid muscle, may receive carotid chemoreceptor mediated inhibitory input from the vagus nerve, decreasing the amount of expiratory laryngeal resistance in hypercapnic conditions (England, Bartlett, & Knuth, 1982).

3.2.3 Respiratory responses to changing blood-gas levels.

The respiratory system is uniquely designed to rapidly respond to a variety of physical, chemical, and cognitive stimuli via increases and decreases in ventilatory frequency. The central chemoreceptor detection of changing CO_2 is crucial to the central and peripheral control of breathing. PCO_2 is low when ventilation is increased, and PCO_2 is high when ventilation is decreased. Minute ventilation (V_E) (the amount of air entering and leaving the body per minute) increases with increasing amounts of CO_2 in inspired air. This effect is most pronounced when inspired CO_2 equals 5-10% of the total gas mixture (CO_2 typically makes up .03% of atmospheric content) (Levitzky, 1995). Inhaling 5-10% CO_2 causes an increase in alveolar PCO_2 between 40-70 torr. At this level, the ventilatory response to increasing CO_2 is linear. Concentrations of CO_2 of 10-15% of inspired air cause additional symptoms beyond increased minute ventilation such as dyspnea, headaches, restlessness, faintness, and cognitive effects. When CO_2 reaches levels greater than 15% in inspired air, unconsciousness, rigidity and tremors are observed. Increasing levels of CO_2 in the lung causes an increase in alveolar ventilation which decreases alveolar (and arterial) PCO_2 to return the body to blood-gas homeostasis.

Severely elevated PCO_2 can cause respiratory depression. Chronic hypercapnia can cause chronic elevations in pH and a less sensitive ventilator response in acute hypercapnic situations.

3.2.4 Respiratory responses to cognitive stimuli.

Non-metabolic respiration, or respiration for speech and emotional expression among other voluntary acts, is mediated by high cortical areas (Hixon & Hoit, 2005). The primary motor cortex, premotor cortex, and supplemental motor area of the frontal lobe, and somatosensory area in the parietal lobe, are all involved in the control of volitional respiration. Structures in the limbic lobe, a phylogenetically older region of the cerebral cortex, are responsible for involuntary respiratory changes due to emotional contexts (Hixon & Hoit, 2005). Some "typical" emotional respiratory responses such as gasping, breath holding when scared, laughing, and crying, are regulated from centers in the hypothalamus (Marieb, 2002). When an individual experiences stress in circumstances requiring phonation, as may occur in stage fright in public speaking or singing, the nervous system has to simultaneously maintain metabolic homeostasis, support the intended communicative output, and overcome the adverse limbic influence (Hixon & Hoit, 2005). Voluntary breath holding will eventually induce a chemical drive to breathe (by inducing high PCO_2, low PO_2, and low pH) (Levitzky, 1995).

3.3 ABNORMAL BLOOD-GAS LEVELS IN PULMONARY AND PSYCHOLOGICAL DISORDERS

Of interest for the present argument, individuals with anxiety disorders are thought to be more sensitive to PCO_2 fluctuations than healthy controls. Those with panic disorder are especially susceptible. A panic attack can be triggered by small increases in PCO_2, which in turn trigger an increase in ventilation (hyperventilation), which then reduces PCO_2. This ventilatory response is known as an individual's CO_2 sensitivity (Zvolensky & Eifert, 2001). In addition, individuals with high trait anxiety have been observed to have shorter expiratory phases in respiration, reduced or erratic tidal volumes, and chronically decreased end-tidal PCO_2 levels compared to those with low trait anxiety (Bass & Gardner, 1985a; Masaoka & Homma, 2001). These characteristics are amplified in situations causing anticipatory anxiety, and during stress-inducing tasks (Masaoka & Homma, 2001).

Even more relevant to the greater public health is the prevalence of respiratory disorders in the population. More than 10% of the US population suffers from a chronic respiratory condition such as asthma or chronic obstructive pulmonary disease (COPD) (Kunik et al., 2005). These obstructive disorders are the second leading reason, behind heart disease, that people seek disability benefits (West, 1995). Both asthma and COPD, particularly when untreated, can cause chronic states of hyperventilation resulting in hypocapnia (Braman, 2007). *Hyper*capnia is also an etiologic factor in the pervasive dyspnea experienced by individuals with pulmonary, cardiac, and neurologic disease (Hoit, et al., 2007). Dyspnea during speech is a common complaint, with up to a third of individuals with COPD complaining of speaking-related dyspnea (Hoit, et al., 2007). Dyspnea in speech has been perceived as worse than dyspnea during rest breathing (Hoit,

et al., 2007). One reason for this complaint is a presumed competition between the respiratory needs of the speech mechanism, and those of the lower airway (Hoit, et al., 2007).

Anxiety, depression, and panic disorder are common co-morbidities in pulmonary disorders (Bass, 1997; Dratcu, 2000; Goodwin & Pine, 2002; Kunik, et al., 2005). Rates of panic disorder in patients with COPD are three times higher than panic disorder rates in the general population (Dratcu, 2000). Anxiety is also common in these individuals, estimated at 34-50% (Kunik, et al., 2005). In addition, and germane to the present proposal, co-morbidity of dysphonia and lower airway disease such as asthma is also common (Cohen, 2010; England, Ho, & Zamel, 1985; Hackenberg, Hacki, Hagen, & Kleinsasser, 2010; Stanton, Sellars, Mackenzie, McConnachie, & Bucknall, 2009), with estimates ranging from 25%-50% of people with pulmonary disease also experiencing voice problems (Cohen, 2010; Hone et al., 1996; Lavy, Wood, Rubin, & Harries, 2000).

As many as 38% of patients with “functional dysphonia” (a common synonym for MTD-1 as noted in 2.1) have asthma (Schalen, Andersson, & Eliasson, 1992). In addition, a significantly greater number of patients have dysphonia and lung disease than have dysphonia and no lung disease (Cohen, 2010). Co-morbid asthma and voice problems affect children as well, with some estimates revealing 7% of children with confirmed asthma are dysphonic (Carding, Roulstone, & Northstone, 2006). Of import, in many cases, dysphonia cannot be attributed to laryngeal mucosal changes from inhaled corticosteroids- a common treatment for asthma (Hone, et al., 1996; Lavy, et al., 2000). Individuals with asthma demonstrate reduced maximum phonation time, increased noise to harmonic ratio, and perceptually dysphonic voices when compared to non-asthmatic speakers (Dogan, Eryuksel, Kocak, Celikel, & Sehitoglu, 2007). Laryngeal resistance during expiration is increased compared to baseline breathing during

bronchoconstriction (both experimentally induced and as a result of spontaneous asthma attack) and in individuals with chronic obstructive lung diseases (England, et al., 1985; Shindoh, Sekizawa, Hida, Sasaki, & Takishima, 1985). This increased resistance is thought to aid in hyperinflation of the lower airways, preventing further alveolar collapse (Shindoh, et al., 1985). In summary, pulmonary disease, psychological illnesses, and voice problems are common co-morbidities. The next chapter addresses the role of the larynx in the respiratory system.

4.0 LARYNGEAL RESISTANCE

The first studies of laryngeal airway resistance during phonation (R_{law}) in voice and speech science were conducted in the 1960s. Investigation of R_{law} was then considered a "pedestrian activity" (p. 5) necessary to better understand the details of vocal fold oscillation (Campbell, Murtagh, & Raber, 1963). At the time, R_{law} was studied using a rudimentary model of plaster casts of the human larynx with metal rectangular slits of known dimensions serving as the glottis (Campbell, et al., 1963). In this chapter we will discuss laryngeal resistance as it is understood from the standpoints of both respiratory physiology and phonatory science.

4.1 BASIC CONCEPTS

Physical resistance refers to an opposition to flow. Laryngeal resistance is the opposition to air flowing into or out of the lower airways. In the respiratory physiology literature, laryngeal resistance is traditionally measured by calculating glottal area in the absence of phonation (1983; England & Bartlett, 1982; Kuna, McCarthy, & Smickley, 1993). In the voice science literature, laryngeal airway resistance (R_{law}) is generally calculated as subglottal pressure (in centimeters of water) divided by glottal flow (in liters per second) *during phonation*. Originally, R_{law} was measured with invasive procedures such as tracheal puncture, or using cumbersome equipment

such as full body plethysmography (for example, Murry, 1971; Savard, Cole, Miljeteig, & Haight, 1993). Non-invasive technology has allowed for estimations of R_{law} relatively non-invasively during phonation (Hillman, Holmberg, Perkell, Walsh, & Vaughan, 1989b; Ma et al., 2007; Rothenberg, 1977). These methods, their development and implementation, are the focus of this chapter.

4.2 LARYNGEAL RESISTANCE IN VOICE PHYSIOLOGY

4.2.1 Measurement of laryngeal resistance

Phonatory laryngeal airway resistance (R_{law}) in humans was first measured in the 1960s (Campbell, et al., 1963). At the start of the 1970s, Murry measured laryngeal resistance associated with various laryngeal configurations during voicing (1971). Specifically, he measured subglottal pressure (P_{sub}) directly below the vocal folds via tracheal puncture, along with simultaneous expired airflow at the mouth, during the production of sustained vowels across the pitch range. P_{sub} was greater during productions of vocal fry than in modal pitch, and in general decreased as subjects progressed from fry to modal, with the greatest decrease observed at 30% of the modal pitch range. Airflow was lower during fry than at modal pitches. The author concluded that high P_{sub} and low airflow observed during vocal fry was due to the long closed phase in fry with reduced glottal opening (Murry, 1971). A related finding was observed in a pre-dissertation research study of P_{sub} (estimated via oral pressure during voiceless stops) and airflow in females with MTD-1 (Gillespie, et al., 2012). In that study, 18.9% of subjects had low airflow

with normal P_{sub}, and 17.8% had normal airflow and high P_{sub}. Stated differently, 36.7% of patients diagnosed with MTD-1 demonstrated pressure/flow relations skewed in the direction of high P_{sub} compared to flow, qualitatively similar to findings reported by Murry for vocal fry (1971).

A less invasive method of gathering R_{law} data was developed by Smitheran and Hixon in 1981. The protocol for collecting R_{law} was as follows. The subject sits with a facemask over the nose and mouth, with a thin pressure tube placed between the lips in the oral cavity. The subject is then asked to produce a stop consonant-vowel syllable (e.g. /pa/, /pi/, or /pae/) five times, smoothly, at a rate of approximately 1.5 repetitions/second, phonating at a constant comfortable pitch and intensity level. The theoretical basis for the task is that oral pressure during a stop consonant should approximately reflect phonatory subglottal pressure during subsequent vowel phonation, which is the phonatory target of interest. The vowel following the stop consonant is selected based on observations that vowels without lip rounding are least likely to interfere with the seal between mask and face (Smitheran & Hixon, 1981). The production rate of 1.5 syllables/second was suggested by a validity study reported by Holmberg and colleagues (Holmberg, Perkell, & Hillman, 1987). This method of collecting R_{law} data has been well vetted in the literature, and is the method used in the current investigation.

4.2.2 Laryngeal airway resistance in populations with voice disorders

In the 1980s, Netsell and colleagues suggested that “to understand complicated voice disorders…..quantitative evaluation at all levels of the ‘speech production chain’ is required.”

(Netsell, Lotz, & Shaughnessy, 1984, p. 397). This author group, using methods developed by Smitheran and Hixon described above (1981), studied the laryngeal resistance of 18 individuals with vocal fold lesions and neurologic voice problems and compared the results to those for 30 normal speakers. Findings indicated that specific voice disorders were not associated with specific R_{law} ratios, but that each patient had at least one measure (either pressure, flow, or both) outside the normal range. With few exceptions, other authors have similarly found that many, but not all, patients with voice problems exhibit values outside the norm for these parameters (Gillespie, et al., 2012; Higgins, Chait, & Schulte, 1999; Hillman, et al., 1989b; Ma, et al., 2007).

4.3 RELIABILITY AND VALIDITY OF LARYNGEAL RESISTANCE MEASURES

According to at least one study, phonatory laryngeal resistance values in typical females (without voice, hearing, or neurologic disorders) obtained over two days at multiple time points were not statistically significantly different (Leeper & Graves, 1984). The individual factors that determine laryngeal resistance, estimated subglottal pressure and transglottal airflow, also do not change significantly across the same time points. Changes in intensity caused the variability in laryngeal resistance, whereas resistance was fairly insensitive to fundamental frequency changes. When intensity was controlled for, resistance variability decreased compared to the uncontrolled condition (Leeper & Graves, 1984). Finally, the effects of auditory perturbations on phonatory laryngeal resistance have shown resistance remains stable in healthy speakers using normal or resonant voice, with and without auditory masking (Grillo & Verdolini, 2007). The effects of

lower airway perturbations on the stability of laryngeal resistance during phonation have not been tested and are the focus of the current investigation.

4.4 LARYNGEAL RESISTANCE IN THE ABSENCE OF PHONATION

During normal tidal breathing, the upper airway provides 25-60% of overall respiratory resistance (England, Bartlett, & Daubenspeck, 1982; Levitzky, 1995; Savard, et al., 1993). The larynx is an important factor in the determination of respiratory resistance, airflow volume, and rate of breathing. During inspiration for tidal breathing, the glottal opening is wide, providing low resistance. For tidal expiration, the vocal folds move slightly towards midline, and resistance increases marginally compared to inspiration (Bartlett, 1979; Brancatisano, Dodd, & Engel, 1991; England, Bartlett, & Daubenspeck, 1982).

4.4.1 Laryngeal resistance across breathing conditions

Relevant for laryngeal ab- and adductory gestures in breathing, abduction is primarily accomplished by the posterior cricoarytenoid (PCA) muscle, and the thyroarytenoid (TA), lateral cricoarytenoid (LCA), cricothyroid (CT), and interarytenoid (IA) muscles all play varying adductory roles.

In hypercapnic conditions (high CO_2) without phonation, laryngeal resistance during breathing, (historically determined by calculation of the distance between the vocal processes

and the anterior-posterior vocal fold length as measured from a still image; intrinsic laryngeal muscle electromyography; and also by calculation of subglottal pressure divided by laryngeal airflow), decreases in both inspiratory and expiratory phases of the cycle, as a function of substantial increase in PCA muscle activation, presumably to allow an increase in airflow to release more CO_2 and inspire more O_2 (Bartlett, 1979; Brancatisano, et al., 1991; England & Bartlett, 1982). An *increase* in laryngeal resistance during breathing in both inspiration and expiration in hypocapnia (low CO_2) has been observed (Bartlett, 1979; Kuna, McCarthy, et al., 1993). In hyperventilation-induced hypocapnia in mechanically ventilated patients, the PCA has shown to cease fire, and the laryngeal adductors increase activation, resulting in vocal fold adduction (Kuna, Insalaco, Villeponteaux, Vanoye, & Smickley, 1993). This mechanism of action is presumed to assist in CO_2 retention and maintenance of alveolar inflation. However, in awake patients, hyperventilation, with and without hypocapnia, has shown to have the opposite effect of decreasing laryngeal resistance and increasing PCA activity (Bartlett & Knuth, 1984; Savard, et al., 1993). In these studies, the laryngeal response to hyperventilation was thought to override the laryngeal response to decreasing CO_2.

Despite changing resistance as a function of level of CO_2, *inspiratory* resistance is typically lower than *expiratory* resistance (Bartlett, 1979; Savard, et al., 1993). Animal and human studies have shown great variability in raw laryngeal resistance during breathing between subjects, but trends in resistance increases and decreases are stable within and between subjects (Bartlett, 1979; Savard, et al., 1993) (Table 4.1).

Respiratory laryngeal resistance in humans with airway disease is particularly relevant to the current investigation. Laryngeal resistance during breathing increases with increasing lower airway resistance, as is experienced, for example, with bronchoconstriction during asthma attack

(England, et al., 1985). Data on respiratory laryngeal resistance in response to lower airway perturbations in patients with respiratory disease are mixed, with some reports indicating an increase in resistance after methacholine challenge, for example, and others reporting no laryngeal change with *induced* provocations, but an increase in resistance during breathing with *spontaneous* asthma attack (Shindoh, et al., 1985; Yanai, Ohrui, Sekizawa, Sasaki, & Takishima, 1989). One explanation for conflicting results may lie with the hypothesized mechanisms by which the larynx is affected by lower airway perturbations- whether induced or spontaneous. It is hypothesized that glottal narrowing is an efferent response to chemicals released during an asthma attack which stimulate laryngeal afferents (Shindoh, et al., 1985). Furthermore, as levels of CO_2 rise in respiratory disease, carotid chemoreceptors -- the primary respiratory sensory mechanisms for alterations in arterial blood-gas levels -- may cause an increase in inhibition of the abductor posterior cricoarytenoid, thereby reducing glottal width and increasing laryngeal resistance in breathing under respiratory disease (England, et al., 1985). In addition, an increase in laryngeal resistance during breathing may occur as a laryngeal compensation to the constriction of the lower airways (England, et al., 1985; Shindoh, et al., 1985). The “braking” of expired air, which occurs from increasing laryngeal resistance, increases lung volume and expands the lower airways, assisting in hyperinflation and combating bronchoconstriction (England, et al., 1985).

Alteration of upper airway resistance and limiting chest wall movement affect laryngeal resistance during respiration. Breathing against external resistance of airflow as well as with a mechanically constricted chest wall cause a decrease in respiratory laryngeal resistance (Sekizawa, Yanai, Sasaki, & Takishima, 1986). In addition, an increase in lower airway resistance, often as a result of disease-induced bronchoconstriction or obstruction, results in an

increase in laryngeal resistance during rest breathing (England, et al., 1985; Higenbottam, 1980; Higenbottam & Payne, 1982; Shindoh, et al., 1985). Table 4.1 details the change in respiratory rate and upper airway resistance as a result of blood-gas fluctuations.

Table 4.1. Upper Airway Response to Changing Blood-Gas Concentrations

Condition	Respiratory rate response	Laryngeal/upper airway response
Hypoxia	Increased ventilation	Upper airway dilation, decreased laryngeal resistance
Hypercapnia	Increased ventilation	Decreased laryngeal resistance
Hypocapnia	Decreased ventilation	Increased laryngeal resistance
Homeostasis	Eupnea	Low laryngeal resistance

5.0 SUMMARY AND HYPOTHESES

The larynx is responsible for the control of gas flow into and out of the lungs for dual purposes of ventilation and voice production. In ventilation, the airways' primary function is to maintain homeostasis between O_2 and CO_2 levels in arterial blood (Hixon & Hoit, 2005; Levitzky, 1995). In healthy humans, gas imbalances are addressed with changes in respiratory volume and frequency. In normal tidal breathing (eupnea), laryngeal resistance decreases during inspiration and increases during expiration (Bartlett, 1979; Brancatisano, et al., 1991; England, Bartlett, & Knuth, 1982). In hypercapnia (increased CO_2), resistance decreases in both inspiratory and expiratory phases of the cycle, thereby facilitating intake of O_2 and release of CO_2 (Bartlett, 1979; Brancatisano, et al., 1991; England & Bartlett, 1982). Conversely, in hypocapnia (decreased CO_2), resistance is increased during both inspiration and expiration, facilitating CO_2 retention (Bartlett, 1979; Kuna, McCarthy, et al., 1993) (Table 5.1). Laryngeal airway resistance is also increased in conditions of increased lower airway resistance, for example, during bronchoconstriction, likely in attempt to assist in alveolar inflation to counteract the constriction (England, et al., 1985; Higenbottam, 1980; Higenbottam & Payne, 1982). Table 5.1 details the ventilation rate and laryngeal resistance response to changing levels of CO_2. It is important to note that while ventilation rate and laryngeal resistance respond to CO_2 changes (as noted in

Table 5.1), these factors also reciprocally alter CO_2 levels. For example, increasing ventilation beyond the body's metabolic needs (i.e. hyperventilation) reduces CO_2. Increasing laryngeal resistance can also increase CO_2.

Table 5.1. Upper Airway Resistance Response to Blood-Gas Levels

Condition	Ventilation Rate	Laryngeal Resistance
Hypercapnia	Increased	Decreased
Hypocapnia	Decreased	Increased
Eupnea	Tidal	Baseline

Of significance is that both hypo- and hypercapnia are clinically common. Abnormal levels of arterial CO_2 are observed in individuals with a wide range of clinical disorders, including respiratory disorders (e.g., asthma, Chronic Obstructive Pulmonary Disease [COPD], and hypoventilation syndrome) (Bass, 1997; Brown, 2010; Devriese et al., 2000; Hoit, et al., 2007), psychological disorders (Generalized Anxiety Disorder and Panic Disorder) (Bass & Gardner, 1985b; Houtveen, Rietveld, & de Geus, 2003; Wientjes & Grossman, 1994), somatic illnesses (chronic fatigue syndrome, multiple chemical sensitivity, and functional gastrointestinal disorders) (Bass, 1997; Devriese, et al., 2000), neuromuscular disorders (e.g. multiple sclerosis, Guillain-Barré, amyotrophic lateral sclerosis), and chest wall disorders (kyphoscoliosis, spondylitis, and fibrothorax) (Brown, 2010; Hoit, et al., 2007). Pathologic levels of CO_2 experienced by patient groups are shown in Table 5.2.

Table 5.2. Arterial CO_2 Levels in Patient Groups

Disorder	Hypercapnia	Hypocapnia
Asthma	X	X
COPD	X	X
Anxiety disorder		X
Panic disorder		X
Somatic Illness	X	
Neuromuscular disorders	X	
Chest Wall disorders	X	
MTD-1	?	?

In addition to the larynx's role as gateway in gas exchange during ventilation, in phonation the larynx serves as an oscillator that modulates pulmonary airflows, transforming them into systematic acoustic air columns propelled into the vocal tract, which in turn exert upstream effects onto vocal fold oscillation itself (Titze, 1988). For voice, phonatory laryngeal resistance is calculated as the ratio of subglottic pressure in cmH_2O to laryngeal airflow in L/sec (Smitheran & Hixon, 1981). The present study was motivated by the observation that although the larynx has indisputably critical functions for both respiration and phonation, until now the reciprocal relations between these functions has only been minimally investigated (Bartlett, 1979; England & Bartlett, 1982; England, Bartlett, & Daubenspeck, 1982; England, Bartlett, & Knuth, 1982; England, et al., 1985; Hixon & Hoit, 2005; Iwarsson, 2001; Iwarsson, et al., 1998; Kuna, McCarthy, et al., 1993). Of particular concern in the present context are the possible etiologic effects that respiratory abnormalities may have for pathogenesis in selected voice disorders. The issue is a non-trivial one. In addition to the estimated 25% of the US population with dyspnea (Hoit, et al., 2007), voice problems affect 3-9% of the general population at any given point in time (Verdolini & Ramig, 2001). Further, up to 40-70% of clinical voice caseloads

are reportedly comprised of individuals with Primary Muscle Tension Dysphonia (MTD-1) (Angsuwarangsee & Morrison, 2002; Roy, 2003), generally defined as a voice disturbance in the absence of known structural or neurologic abnormalities (Roy, 2003), but often with at least partial presumed etiology in abnormal respiration (Behrman, 2005; Hixon & Hoit, 2005; Hixon & Putnam, 1983; Iwarsson, 2001; Koufman & Blalock, 1988; Morrison, 1997; Morrison & Rammage, 1993). Respiratory complaints, such as phonatory dyspnea, are common among individuals with MTD-1 (Nguyen, et al., 2009). Published reports have pervasively described a general "dyscoordination" between respiration and phonation, and problems with breath "control," in individuals with MTD-1 (Behrman, 2005; Hixon & Hoit, 2005; Hočevar-Boltežar, et al., 1998; Koufman & Blalock, 1988; Morrison, 1997; Morrison & Rammage, 1993). In addition to such general observations, the literature also specifically describes paradoxical breathing (Iwarsson, 2001), shallow breathing (Koufman & Blalock, 1988), and use of low lung volumes in MTD-1 (Iwarsson, et al., 1998). During phonation in some individuals, the larynx may act as more of a gross respiratory control valve than as a finely-tuned modulator needed to transform pulmonary airflows into systematic acoustic waves (Morrison & Rammage, 1993). Many of these observations are also made in people with lower airway disease.

Of note, to date the primary etiologic factors that have been systematically investigated in MTD-1 are psychological. For example, individuals with MTD-1 are likely to have greater *anxiety* than healthy controls or individuals with other voice problems (Anbar, 2002; Dietrich, Verdolini Abbott, Gartner-Schmidt, & Rosen, 2008; Homnick & Pratt, 2000; House & Andrews, 1987; Kinzl, et al., 1988; Leo & Konakanchi, 1999; Millar, et al., 1999; Powell et al., 2007; Roy, Bless, & Heisey, 2000; Roy, McGrory, et al., 1997; van Mersbergen, Patrick, & Glaze, 2008; Willinger, et al., 2005). Furthermore, abnormal laryngeal and perceptual voice quality findings

are common in individuals with respiratory disorders (Cohen, 2010; Dogan, et al., 2007; England, et al., 1985; Hackenberg, et al., 2010; Stanton, et al., 2009). Laryngeal resistance *during breathing* changes with varying levels of arterial CO_2, conditions experienced by people with lower airway disorders (Brancatisano, et al., 1983; England, Bartlett, & Knuth, 1982; England, et al., 1985). Laryngeal resistance *during phonation* is known to be stable in normal speakers (Grillo & Verdolini, 2008; Leeper & Graves, 1984), however resistance measures are shown to be abnormal in individuals with voice problems (Gillespie, et al., 2012; Higgins, et al., 1999; Hillman, et al., 1989b). The mechanism by which the apparently regulated stability of the voice motor system-- and within that system, laryngeal resistance during phonation -- is altered in individuals with voice problems remains unknown. The driving hypothesis for the present study is that respiratory issues involved in such voice problems represent a common pathway in people with voice problems, whether they originate in psychological disorders or in physical disease.

5.1 HYPOTHESES

The current study expands on well-established knowledge regarding the role of the larynx in homeostatic regulation of blood-gas concentrations (Bartlett, 1979; Brancatisano, et al., 1991; England, Bartlett, & Daubenspeck, 1982; England, Bartlett, & Knuth, 1982; Kuna, McCarthy, et al., 1993). Unfortunately, to date, studies on the respiratory versus phonatory functions of the larynx have demonstrated remarkably little cross-talk, which this series hopes to incorporate. The

theoretical hypothesis was that when challenged to produce voice under conditions of varying CO_2 levels, the larynx would sacrifice *phonatory* resistance stability in favor of *respiratory* resistance mechanisms tending to return the system to physiologic homeostatic baseline. It was hypothesized that the respiratory perturbations would overcome the apparently inherent stability of phonatory laryngeal resistance and revert the larynx's function to one primarily responsible for responding to respiratory needs. This study was the first to study the effects of respiratory condition on laryngeal physiology during phonation and to identify the larynx's varying functions at the intersection of respiration and communication. Specifically, this study represented the second step in a programmatic line of research that will ultimately lead to future studies investigating causal relations between respiratory-induced adjustments in laryngeal resistance, phonatory adaptations in individuals with respiratory disorders such as COPD and asthma, psychological disorders such as anxiety and panic, and specific voice disorders such as MTD-1. Future studies will also investigate respiratory bio-markers useful for identifying individuals at risk for MTD-1 and other pathologies affecting voice, and will establish new approaches to their evaluation and treatment.

Specific hypotheses were: phonatory laryngeal resistance will increase during hypocapnia (SA1a) and decrease during hypercapnia (SA1b), favoring a return towards respiratory homeostasis, and amount of phonatory laryngeal resistance will in turn attempt to return the respiratory system to homeostasis (SA2). Data to this effect would provide critical evidence on how respiratory and laryngeal functions may interact in phonation under conditions of respiratory abnormality. Data would also provide evidence on the stability of phonatory laryngeal resistance during substantial respiratory perturbations similar to those experienced by individuals with respiratory disorders.

6.0 METHODS

6.1 PARTICIPANTS

Healthy females, ages 18-45 years, were recruited from the Pittsburgh metropolitan region. Based on estimates from past research on phonatory laryngeal airway resistance (Grillo & Verdolini, 2008), using a repeated measures design with an alpha of .05, and an anticipated moderate effect size, a sample size of 20 participants would be necessary to achieve 80% statistical power for SA1, the primary aim of interest. However, because the experiment utilized counterbalancing across 3 conditions, the total number of participants required to complete the experiment had to be a multiple of 6. Therefore, we targeted a total of 24 participants to complete the experimental procedures. Females were the focus for this initial study, as women experience voice problems in general, and MTD-1 specifically, more commonly than males (Roy, 2003).

6.2 INCLUSION/EXCLUSION CRITERIA

Inclusion/Exclusion criteria were:

By self-report: Female, non-smoker, age 18-45 years (to limit hormonal influences on voice); negative history of voice problems (voice disturbance lasting for greater than 2 weeks, or recurring greater than 3 times during the preceding year) or history of any prior voice treatment; negative history of respiratory disorders including asthma, COPD, emphysema, sleep apnea; negative history of psychological disorders including depression, anxiety, panic disorder; negative history of vocal training (defined as any private study in vocal performance); and negative history of use of any medication that might affect voice.

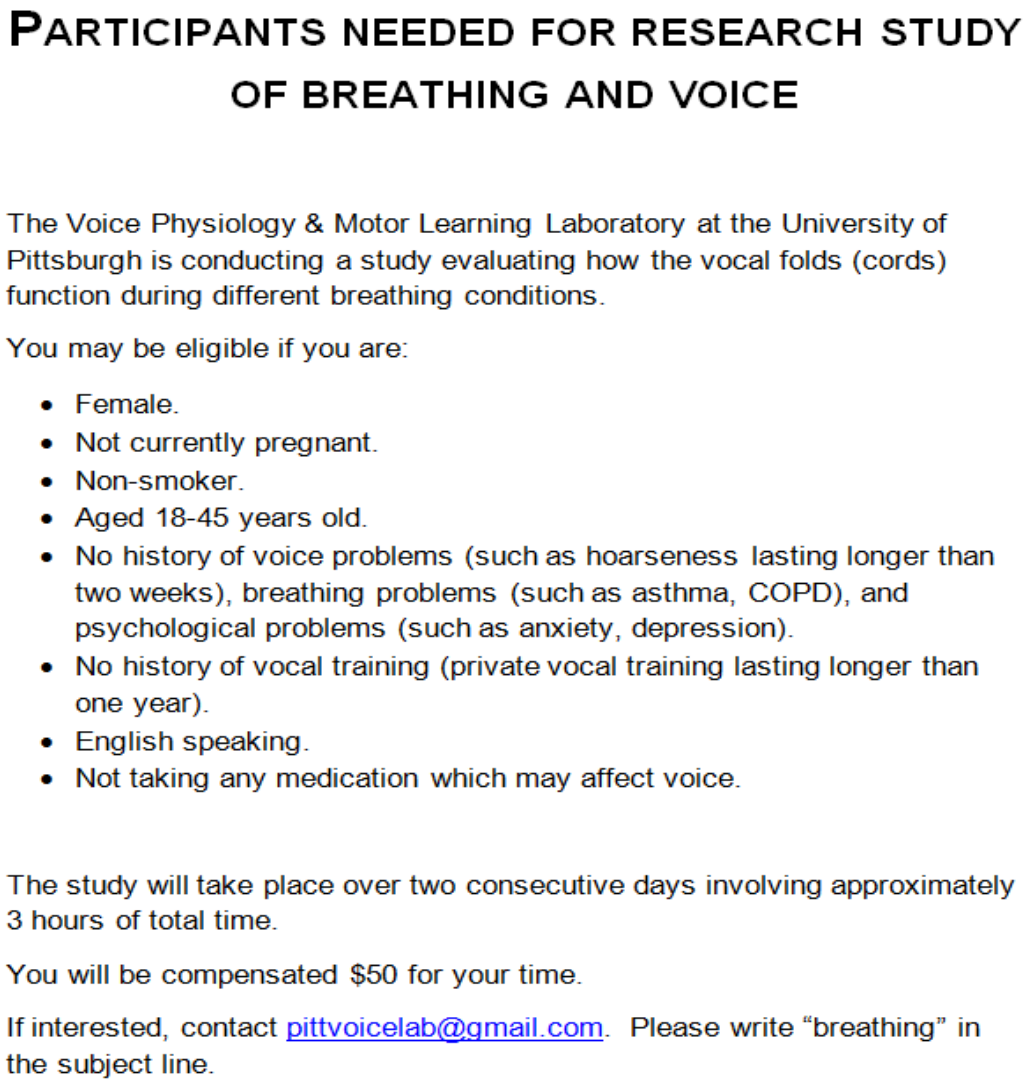
By clinical judgment (PI): English comprehension and hearing sufficient to provide fully informed consent and follow study instructions; speech production sufficient to produce the target phoneme /pa/.

By instrumental assessment: Not pregnant (by administration of a urine pregnancy test); normal vocal quality as judged by CAPE-V score independently judged by a rater not otherwise involved in the study (masters level speech-language pathologist specializing in voice); normal hearing as determined by hearing screening (30dB at 500, 1000, 1500, and 2000 Hz bilaterally); no self-perceived voice problem as determined by Voice Handicap Index-10 (VHI-10) score < 11 (Arffa, Krishna, Gartner-Schmidt, & Rosen, 2012; Rosen, Lee, Osborne, Zullo, & Murry, 2004), no indication of laryngopharyngeal reflux affecting voice as determined by a Reflux Severity Index (RSI) score <13 (Belafsky, Postma, & Koufman, 2002); normal larynx as judged independently by the PI and a fellowship trained laryngologist, based on rigid or flexible endoscopy; normal pulmonary function as determined by flow-volume loop spirometry

performed by the PI and confirmed by a senior pulmonary lab technician (Miller et al., 2005). Further details regarding instrumented assessment criteria are provided shortly.

6.3 RECRUITMENT

Potential participants were recruited with flyers distributed on the University of Pittsburgh's Pittsburgh campus and on the internet (www.craigslist.org) (Figure 6.1).



PARTICIPANTS NEEDED FOR RESEARCH STUDY OF BREATHING AND VOICE

The Voice Physiology & Motor Learning Laboratory at the University of Pittsburgh is conducting a study evaluating how the vocal folds (cords) function during different breathing conditions.

You may be eligible if you are:

- Female.
- Not currently pregnant.
- Non-smoker.
- Aged 18-45 years old.
- No history of voice problems (such as hoarseness lasting longer than two weeks), breathing problems (such as asthma, COPD), and psychological problems (such as anxiety, depression).
- No history of vocal training (private vocal training lasting longer than one year).
- English speaking.
- Not taking any medication which may affect voice.

The study will take place over two consecutive days involving approximately 3 hours of total time.

You will be compensated $50 for your time.

If interested, contact pittvoicelab@gmail.com. Please write "breathing" in the subject line.

Figure 6.1. Recruitment flyer.

6.4 EXPERIMENTAL DESIGN

The primary focus of the study, addressed in SA1, involved a within-subjects repeated measures design. The independent variable was experimental respiratory condition (eupnea, hypocapnia, and hypercapnia). The dependent variable was laryngeal resistance (estimated P_{sub} in cmH_2O/ glottal airflow in L/sec) (SA1a, b). Secondary outcome measures of this aim included fundamental frequency (F_0) and vocal intensity (dB SPL). A secondary focus, addressed in SA2 but derived from the same within-subjects experimental run, involved a 3 x 2 within-subjects design. Independent variables were experimental condition (eupnea, hypocapnia and hypercapnia) and phonation (yes/no). The dependent variable was end-tidal CO_2 ($P_{et}CO_2$).

6.5 EQUIPMENT

Screening Phase. Equipment used for the screening phase included flexible nasendoscope (Olympus Medical, Center Valley, PA), Koko spirometer (Grace Medical, Kennesaw, GA), Audiometer (Maico Diagnostics, Eden Prarie, MN), Pregnancy Test Strips (Wondfo, Willowbrook, IL), Matrix MR500 Metronome, and Computerized Speech Laboratory (KayPENTAX, Montvale, NJ, USA).

Pre-experimental training. The Phonatory Aerodynamic System 6600 (PAS6600) (KayPENTAX, Montvale, NJ, USA) was used during pre-experimental training.

Experiment. For the experiment proper, equipment included the PAS6600 (KayPENTAX, Montvale, NJ, USA), Matrix MR500 Metronome , Hans-Rudolph Model 2700 low-resistance

non-rebreathing valve used for each subject and sanitized between subjects per manufacturer's instructions (Hans Rudolph, Inc., Shawnee, KS, USA), the Viasys Data Acquisition System (SensorMedics/Viasys Corp, Yorba Linda, CA, USA), ECG-gated pulse oximetry (Model 504-USP, Criticare Systems Inc., Waukesha, WI, USA) and 5-lead ECG/BP monitor/recorder (Model Sirecust 732, Siemens Medical Systems, Inc. Danvers, MA, USA), room-air H cylinder, 7% CO_2 cylinder (Praxair Inc., Danbury, CT, USA), and balloon collection bags (Vacumetrics, Inc., Ventura, CA, USA).

6.6 EQUIPMENT CALIBRATION

For the PAS6600, the air-pressure tube and pneumotach were calibrated daily, per manufacturer instructions. Specifically, the system automatically calibrated the air-pressure tube upon the experimenter's selection of *Calibrate Air Pressure Zero Level* from the system's Options menu. To calibrate the pneumotach, a 1.0L syringe was coupled to the airflow head. The syringe plunger was depressed in one continuous motion for 2-4 seconds, emptying the syringe air into the pneumotach. The system then provided a calibration value. Proper calibration resulted in a value of 1.0L +/- 1-2%. Calibration of the Viasys Data Acquisition System was also required, and was completed before running each new participant. In a similar fashion to calibration for the PAS6600, a 3.0L syringe was coupled to the Viasys pneumotach and depressed. Proper calibration resulted in a value of 3.0L +/- 1-2%.

The gas sensors were also calibrated and the gas-sensor tubing was replaced to avoid contamination due to condensation prior to the start of each new participant in the experiment.

The gases were calibrated by two-point certified calibration gas mixtures prior to each study. Finally, the microphone was calibrated daily. Given the connection of valves and tubing to the terminal end of the PAS6600 pneumotach, the microphone had to be repositioned from the terminal end of the pneumotach, to the left side of the facemask. The change of microphone position is shown in Figure 6.2. Because the microphone is calibrated internally by the manufacturer (KayPENTAX) to represent 15cm from the speaker's mouth, it had to be re-calibrated to account for any recorded dB SPL changes in its new position. Per the manufacturer's direction (personal communication, Steve Crump, 6/12/2012) a 200Hz pure tone was generated via standard computer speakers against the facemask. The dB SPL value was first recorded with the microphone in the original position. Then, without changing the audio input, the microphone was moved to the experimental position and the dB SPL value was again recorded. The difference between the dB SPL of the original and experimental microphone positions was recorded and used to adjust the dB SPL values of the experimental task in later analysis.

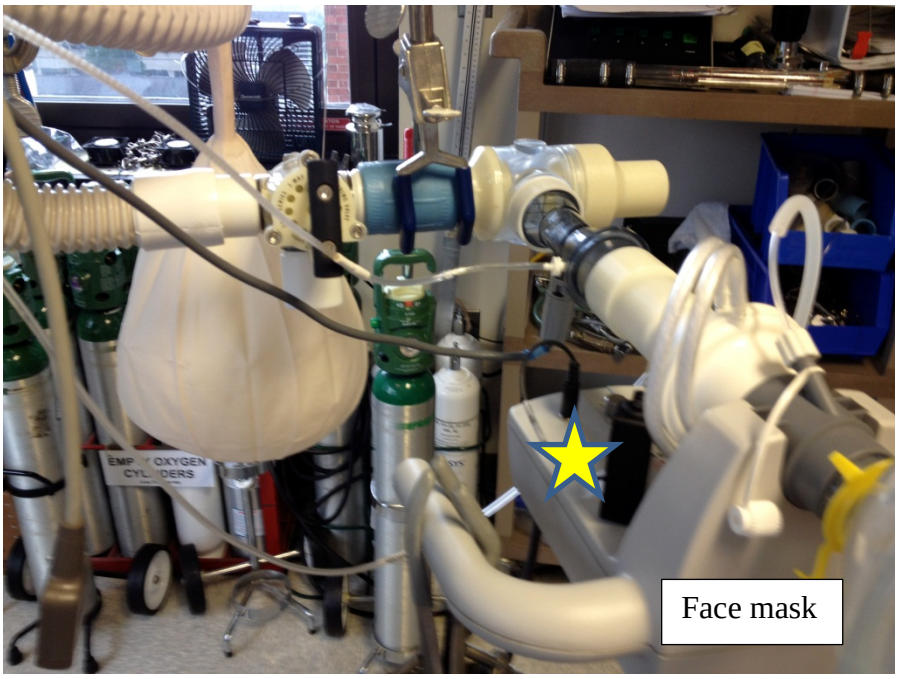


Figure 6.2. Re-positioned microphone (to the right of the yellow star). Facemask is visible in lower right corner.

7.0 EXPERIMENTAL PROCEDURES

7.1 SCREENING

Each participant provided informed consent before proceeding with any of the instrumental screening or experimental procedures. After consent had been obtained, first, satisfaction of self-report and clinician rated inclusion/exclusion were confirmed (see 6.2). Next, the participant completed the Voice Handicap Index-10 (VHI-10) and Reflux Symptom Index (RSI) via an online secure form (see Appendix). Scores on the VHI-10 of less than 11, and RSI less than 13 were considered normal (Arffa, et al., 2012; Belafsky, et al., 2002; Rosen, et al., 2004). If eligible based on these criteria, the participant was invited to attend an in-clinic screening. At the in-clinic screening, participants first completed a urine-pregnancy test to confirm non-pregnant status. Then, the participant's height and weight were recorded using a standard scale and stadiometer. Next, the participant's voice was recorded following the standard protocol for the Consensus Auditory Perceptual Evaluation of Voice (CAPE-V) and judged for normalcy by a master's level speech-language pathologist specializing in voice, with no knowledge of the experimental hypotheses. Then, a hearing screening was performed to confirm normal hearing bilaterally at 30dB at 500, 1000, 1500, 2000 Hz. Next, laryngeal examination was performed by a fellowship trained laryngologist, using flexible endoscopy. The participant was positioned upright, and both nasal passages were sprayed with a local anesthetic (e.g. Cetacaine®). When

sufficient numbing had been achieved based on participant report and clinician evaluation, the flexible nasendoscope was passed through the most patent naris into the laryngopharynx, until the larynx was fully visualized. The initial portion of the examination involved halogen lighting of the larynx at rest during breathing. The larynx was then visualized under both halogen and stroboscopic illumination during sustained /i/ at comfortable (spontaneous/modal) and high pitches (about one octave higher than spontaneous comfortable pitch) and quiet and comfortable intensity, empirically determined, at both pitches. The laryngologist made a subjective determination about laryngeal normalcy. A normal laryngeal appearance was defined as no visible lesions, normal arytenoid dynamics on ab/adduction, normal vocal fold shortening and lengthening with pitch changes, and expected phonatory glottic closure. Impression of normal larynx was confirmed by the PI. Normal pulmonary functioning was determined with spirometric testing, per American Thoracic Society (ATS) standards (Miller, et al., 2005). This testing was conducted by the PI using the KoKo Spirometer, following ATS guidelines. Specifically, participants performed a sequence of four tidal breaths into a mouthpiece, followed by a deep inspiration and forceful, prolonged expiration. This procedure was repeated 3-8 times. Participants were required to achieve 3 normal results. Results judged as “normal” were free from artifacts, including observation or evidence of cough, glottic closure, early termination, or perceived sub-maximal effort; results confirm expiration for at least 6 seconds, and forced vital capacity (FVC) and forced expiratory volume in one second (FEV-1) were greater than 80% of predicted normal value (Miller, et al., 2005). Participants who failed to achieve three normal results after eight attempts were dismissed and excluded from further study participation.

7.2 PRE-EXPERIMENTAL SET-UP AND TRAINING FOR R_{LAW} PROCEDURES

After eligibility criteria were satisfied, the participant was trained in data collection procedures that were to be used for the subsequent experiment, specifically for the collection of phonatory laryngeal airway resistance measures (R_{law}). Although R_{law}, calculated as estimated P_{sub} (cmH20) /average phonatory airflow (L/sec), has been shown to not significantly change when measured over two days at multiple time points (Leeper & Graves, 1984), some data do indicate that one component aspect of R_{law} (P_{sub}) may be susceptible to practice effects. Specifically, according to one report, a threshold permutation of P_{sub}, phonation *threshold* pressure, varies across performance days, whereby the best performance is typically not shown until Day 2 of performance (Dastolfo, 2011). Therefore, to err on the side of caution, and to avoid practice effects during the experiment proper, participants were trained in R_{law} data collection procedures the day prior to the experiment, at the time of screening. The R_{law} task utilized the PAS6600. This system's hardware consists of a pneumotach coupled to a facemask with an integral intra-oral pressure tube, and external microphone. The speaker phonates into the facemask with the intra-oral pressure tube placed in the mouth, on top of the tongue. Expired air flows to the pneumotach, which consists of a stainless steel mesh screen with pressure transducers on either side. The system calculates the pressure difference on either side of the screen to determine airflow rate. P_{sub} is estimated by calculating intra-oral pressure in the pressure tube in the mouth during the production of a voiceless stop (e.g. /p/) in a consonant-vowel sequence. Sound is captured by a microphone re-located to the left of the facemask (see Figure 6.2) approximately 15 cm from the participant's mouth.

For the R_{law} task itself, the PI trained the participant to produce a string of five consonant-vowel (CV) syllables (/pa pa pa pa pa/) on one breath, at a rate of 1.5 syllables/second (or 90 beats per minute, guided via metronome). Care was taken by the PI to assure the participant fit the facemask snugly over nose and mouth during task production, and that the pressure tube was sitting lightly on top of the tongue. For the experiment only, the face mask was secured with an elastic strap around the participant's head to avoid any change in mask position. This set-up is shown in Figure 7.1. The morphology of pressure peaks and airflow plateaus was inspected visually for each syllable. Morphology was considered acceptable if pressure peaks were not pointy or jagged, and flow minima corresponded with pressure maxima, based on visual inspection (Helou & Solomon, 2011). Training ceased when the participant produced the CV string with peaks 2-4 showing acceptable morphology, as verified by the PI based on output from the PAS6600 (Helou & Solomon, 2011; Smitheran & Hixon, 1981). The participant was then sent home and asked to return the following day for the experiment proper.

7.3 GENERAL SET-UP FOR EXPERIMENTAL DAY

Participants were seated comfortably in a standard desk chair. Heart rate and oxygen (O_2) saturation were measured using a 5-lead ECG and transcutaneous O_2 saturation monitor, respectively. The participant's nose and mouth were fit snugly into a standard anesthesia facemask, connected to the PAS6600. The facemask was secured around the participant's head with an elastic band. The mask was visually inspected by the PI to ensure a leak-free seal on the

face. Figure 7.1shows the positioning of the facemask on one participant. Written permission to use the participant's photo was received.

Figure 7.1. Facemask positioned with elastic around participant's head (figure used with participant's permission).

The PAS6600 system was connected to a Hans-Rudolph valve. The Hans-Rudolph valve is a one-way valve that allowed the participant to inspire a given concentration and volume of air, and expire into the atmosphere to prevent re-breathing of expired air. At the point of connection between the PAS6600 and Hans-Rudolph valve were integral ports for airflow and gas concentration sensors. These connections are shown in Figure 7.2. These sensor lines connected into the Viasys Data Acquisition System. This system provided real-time breath-by-breath analyses of respiratory data.

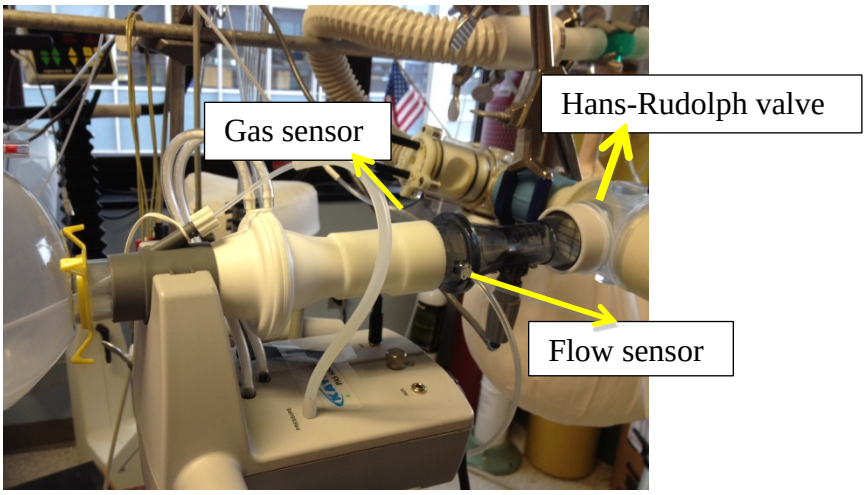


Figure 7.2. Connection of PAS6600 to Hans Rudolph valve with airflow and gas sensor lines.

For the purposes of the current study, arterial CO_2 was estimated by measuring the partial pressure of CO_2 in expired air- the end-tidal partial pressure of CO_2 ($P_{et}CO_2$) (Levitzky, 1995). Plastic tubing connected the Hans-Rudolph valve to a rubber balloon collection bag, which was connected to one of two H-cylinders. The H-cylinders were identical in size and shape and contained either room air (21% O_2, .05% CO_2, 78% Nitrogen) or enhanced CO_2 (7% CO_2, 21% O_2, balanced Nitrogen). A rotameter connected to the cylinders allowed the examiner (Mr. Slivka, senior pulmonary technician and project consultant) to select the appropriate cylinder and control the flow rate of gases from that cylinder to the participant. The examiner, blinded to experimental hypotheses, controlled the rotameter to meter either the room air or CO_2 enriched air to the participant and to change the volume of airflow being inspired. Figure 7.3 shows the experimental set-up on one participant. All three conditions utilized air from one of the two cylinders. The order of eupneic, hypo- and hypercapnic conditions was counterbalanced across participants to control for order effects. Then, each counterbalanced set of conditions was randomly assigned to each participant. Each experimental condition lasted approximately five

minutes (Table 7.1). Baseline data collection, described next, lasted five minutes. There were also three 15-minute breaks after each condition (Gorman et al., 1994; Papp et al., 1997), for a total of approximately 65 minutes experimental time per participant. Subjects were compensated $50 for completing the entire protocol, or $10 for any partial completion.

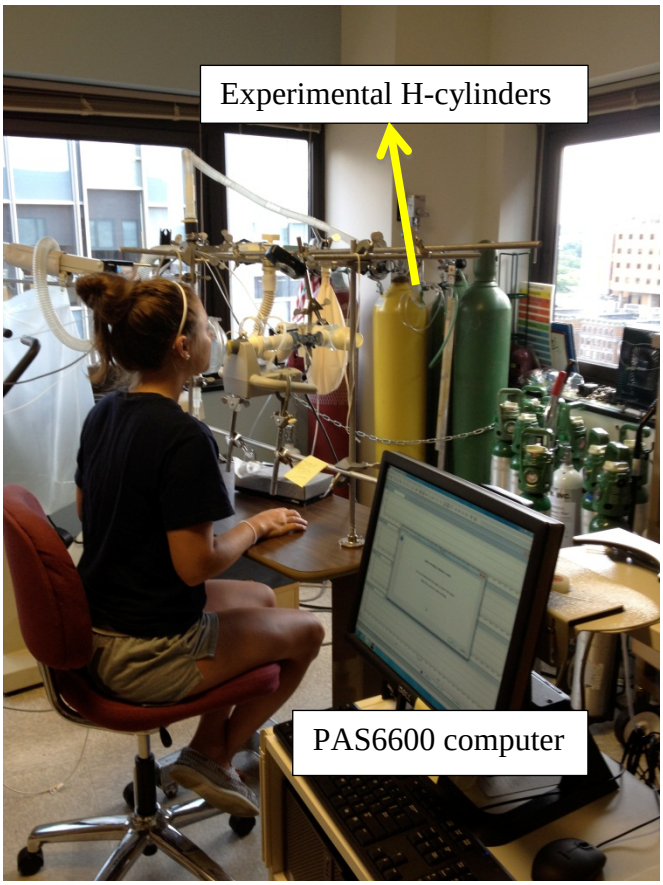


Figure 7.3. Participant in experimental chair with gas cylinders in background (figure used with participant's permission).

Table 7.1. Template for Experimental Procedures. The Order of Breathing Conditions was Counterbalanced and Randomized Across Participants

Condition	Activity	Duration
Baseline	Participant performs normal, tidal breathing, no phonation	5 minutes
Eupnea	Participant breathes room air	5 minutes
	R_{law} task	
Rest	Rest	15 minutes
Hypocapnia	Participant breathes room air metered from an H-cylinder at 2x the participant's resting respiratory rate	5 minutes
	R_{law} task	
Rest	Rest	15 minutes
Hypercapnia	Participant breathes CO_2 enriched air metered from an H-cylinder	5 minutes
	R_{law} task	
Rest	Rest	15 minutes

7.4 EXPERIMENTAL PROCEDURES

7.4.1 Baseline

For the experimental procedures proper, first a respiratory baseline was obtained for each participant. For baseline data collection, the participant sat with the facemask in place as previously described and breathed room air. Baseline tidal volume (Vt), minute volume (VE), inspiratory time (Ti), expiratory time (Te), respiratory rate (RR), $P_{et}CO_2$, heart rate (HR), and transcutaneous O_2 saturation (O_2) data were collected using the Viasys pulmonary data

acquisition system, 5-lead ECG, and ECG-gated pulse oximetry, respectively. Baseline data were collected and analyzed on a breath-by-breath basis for 5 minutes of tidal breathing. Sixty-second averages of the third, fourth and fifth minute of baseline breath-by-breath values of $P_{et}CO_2$, resting respiratory rate, and resting tidal volume were calculated by the computer, and then those three values were averaged to provide one baseline value for each variable of interest. Baseline $P_{et}CO_2$ values were used to determine the target hypocapnic range for each participant, described shortly. Baseline minute volume was used to determine the starting minute volume rate required to induce hyperventilation for the hypocapnic condition described next.

7.4.2 Intervention

Following collection of baseline data, each participant underwent the following interventions, counterbalanced across subjects (See Table 7.1). First, the voice task (three sets of five productions of /pa/) was reviewed and the participant practiced the task to conform to criteria (pressure peaks not pointy or jagged, and flow minima corresponded with pressure maxima, based on visual inspection). For the eupneic condition, the participant breathed room air through the cylinder, metered in at the participant's baseline minute volume, and produced the R_{law} task five times, with a breath between each production. As for all experimental conditions, a 15-minute break followed, during which time the participant removed the face mask and breathed normally. Fifteen minutes was selected as the rest time, as the literature indicates that 15 minutes of normal breathing is sufficient to allow respiratory variables to return to baseline following experimental challenge (Gorman, et al., 1994; Papp, et al., 1997). During the final five minutes

of rest, the participant again breathed through the mask, open to the atmosphere, to capture the same respiratory data as previously ($P_{et}CO_2$, VE, RR, HR, and O_2) and confirm return to baseline.

For the hypocapnic condition, the examiner metered room air at a rate triple the participant's resting respiratory minute volume as determined during baseline procedures, via the cylinder containing room air, to the participant. The participant was instructed to visually monitor the rubber balloon collection bag positioned between the facemask and the cylinder, as it filled with air, and to breathe as quickly as possible to keep the bag from fully inflating or completely deflating. The minute volume was increased in 5L increments until the participant's $P_{et}CO_2$ reached 50% +/- 2 mmHg of the baseline value. This procedure resulted in spontaneous hyperventilation for each participant. This method of achieving hypocapnia has been demonstrated in the pulmonary literature, and was also confirmed with our pre-experimental feasibility testing (Antony, Brown, & Barlow, 1997; Rapee, Brown, Antony, & Barlow, 1992; Zvolensky & Eifert, 2001). The PI monitored the breath by breath analysis to determine when the participant had reached the hypocapnic state. Once the participant's $P_{et}CO_2$ was 50% +/- 2 mmHg of baseline for 30 seconds (steady state), the participant was instructed to perform the R_{law} task five times, as previously trained. The gas was turned off during each voiced production to reduce noise interference with the voiced data collection. Again, a 15-minute break followed.

For the hypercapnic condition, the 7% CO_2 gas was delivered via a 60 liter Douglas bag. The participant first breathed room air and the CO_2 gas mixture was silently switched to the inspiratory limb of the breathing circuit (Antony, et al., 1997; Gorman, et al., 1994; Hoit, et al., 2007; Papp, et al., 1997). The PI monitored the breath-by-breath analyses to determine when the participant had reached the hypercapnic state. Once the participant's $P_{et}CO_2$ reached 50

mmHg (+/- 2 mmHg) for 30 seconds (steady state), the participant was instructed to perform the R_{law} task five times. The hypercapnic gas was delivered continuously during the trial. Another-15 minute break of breathing room air followed with the final five minutes of rest recorded to ensure return to baseline of all variables.

During the steady state portions of the eupneic, hypocapnic, and hypercapnic trials, i.e., 30 seconds prior to the onset of phonation, $P_{et}CO_2$, VE, RR, HR, Ti, Te, and O_2 were values recorded. These variables were also recorded during the R_{law} task performance in each of the conditions. The difference between $P_{et}CO_2$ in each condition (without phonation during steady state, and with phonation during R_{law}) was later analyzed for statistically significant differences.

7.4.2.1 Blinding

All experimental conditions required the participant to breathe gas from a cylinder through a facemask. The participants were not informed which gas concentration they were breathing during the experiment. However, each condition produced a change in breathing pattern, so participants were aware the conditions were different. All collected data were saved under an alpha-numeric code linked to the condition name (e.g. “A” was always the first condition, which, based on counter-balancing, might have involved hypocapnia, hypercapnia, or eupnea). All R_{law} analyses were conducted by a master’s level speech-language pathologist specializing in voice with substantial experience analyzing aerodynamic data, and who was blinded to subjects’ condition.

7.5 DATA REDUCTION

All data reduction was completed by the PI and another member of the PI's doctoral lab, a master's level speech-language pathologist specializing in voice with substantial experience analyzing aerodynamic data, who was blinded to experimental condition. Five repetitions of the /pa/ utterance were completed for each condition. Following each experimental condition, the entire aerodynamic signal was saved. Then, the R_{law} signal was magnified and the middle three (of five) tokens were trimmed and saved for analysis. For calculation of R_{law}, first the middle three pressure peaks generated during the /p/ of each five-syllable /pa/ string were manually selected and examined for acceptable morphology (Smitheran & Hixon, 1981). The corresponding flow signal during the voiced /a/ was also manually selected and inspected. Specifically, the signals were inspected to ensure pressure returned to baseline during the vowel, and airflow returned to baseline during the consonant (Smitheran & Hixon, 1981). The middle three tokens were selected as default for analysis unless the morphology of the pressure peaks and airflow plateaus were deemed unacceptable, in which case the best three of five tokens (of any set) were saved and analyzed. Of note, a build-up of pressure from an increase in dead space due to the lengthening of the tube beyond the terminal end of the PAS6600 pneumotach prevented the pressure signal from returning to zero between syllables. Therefore, the pressure value was calculated by subtracting the baseline pressure (above zero) from the peak pressure. The physical experimental set-up disallowed the PI from monitoring sample requirements during collection. Therefore, though three syllable strings were required for analysis, five sets of data were collected in order to discard any syllable strings not satisfying the criteria. An example of one R_{law} set with baseline pressure above zero is shown in Figure 7.4. Once deemed acceptable,

the average pressure and airflow signals from the respective syllables were selected and the values manually recorded. The same procedure was repeated for the middle three trials (of five) for each condition (eupnea, hypocapnia, hypercapnia), and data used in statistical analyses. The $P_{et}CO_2$ values recorded via breath-by-breath analysis were also recorded during the periods of interest, that is, during the 30-sec non-phonated steady state portions of the eupneic, hypocapnic, and hypercapnic trials, as well as during the R_{law} task for each condition. The breath-by-breath values were averaged over the time of interest, and the averaged values used for analyses. Ten percent of data were reanalyzed by the PI for evaluation of reliability.

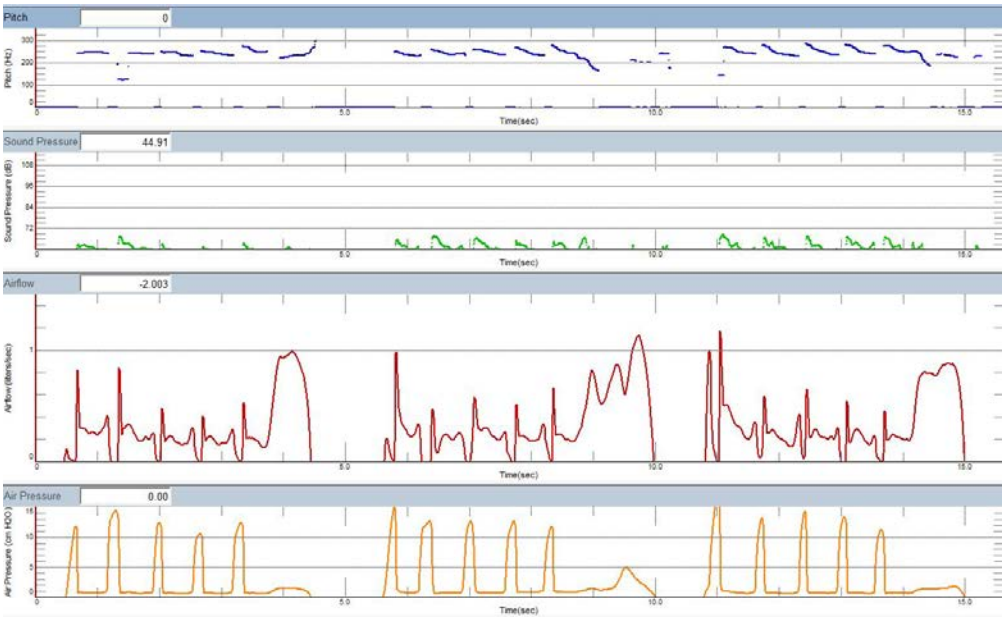


Figure 7.4. Example of PAS6600 data for 3 sets of /pa/ for one participant.

7.6 STATISTICAL ANALYSES

All statistical analyses were conducted using SPSS19.0. For primary statistical analyses, the independent variable was experimental condition (eupnea, hypocapnia, and hypercapnia) and the

dependent variable was laryngeal resistance (estimated P_{sub} in cmH_2O/ glottal airflow in L/sec). A within-subject Analysis of Variance (ANOVA) was run on these data. For secondary analyses, independent variables were experimental condition (eupnea, hypocapnia and hypercapnia) and phonation (yes/no), and the dependent variable was $P_{et}CO_2$. A 2-way within-subjects ANOVA (condition x phonation) was performed on these data. The alpha level was set to .05 for each test, without protection for alpha inflation due to the preliminary nature of the study. Interaction effects were tested first, if an interaction was found, main effects were then investigated. If a significant main effect of condition was found, post-hoc comparisons using Bonferroni adjustment were performed. The components of R_{law} – estimated subglottal pressure (P_{sub}) and laryngeal airflow -- were also analyzed with a one-way within-subjects ANOVA, to determine each part's contribution to the overall result. Due to the possible influence of subjective human error and variability in selection of tokens for R_{law} analysis, R_{law} reliability was assessed by the Interclass Correlation Coefficient.

7.7 INNOVATION

This study represented the first to analyze laryngeal airway resistance during phonation in conditions of hyper- and hypocapnia. These respiratory conditions are experienced daily by individuals with respiratory, psychological, and other disorders. Speech characteristics in hypercapnia (Hoit, et al., 2007; Russell, Cerny, & Stathopoulos, 1998), and laryngeal resistance during breathing but not during phonation have been studied by other authors (Bartlett, 1979; Brancatisano, et al., 1991; England & Bartlett, 1982; England, Bartlett, & Knuth, 1982; England,

et al., 1985; Kuna, McCarthy, et al., 1993). The influence of phonation on the larynx's role as a part of the respiratory system has not previously been investigated.

8.0 RESULTS

8.1 PARTICIPANTS

Fifty-seven potential participants were screened. Figure 8.1 provides information on all screened participants. Twenty-four satisfied all of the inclusion/exclusion criteria and participated in the study. All individuals who initiated the study completed it. R_{law} Data from two participants were excluded from final R_{law} analyses due to task violations (explained shortly). Table 8.1 and Table 8.2 provide demographic information including age, ethnic and racial category, participant perceived voice handicap (VHI-10), participant perceived reflux symptoms (RSI), and clinician-rated perceptual overall voice quality (CAPE-V) for each subject who participated.

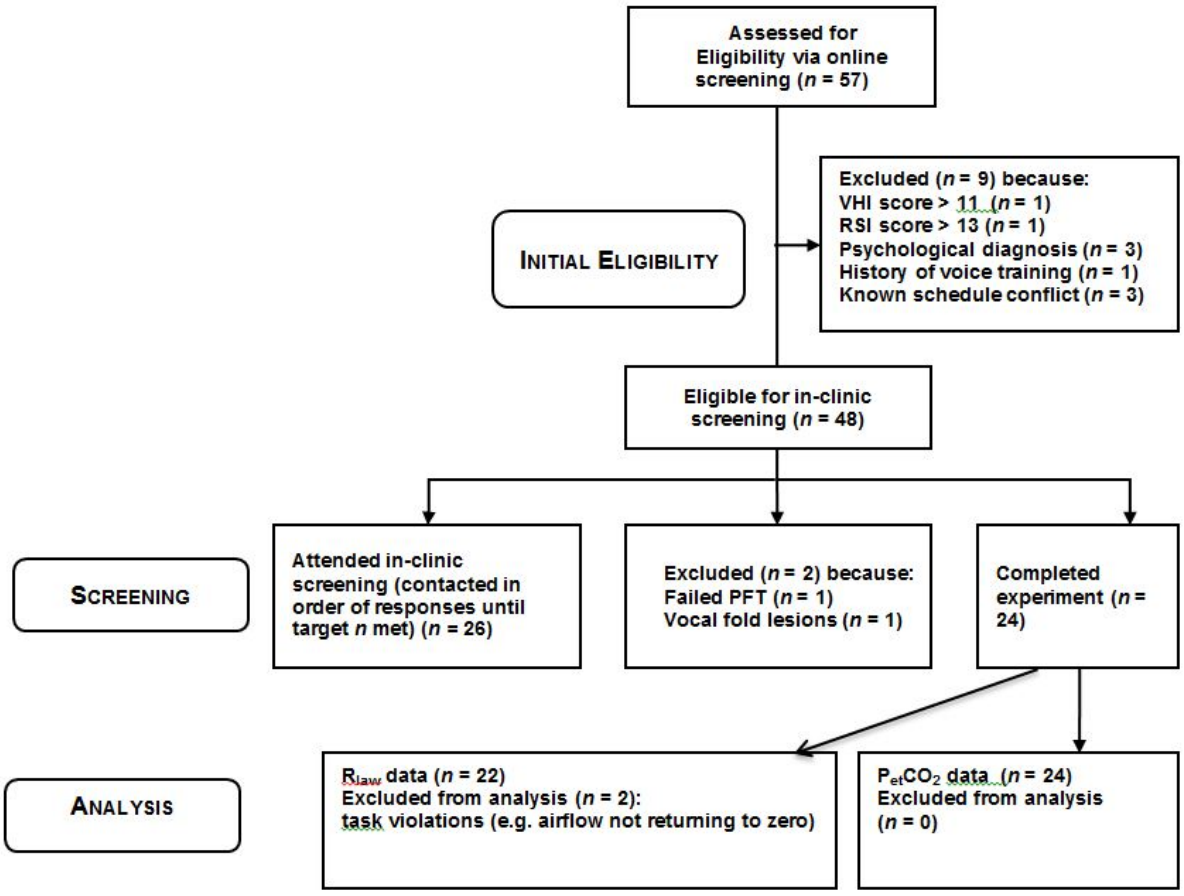


Figure 8.1 Participant flow chart

Table 8.1. Number of Participants in Each Racial and Ethnic Category; Range and Mean of Participant Ages

Hispanic	Non-Hispanic	White/Caucasian	Black/African American	Asian	Age range	Age mean
2	22	15	3	6	19-45	24.58

Table 8.2. Range and Means for CAPE-V, VHI-10 and RSI Scores for Participants

CAPE-V (max score: 100, higher score = worse)			VHI-10 (max score: 40, higher score = worse); Normative *M* = 7, *SD* = 2			RSI (max score: 50, higher score = worse); Normative *M* = 11, *SD* = 2		
range	mean	*SD*	range	mean	*SD*	range	mean	*SD*
0-3	0.75	0.79	0-6	1.08	1.61	0-10	2.13	2.63

8.2 PRIMARY OUTCOMES

8.2.1 Phonatory Laryngeal Resistance (R_{law})

The first hypothesis was that the larynx would sacrifice its *phonatory* resistance mechanisms in favor of *respiratory* resistance mechanisms, tending to return the system to physiologic homeostatic baseline under conditions of ventilatory perturbation. For investigation of this aim, phonatory laryngeal resistance (R_{law}) was measured during conditions of induced hypocapnia, hypercapnia, and eupnea. R_{law} data for 22/24 participants were available for final analyses. Two participants' data were excluded due to data violations in at least one condition (e.g., airflow not

returning to zero baseline during the voiceless pressure build). For analysis, these data were considered missing at random. Because the study used a repeated measures design, when one condition contained no analyzable data, the other conditions were therefore not useable, and all data for that participant were excluded from the final analysis.

Descriptively, participants' R_{law} responses to the breathing conditions demonstrated mutually opposing contrasting results. R_{law} was greater in the hypocapnic condition than the hypercapnic condition in 12/22 (55%) participants. The opposite result, showing greater R_{law} in hyper- as opposed to hypocapnia – occurred in 10/22 (45%) participants (Figure 8.2).

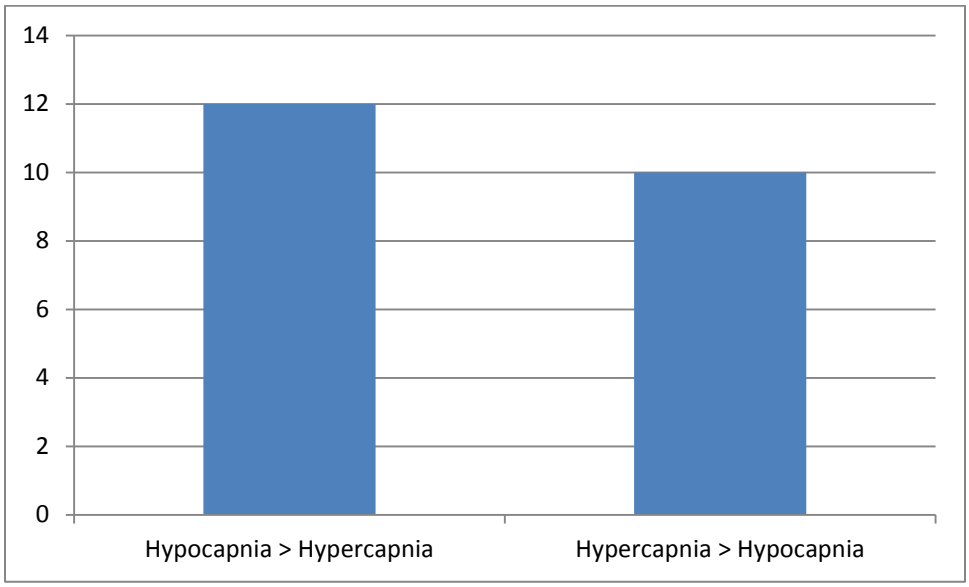


Figure 8.2. Number of participants in each breathing condition with greater R_{law}.

When eupnea R_{law} values were added to the descriptive analysis, the values represented all six possible combinations of R_{law} directions (Table 8.3). The group with the most participants (n = 6) demonstrated the lowest R_{law} values in the eupnea condition, followed by hypercapnia, then hypocapnia. Results for a second group two (n = 5) were consistent with the experimental hypothesis, that R_{law} values would be lowest in the hypercapnic condition, intermediate during

eupnea, and greatest in the hypocapnia condition. R_{law} values for a third group (n = 5) were lowest in eupnea, followed by hypocapnia, then hypercapnia. Group four's R_{law} values (n = 3) were lowest in hypocapnia, followed by hypercapnia, then eupnea. Group five (n = 2) demonstrated the lowest R_{law} values for hypocapnia, followed by eupnea, then hypercapnia. The final group (n = 1) had the lowest R_{law} values for hypercapnia, then hypocapnia, followed by eupnea.

Table 8.3. Combinations of R_{law} Directions

# of participants	Direction of R_{law} values (lowest – middle – greatest)		
6	Eupnea	Hypercapnia	Hypocapnia
5	Hypercapnia	Eupnea	Hypocapnia
5	Eupnea	Hypocapnia	Hypercapnia
3	Hypocapnia	Hypercapnia	Eupnea
2	Hypocapnia	Eupnea	Hypercapnia
1	Hypercapnia	Hypocapnia	Eupnea

A within-subjects Analysis of Variance was performed on phonatory laryngeal resistance (R_{law}) data as a function of breathing condition (eupnea, hypocapnia, hypercapnia). The assumption of sphericity was met (Mauchly's W = .964, $X^2(2)$ = .723, p = .697). The assumption of normality was not met (Table 8.4). Therefore the data were transformed using natural logarithmic transformation. The transformed data met the assumption of normality (Table 8.5). However, the results of the analysis of variance were not affected by the transformation (F[2, 42] = 1.130, p = .333, partial η^2 = .051). Therefore, results from analyses of the original un-transformed data are reported here.

Table 8.4. Test of Normality of the Untransformed Phonatory Laryngeal Resistance Values for Each Breathing Condition.

Condition	Shapiro-Wilk W	df	p
Eupnea	.818	22	.001
Hypocapnia	.839	22	.002
Hypercapnia	.707	22	.000

Table 8.5. Test of Normality of Phonatory Laryngeal Resistance Values after Natural Logarithmic Transformation for Each Breathing Condition

Condition	Shapiro-Wilk *W*	*df*	*p*
Log Eupnea	.958	22	.441
Log Hypocapnia	.951	22	.323
Log Hypercapnia	.938	22	.179

The main effect of breathing condition was not significant for $R_{law,}$ (F[2, 42] = .274, p = .762, partial η^2 = .013). Means, standard deviations, and ranges for R_{law} in each breathing condition are displayed in Table 8.6. Inter-rater reliability of R_{law} data showed excellent correlation between raters (r = .988).

Table 8.6. Means, Standard Deviations, and Ranges of R_{law} Values ($cmH_2O/L/sec$) for Each Breathing Condition

Condition	Mean	SD	Range
Eupnea	50.27	28.43	12.17-139.20
Hypocapnia	54.77	25.66	24.87-125.42
Hypercapnia	52.31	30.67	17.76-168.29

As previously noted, 6 combinations of R_{law} changes were observed across experimental conditions, indicating a substantial amount of variability in the data, and standard deviations were large. Inspection of individual R_{law} data (Figure 8.3) in each condition speaks to this variability. Two participants had at least one R_{law} value that fell 4 standard deviations outside the mean for that condition, two participants had values 3 standard deviations from the mean, and four had values 2 standard deviations from the mean across all conditions. The remaining 14 participants' values were within 1 standard deviation of the mean. Interestingly, the mean R_{law}

values observed in these data for each breathing condition were well within 1 standard deviation of R_{law} values for normal speakers (Zraick, Smith-Olinde, & Shotts, 2012). However, the failure to confirm the experimental hypothesis (lowest R_{law} values in hypercapnia and greatest values in hypocapnia) was not due to variability in the data: mean values were not positioned in the anticipated order, even descriptively.

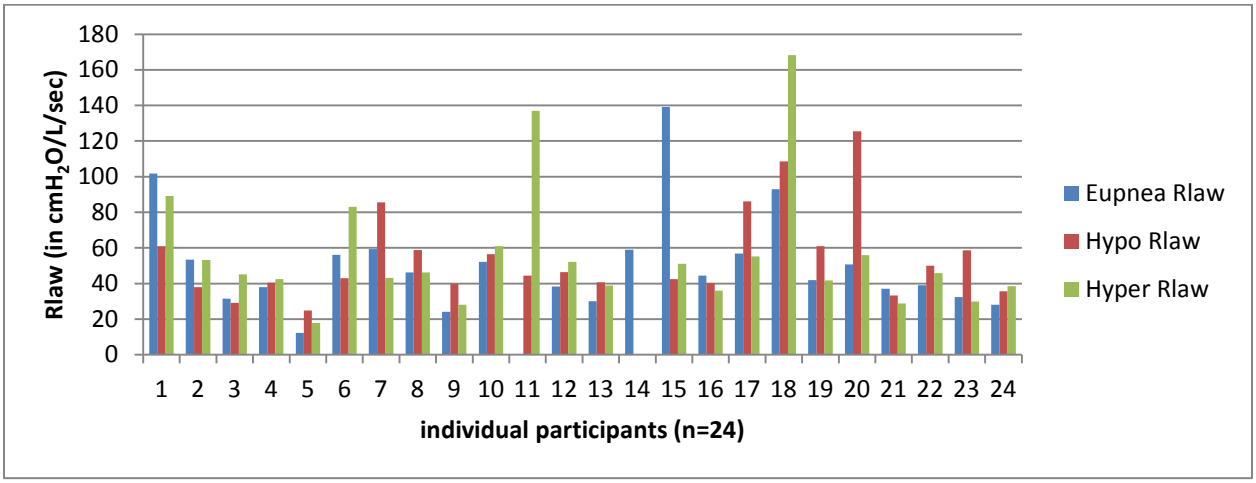


Figure 8.3. R_{law} values ($cmH_2O/L/sec$) for each participant in each condition. *Note: Eupnea Rlaw = R_{law} in the eupneic condition; Hypo Rlaw = R_{law} in the hypocapnic condition; Hyper Rlaw = R_{law} in the hypercapnic condition.*

Figure 8.4 displays the relatively stable mean value of R_{law} throughout the range of $P_{et}CO_2$ values (with a slight decrease in R_{law} as $P_{et}CO_2$ values increased).

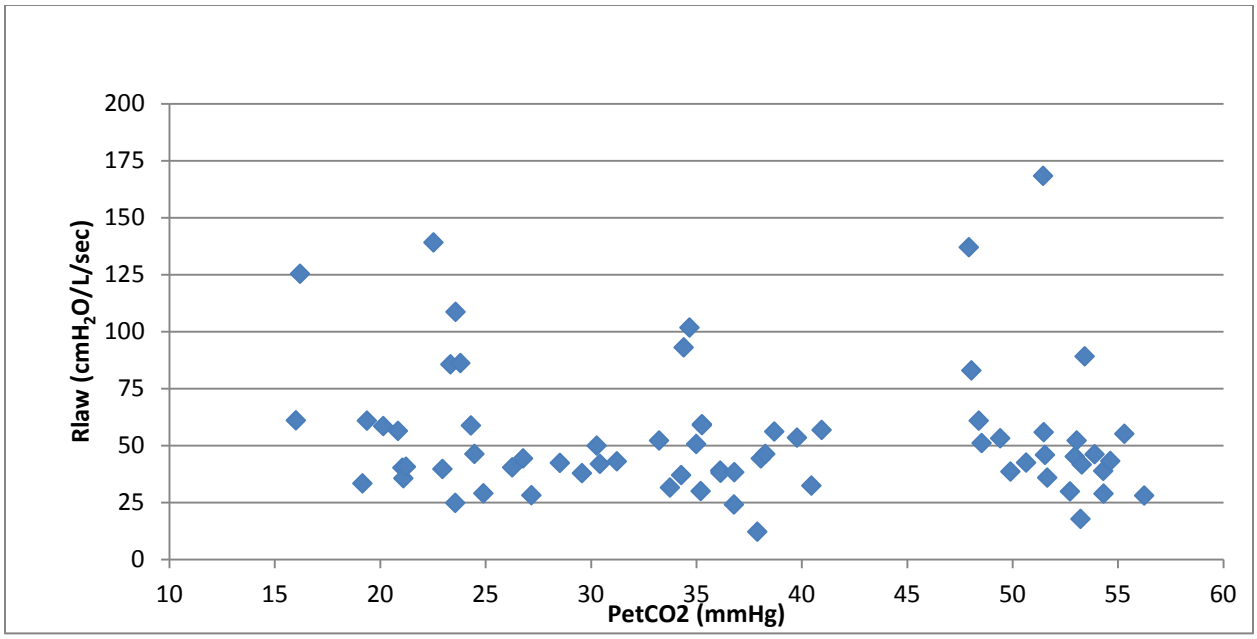


Figure 8.4. $P_{et}CO_2$ (mmHg) as a function of R_{law} (cmH_2O/L/sec).

8.2.2 Component Parts of Laryngeal Resistance

In order to further explore the results for R_{law}, the component parts of the R_{law} ratio - mean translaryngeal airflow (airflow) and mean estimated sub-glottal pressure (P_{sub}) -- were analyzed for differences across the breathing conditions.

8.2.2.1 Translaryngeal airflow

Nine total combinations of airflow directional patterns were observed. In 13/22 participants, airflow was greatest in the hypocapnic condition. For those participants, eupnea was associated with the lowest flow in 3/13, hypercapnia was associated with the lowest flow in 7/13, and eupnea and hypercapnia produced identical flows for the remaining 3/13 participants. In 5/22 subjects, hypercapnia produced the greatest flow. For those individuals, hypocapnia and eupnea produced identical flow values for two participants; flow in eupnea was less than hypocapnia in

two subjects; and hypocapnia was less than eupnea in one subject. Finally, for 2/22 participants, flow was greatest in the eupneic condition, and identical flows were observed in the hyper- and hypocapnic conditions.

A within-subjects Analysis of Variance was performed on translaryngeal airflow as a function of breathing condition (eupnea, hypocapnia, hypercapnia). The assumption of sphericity was met (Mauchly's W = .887, $X^2(2)$ = 2.41, p = .300). The assumption of normality was also met (Table 8.7). Means, standard deviations, and ranges of airflow values for each breathing condition are shown in Table 8.8.

Table 8.7. Test of Normality of Airflow Data

Condition	Shapiro-Wilk *W*	*df*	*p*
Airflow	.973	69	.139

Table 8.8. Mean, Standard Deviation, and Range of Airflow Values for Each Breathing Condition (L/sec)

Condition	*Mean*	*SD*	*Range*
Eupnea	.182	.076	.05-.39
Hypocapnia	.213	.067	.11-.35
Hypercapnia	.176	.058	.07-.26

The main effect of breathing condition on airflow values was significant: $F(2, 42)$ = 5.225, p = .009, partial η^2 = .199. Pairwise comparisons revealed airflow values were significantly higher in the hypocapnic condition than in the hypercapnic condition (p = .021). None of the other comparisons achieved significance. Figure 8.5 displays the airflow measurements for each subject in each experimental condition.

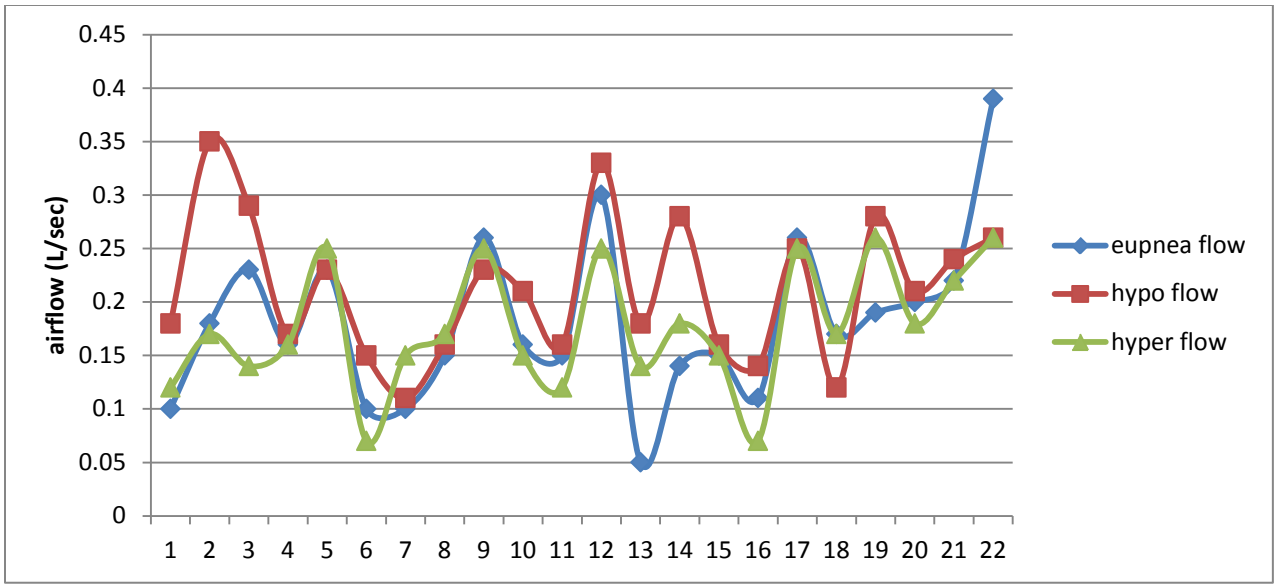


Figure 8.5. Airflow measurements (L/sec) for each participant in each experimental condition. *Note: eupnea flow = flow in the eupneic condition; hypo flow = flow in the hypocapnic condition; hyper flow = flow in the hypercapnic condition.*

8.2.2.2 Minute ventilation

In order to further explore the noted changes across conditions, changes in minute ventilation (L/min) in each breathing condition were also assessed. Minute ventilation is the product of tidal volume and respiratory rate. On average, participants increased minute ventilation by 43 L/min in the hypocapnic over the eupneic condition. Similarly, the hypercapnic condition caused an increase in minute ventilation as a response to elevating levels of CO_2. On average, participants increased their minute ventilation by 12 L/min in the hypercapnic over the eupneic condition (Table 8.10).

A within-subjects Analysis of Variance was performed on minute ventilation as a function of breathing condition (eupnea, hypocapnia, hypercapnia). The assumption of sphericity was met (Mauchly's W = .841, $X^2(2)$ = 3.819, p = .148). The assumption of normality was met for the hyper- and hypocapnic conditions, but not for the eupneic condition (Table 8.9).

Table 8.9. Test of Normality for Minute Ventilation in Each Breathing Condition

	Shapiro-Wilk *W*	df	Sig.
Eupnea	.486	24	.000*
Hypocapnia	.950	24	.275
Hypercapnia	.966	24	.578

*Note: * indicates significance at the .05 level.*

A significant difference in minute ventilation across breathing conditions was found: $F(2, 46) = 133.34$, $p < .001$, partial $\eta^2 = .853$. Pairwise comparisons showed minute ventilation values were significantly higher in the hypocapnic condition than in the hypercapnic condition, which were both significantly greater than in the eupneic condition ($p < .001$ for all comparisons) (Table 8.10).

Due to the violation of the normality assumption for the eupnea condition, all three conditions were subjected to non-parametric testing with the Friedman Test for significance, as well as the parametric testing with ANOVA. The Friedman Test also detected a significant difference in VE among breathing conditions: $\chi^2(2) = 40.583$, $p < .001$.

Post-hoc analysis with Wilcoxon Signed-Rank Tests was conducted with a Bonferroni adjustment, resulting in a significance level set at $p < 0.017$ for each test. There was a significant difference between VE for eupnea and hypocapnia ($Z = -4.257$, $p < .001$), between hypocapnia and hypercapnia ($Z = -4.286$, $p < .001$), and between hypercapnia and eupnea ($Z = -3.714$, $p < .001$). VE was greatest in the hypocapnic condition, followed by hypercapnic, and finally eupneic.

Table 8.10. Minute Ventilation in Each Condition

Condition	*M* Minute Volume (V_E) (L/min)	*SD*
Eupnea	12.26	9.93
Hypercapnia	23.92	10.77
Hypocapnia	55.88	12.93

8.2.2.3 Estimated sub-glottic pressure

Unlike the variability observed in the airflow data, only two directional combinations of estimated sub-glottic pressure (P_{sub}) data were found in the data set. For all participants, hypocapnia produced greater P_{sub} than hypercapnia or eupnea. For 16/22 participants, the lowest P_{sub} was observed during eupnea, followed by hypercapnia. For 6/22 participants, hypercapnia produced the lowest P_{sub}, then eupnea.

A within-subjects analysis of variance was performed on estimated sub-glottic pressure (P_{sub}) as a function of breathing condition (eupnea, hypocapnia, hypercapnia). The assumption of sphericity was not met (Mauchly's $W = .293$, $X^2(2) = 24.53$, $p < .001$), therefore Huynh-Feldt values are reported for tests of significance. The assumption of normality was met (Table 8.11). Means, standard deviations, and ranges of P_{sub} values for each breathing condition are shown in Table 8.12.

Table 8.11. Test of Normality of P_{sub} Data by Experimental Condition

Condition	Shapiro-Wilk *W*	df	*p*
Eupnea	.958	22	.455
Hypocapnia	.942	22	.221
Hypercapnia	.969	22	.679

Table 8.12. Mean, Standard Deviation, and Range of P_{sub} Values (cmH_2O) for Each Breathing Condition

Condition	*Mean*	*SD*	*Range*
Eupnea	7.65	2.01	2.80-10.97
Hypocapnia	10.62	3.00	5.72-15.25
Hypercapnia	7.97	1.86	4.44-11.78

**Note: all values in cmH_2O*

A significant difference in P_{sub} values across breathing conditions was found: $F(1.19, 25.18) = 37.130$, $p < .001$, partial $\eta^2 = .639$. Pairwise comparisons revealed significantly greater P_{sub} values in the hypocapnic condition (M = 10.62 cmH_2O, SE = .641) than the eupneic (M = 7.65 cmH_2O, SE = .429) and the hypercapnic (M = 7.97, SE = .397) conditions. None of the other comparisons achieved significance. The differences in P_{sub} among the breathing conditions are graphed in Figure 8.6.

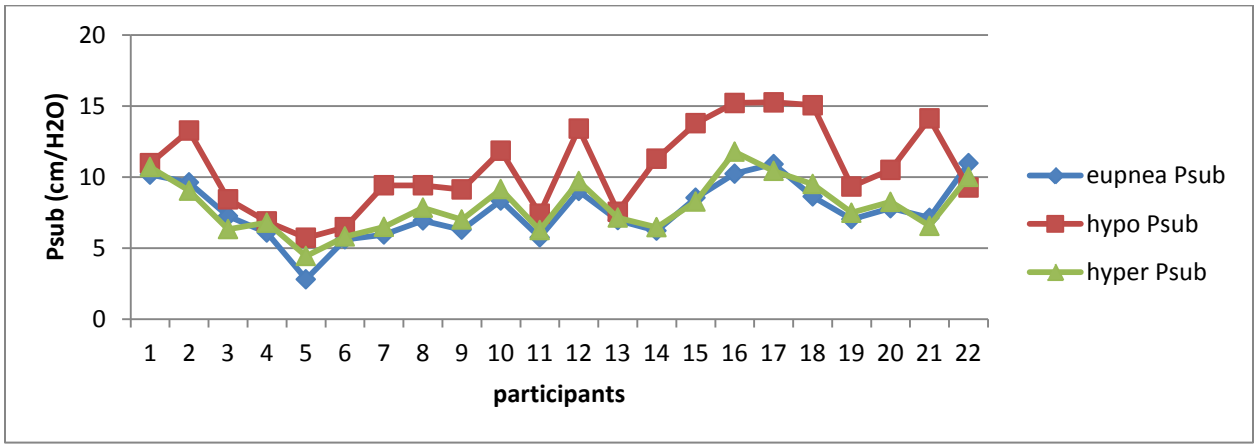


Figure 8.6. P_{sub} (cmH_2O) values for each participant.

Airflow and corresponding P_{sub} values are displayed for each participant in each breathing condition in Figure 8.7,Figure 8.8Figure 8.9.

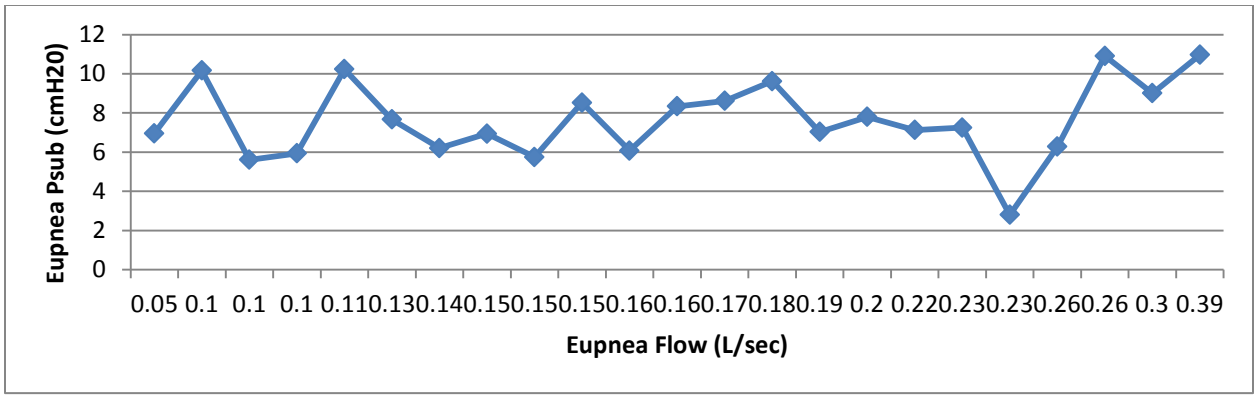


Figure 8.7 Airflow (L/sec) and P_{sub} (cmH_2O) for each participant in eupneic condition.

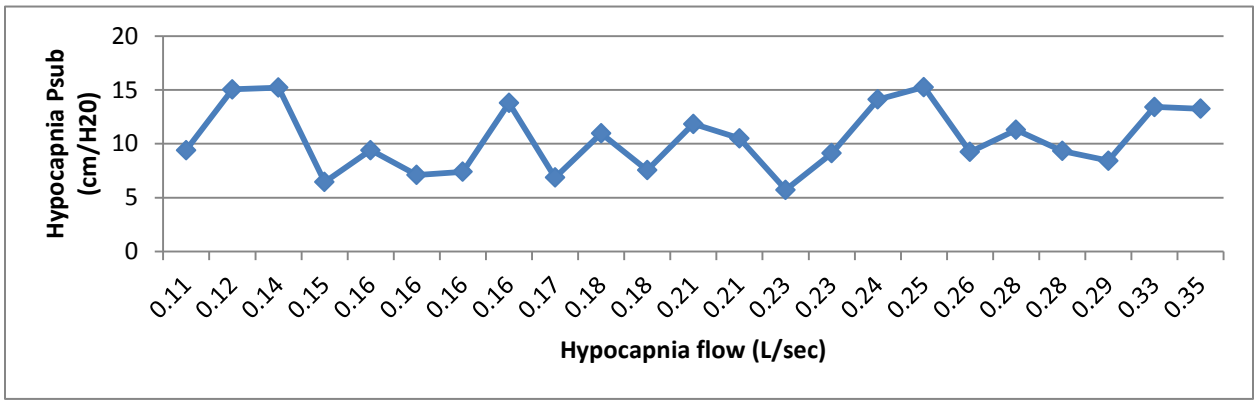


Figure 8.8 Airflow (L/sec) and P_{sub} (cmH_2O) for each participant in hypocapnic condition.

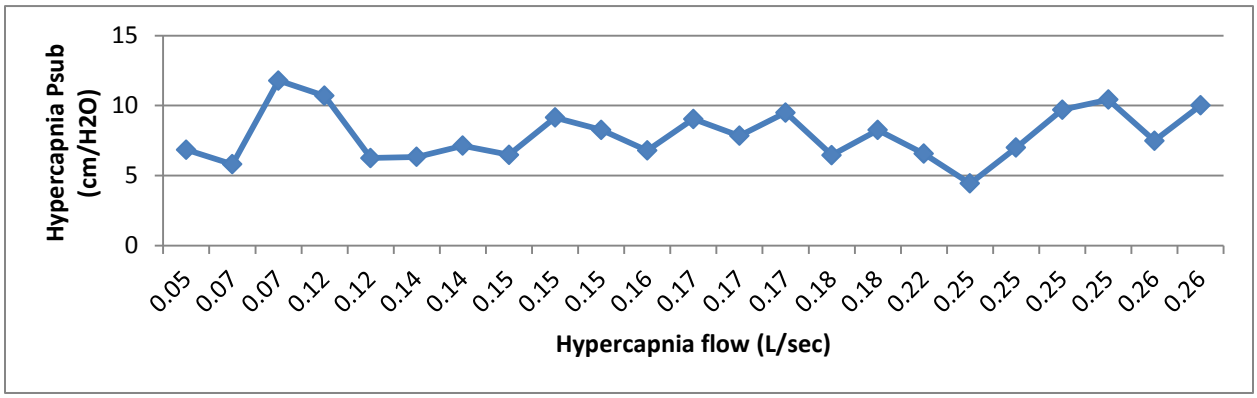


Figure 8.9 Airflow (L/sec) and P_{sub} (cmH_2O) for each participant in hypercapnic condition.

8.2.3 End-tidal Carbon Dioxide ($P_{et}CO_2$).

End-tidal carbon dioxide ($P_{et}CO_2$) data for all 24 participants were acceptable for analysis. For all participants, $P_{et}CO_2$ values were lowest for hypocapnia, intermediate for eupnea, and greatest for hypercapnia.

A 2x3 within-subjects Analysis of Variance was performed on $P_{et}CO_2$ values as a function of phonation (no phonation during steady-state and phonation during voice task) and breathing condition (eupnea, hypocapnia, hypercapnia). The assumption of sphericity was met for all effects independently (phonation: Mauchly's $W = 1.00$; breathing condition: Mauchly's $W = .827$, $X^2(2) = 4.174$, $p = .124$; phonation by breathing condition: Mauchly's $W = .981$, $X^2(2) = .420$, $p = .811$). However, the assumption of normality was not met for pooled P_{et}-CO_2 values (Table 8.13). The data were therefore transformed using a natural logarithmic transformation. The assumption of normality of the transformed data was still not met **(**Table 8.14).

Table 8.13. Normality Test for $P_{et}CO_2$ Variable

Variable	Shapiro-Wilk *W*	df	*p*
$P_{et}CO_2$	.915	144	.000

Table 8.14. Normality Test for $P_{et}CO_2$ Variable after Natural Logarithmic Transformation

Variable	Shapiro-Wilk *W*	df	*p*
Log- $P_{et}CO_2$	.918	144	.000

The original un-transformed data were then split by their grouping variables and normality was tested again. The assumption of normality was met for all conditions except eupnea during phonation and hypercapnia during steady state (Table 8.15). Means and standard deviations for all conditions are reported in Table 8.16.

Table 8.15. Test of Normality for Breathing Condition Data as a function of Phonation

Condition	*Shapiro-Wilk W*	*df*	*p*
Eupnea steady state	.970	24	.661
Eupnea phonation	.870	24	.005*
Hypocapnia steady state	.979	24	.877
Hypocapnia phonation	.973	24	.745
Hypercapnia steady state	.917	24	.050*
Hypercapnia phonation	.945	24	.212

*Note: * indicates significance at the .05 level.*

Because the largest standard deviation of the grouped variables (steady state and phonation) was not greater than twice the smallest standard deviation, the untransformed data (not the transformed data) were subjected to the Analysis of Variance (Moore, 2008).

The interaction of breathing condition and phonation groups was significant (F[2,46] = 8.165, p = .001, partial η^2 = .262). In order to find the pattern of differences on $P_{et}CO_2$ values across breathing condition and phonation separately, the main effects for one variable were evaluated at individual levels of the other variable. Results were as follows.

The simple main effect of breathing condition for phonated segments was significant: F(2,46) = 376.237, p < .001, partial η^2 = .942. Pairwise comparisons using Bonferroni adjustment revealed significantly greater $P_{et}CO_2$ with phonation during hypercapnia than eupnea (p < .01) and hypocapnia (p < .01). The simple main effect of breathing condition for non-phonated steady state was also significant: F(2,46) = 1456.583, p < .001, partial η^2 = .984. Again, pairwise comparisons using Bonferroni adjustment revealed significantly greater $P_{et}CO_2$ during non-phonated steady-state in hypercapnia than eupnea (p < .01) and hypocapnia (p < .01). These results are consistent with physiologic expectations and confirm the success of the intended breathing perturbation.

In order to find the pattern of differences on phonation for each level of breathing condition, simple main effects of phonation at eupnea, hypocapnia, and hypercapnia were also performed. The simple main effect of phonation during eupnea was significant: $F(1,23) = 15.342$, $p = .001$, partial $\eta^2 = .400$. Similarly, the simple main effect of phonation during hypocapnia was also significant: $F(1,23) = 65.081$, $p < .001$, partial $\eta^2 = .739$. Finally, the simple main effect of phonation at hypercapnia was also significant: $F(1,23) = 19.433$, $p < .001$, partial $\eta^2 = .458$. The results revealed greater $P_{et}CO_2$ values during phonation than non-phonated steady state in each of the breathing conditions.

The interaction between phonation and breathing condition was seen by disproportionately large increases in $P_{et}CO_2$ from steady state to phonation during hypocapnia (+ 5mmHg) as compared to eupnea (+3 mmHg), with hypercapnia (+1 mmHg) showing the smallest increase (Figure 8.10).

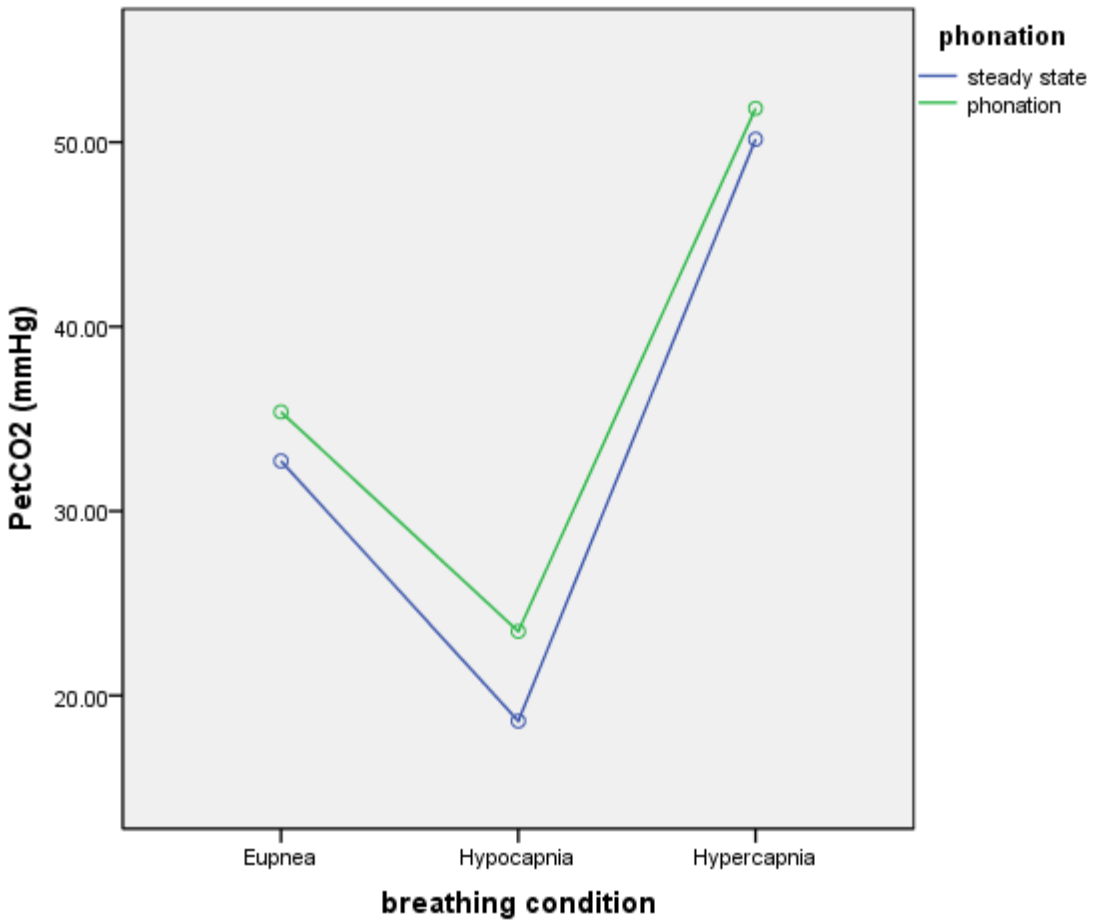


Figure 8.10 Interaction of breathing condition and phonation.

Table 8.16 displays the means, standard deviations and range of $P_{et}CO_2$ values for each breathing condition during steady state and phonation. Figure 8.11 displays the raw $P_{et}CO_2$ values for each observation in each condition (1 observation per breathing condition for both steady state and phonation, multiplied by 24 participants = 72 observations for steady state and 72 for phonation).

Table 8.16. Mean, Standard Deviation, and Range of $P_{et}CO_2$ Values (mmHg) for Steady State and Phonated Portions of Each Breathing Condition

Condition	*Mean*	*SD*	*Range*
Eupnea steady state	32.72	2.72	26.53-37.46
Eupnea phonation	35.38	4.10	22.53-40.95
Hypocapnia steady state	18.61	1.89	14.28-22.46
Hypocapnia phonation	23.48	4.02	16.00-31.23
Hypercapnia steady state	50.16	1.50	47.10-52.50
Hypercapnia phonation	51.84	2.52	47.74-56.25
Note: all values in mmHg			

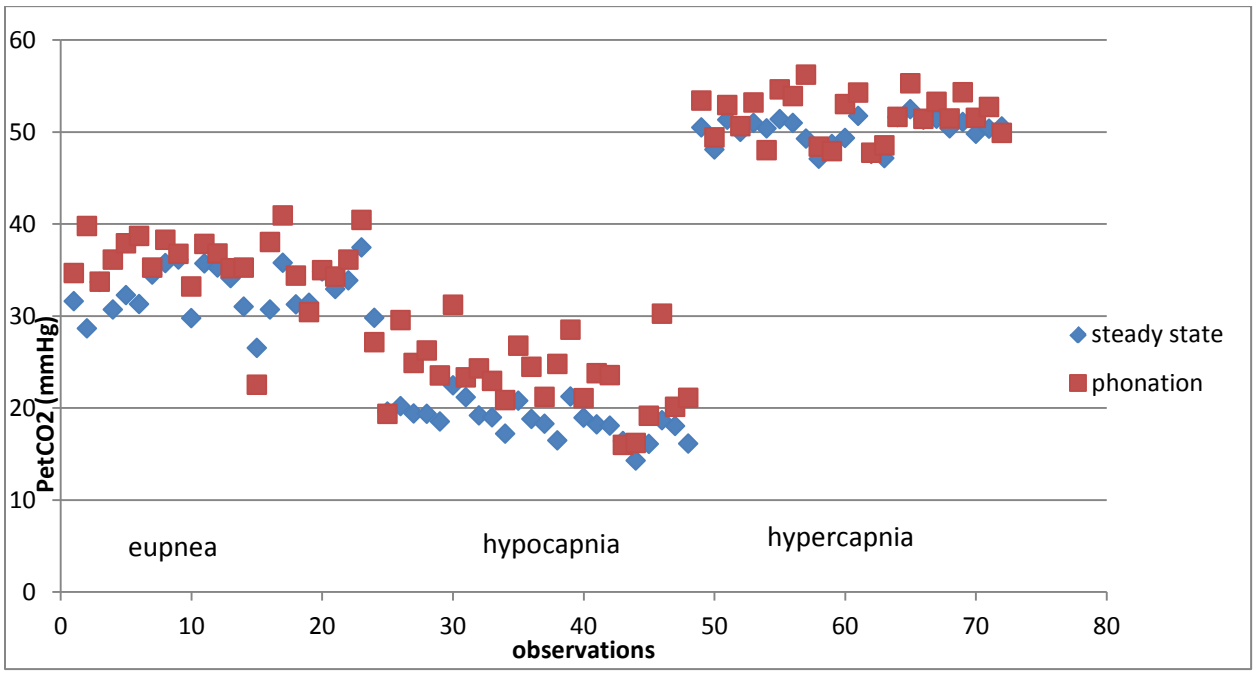


Figure 8.11. $P_{et}CO_2$ values (mmHg) for each observation during steady state respiration and phonation.

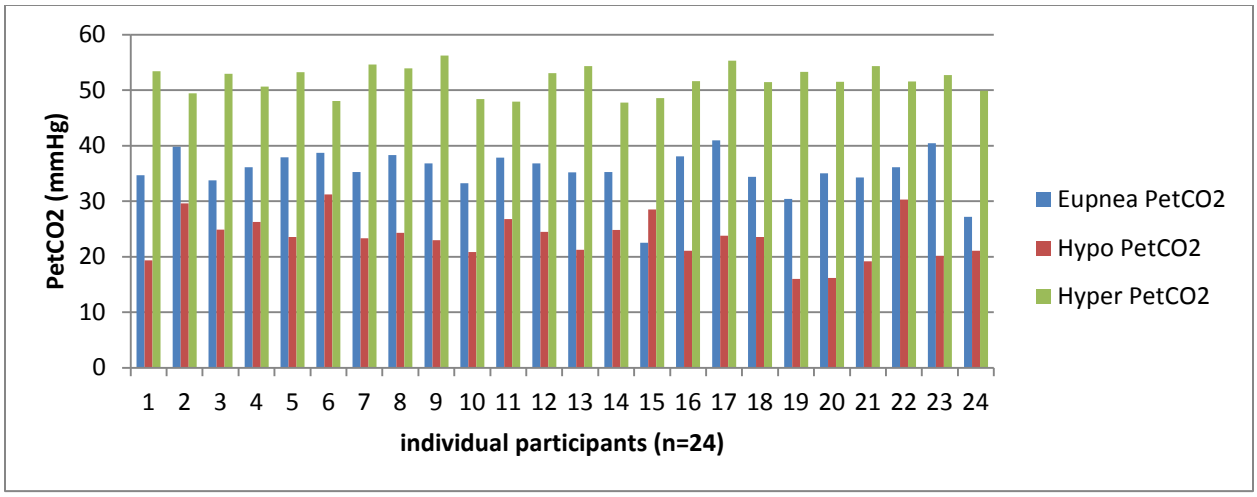


Figure 8.12. $P_{et}CO_2$ values (mmHg) during phonation for each participant in each breathing condition.
Note: Eupnea $P_{et}CO_2$ = $P_{et}CO_2$ in the eupneic condition; Hypo $P_{et}CO_2$= $P_{et}CO_2$ in the hypocapnic condition; Hyper $P_{et}CO_2$= $P_{et}CO_2$ in the hypercapnic condition.

8.3 SECONDARY OUTCOMES

8.3.1 Vocal intensity.

Due to a combination of factors including microphone positioning, noise produced by the gas cylinders, and overall trend towards lower than expected vocal intensities produced by the participants, dB SPL values were only available for 36/72 possible tokens. Of the 12 participants with complete intensity data sets, five had the greatest intensity in the hypocapnic condition, followed by hypercapnic and then eupneic; for 4 participants eupnea evoked the greatest intensity, followed by hypercapnia then hypocapnia; 2 participants had the greatest intensity in

hypercapnia followed by hypocapnia then eupnea; and one participant had the greatest intensity in the hypocapnic condition, then eupneic, and finally hypercapnic.

A one-way within subjects Analysis of Variance (ANOVA) on dB SPL in each breathing condition was performed. The assumption of sphericity was met (Mauchly's W = .822, $X^2(2)$ = 1.955, p = .376), as was the assumption of normality (Table 8.17).

Table 8.17. Test of Normality for dB SPL Values in Each Breathing Condition

Condition	Shapiro-Wilk *W*	df	*p*
dB SPL Eupnea	.908	12	.203
dB SPL Hypocapnia	.949	12	.625
dB SPL Hypercapnia	.958	12	.748

No significant difference in dB SPL values among breathing conditions was found: $F(2, 22)$ = 3.367, p = .053, partial η^2 = .234. Means and standard deviations of dB SPL values are shown in Table 8.18.

Table 8.18. Mean, Standard Deviation, and Range of Corrected dB SPL Values for Each Breathing Condition

Condition	*Mean*	*SD*	*Range*
Eupnea	63.57 dB	2.06	60.83-68.40
Hypocapnia	64.61 dB	3.43	57.86-72.01
Hypercapnia	64.41 dB	2.17	61.19-67.96

8.3.2 Fundamental frequency

As with the dB SPL values, only 36/72 possible tokens were available for analysis of fundamental frequency (F_0). Of the 12 participants with complete frequency data sets, 6 had the highest F_0 during hypocapnia and the lowest in eupnea; 4 had the highest F_0 in hypocapnia and the lowest in hypercapnia; 1 had the highest F_0 in hypercapnia and the lowest in eupnea; and finally, 1 had the highest F_0 in hypercapnia and the lowest in hypocapnia. More simply, the largest trend in F_0 data appeared in hypocapnia, which produced the greatest F_0 in 10/12 participants.

A within-subjects Analysis of Variance (ANOVA) was performed on fundamental frequency values as a function of breathing condition (eupnea, hypocapnia, hypercapnia). The assumption of sphericity was met (Mauchly's W = .959, $X^2(2)$ = .416, p = .812), as was the assumption of normality (Table 8.19).

Table 8.19. Test of Normality for F_0 Data for Each Breathing Condition

Condition	Shapiro-Wilk *W*	df	*p*
F_0 Eupnea	.948	12	.602
F_0 Hypocapnia	.939	12	.479
F_0 Hypercapnia	.951	12	.658

Despite distributional differences just noted, a significant difference in F_0 values across breathing conditions was found $F(2, 22)$ = 17.365, p < .001, partial η^2 = .612. Pairwise comparisons revealed significantly higher F_0 values during phonation in the hypocapnic condition (M = 229.74 Hz) than in hypercapnic (M = 218.29 Hz) or eupneic conditions (M = 210.19 Hz); p = .029 and < .001 respectively. No significant difference was found in F_0 between

eupnea and hypercapnia (p = .076). Figure 8.13 displays the available F_0 data for each participant in each breathing condition.

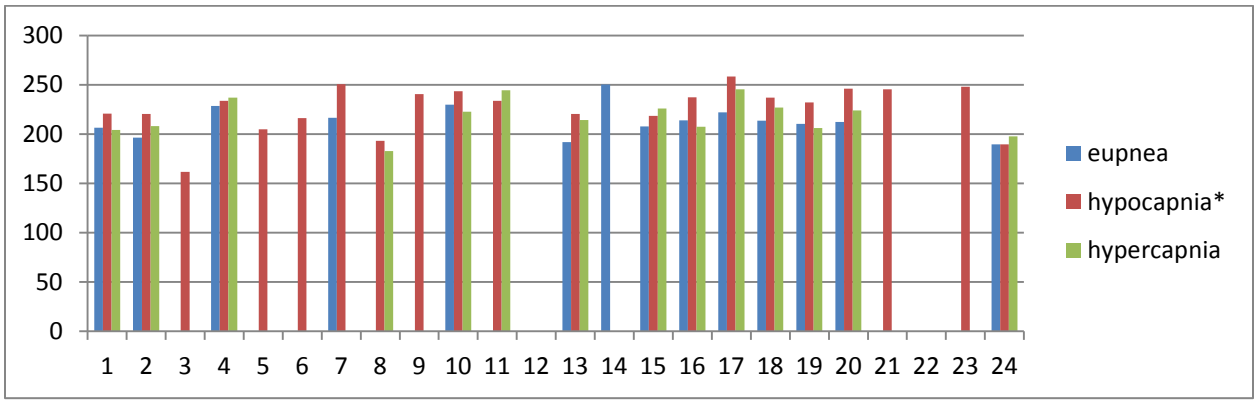


Figure 8.13. Available F_0 data (Hz) for each participant in each breathing condition.

8.3.3 Order effects

The success of the counterbalanced and randomized design was tested using a Latin Square ANOVA. Six orders of condition presentation (eupnea, hypocapnia and hypercapnia) were possible, with four participants randomly assigned to each order. No significant order effects of R_{law} in the breathing conditions were found (p = .268). Order effects of $P_{et}CO_2$ were not tested, because each participant's $P_{et}CO_2$ level was an experimental criteria for initiation of the phonation task in SA1a,b.

9.0 DISCUSSION

9.1 PRIMARY OUTCOMES

9.1.1 Laryngeal resistance

The rather straightforward expectation that prompted this study was that when confronted with conflicting phonatory and respiratory needs, laryngeal resistance (R_{law}) would fluctuate in a direction that favors respiratory homeostasis, sacrificing its normal role in phonatory control. That is, the expectation was that R_{law} would be greatest in the hypo- as compared to the hypercapnic condition, thus assisting with a return to respiratory homeostatic baseline following perturbation. Counter to expectations, no evidence whatsoever was found for such vulnerability in R_{law}, which remained remarkably stable. Moreover, R_{law} remained within the range of normal values for healthy speakers under conditions of fairly substantial ventilatory disruptions. Not only did results of statistical analyses fail to reveal fluctuations in R_{law} under respiratory perturbation, inspection of individual data was also fruitless: 55% percent of participants showed greater R_{law} in the hypo- as compared to the hypercapnic condition, as hypothesized, whereas 45% had the opposite response: greater R_{law} in hyper- as opposed to hypocapnia. Thus, even individual data provided no more clarity than a coin toss.

However, equally interestingly, attempts to examine the data more fully provided some insights. Separate analyses of airflow and estimated subglottic pressure (P_{sub}) data, the component parameters of R_{law}, revealed that these parameters *were* modulated by respiratory condition. Both airflow and P_{sub} values increased robustly under the hypo- as compared with hypercapnic condition. Thus, phonatory control parameters *were* affected by the breathing conditions; they were simply affected in a way that maintained the constancy of R_{law}. Oddly, their modulation appeared paradoxical. It would seem that under conditions of hypocapnia (reduced CO_2), simultaneous increases in airflow and P_{sub} during phonation would serve to further expel CO_2, thus exacerbating CO_2 deprivation. More interesting for the present context, airflow and P_{sub} increased in tandem in such a way as to *maintain constancy in laryngeal resistance values.*

This observation leads to a crucial point for interpretation of the data. Although the study was designed anticipating a demonstration of ventilatory supremacy over phonatory functions, the data imply it ended up being about another issue entirely: *voice motor control.* Specifically, results can be interpreted within a framework that considers voice motor control as part of a *regulated system.* A system is considered physiologically regulated if it detects perturbations and makes adaptive changes to achieve system goals (Brobeck, 1965; Hammond, Warren, Mayo, & Zajac, 1999; Kim, Zajac, Warren, Mayo, & Essick, 1997; Warren, Dalston, Morr, Hairfield, & Smith, 1989; Warren, Morr, Rochet, & Dalston, 1989; Warren, Rochet, Dalston, & Mayo, 1992; Zajac, 1995).

As a case in point, research on upper airway perturbations of the *speech* motor control system has found that despite induced oral or nasal pressure bleeds, human subjects maintain oral pressures at adequate levels for consonant production (Warren, Dalston, et al., 1989;

Warren, Morr, et al., 1989; Warren, et al., 1992). Pressures are maintained generally by a change in articulatory strategy (Zajac, 1995), sometimes at the expense of articulatory precision and intelligibility, as well as through increased respiratory effort or changes in constrictions elsewhere in the upper vocal tract (Hammond, et al., 1999; Kim, et al., 1997; Warren, Dalston, et al., 1989; Warren, et al., 1992).

Similarly, in the present study, the system appeared resistant to threats to one of its apparent system goals, R_{law}. Suggestions have been made that speakers make coordinated changes to maintain "aerodynamic integrity" (p. 566) in the face of perturbations (Warren, Dalston, et al., 1989). What remains largely unknown is to what change the speech (or voice) motor control system responds in order to adjust for intra-oral pressure drops or other potential perturbations. Stated differently, what is the goal that speech and voice regulating systems are attempting to meet? In the speech literature, two competing hypotheses have been proposed; one view is that the goal is tactile; the other is that the goal is acoustic (Guenther, Hampson, & Johnson, 1998; Villacorta, Perkell, & Guenther, 2007; Warren, Dalston, et al., 1989; Warren, Morr, et al., 1989). These hypotheses, along with their potential relevance for the present findings, are discussed in turn next.

Tactile goal hypothesis. In support of a tactile goal hypothesis in speech motor control, evidence exists that speakers maintain oral air pressures during speech above 3cm H_2O (necessary for consonant production) despite oral and nasal pressure bleeds (Warren, Dalston, et al., 1989; Warren, Morr, et al., 1989; Warren, et al., 1992). Furthermore, speakers are aware of a minimum change in oral pressure of 1cm H_2O (Warren, Dalston, et al., 1989). Target pressures are maintained even when acoustic and articulatory accuracy are neglected (Warren, Morr, et al., 1989). Across studies, an increase in oral airflow and respiratory effort have been observed as a

response to oral pressure leaks, and interpreted as one representation of an adaptation performed by the speech regulating system to maintain adequate intra-oral pressure for consonant production (Hammond, et al., 1999; Kim, et al., 1997; Warren, et al., 1992). Arguments are that tactile goals of this type are maintained via oral, nasal, pharyngeal, laryngeal, and tracheal mechanoreceptors, which provide feedback on changing pressure and airflow values (e.g.Warren, Dalston, et al., 1989).

Extending the arguments to the case of aerodynamic *resistance* more broadly, of which pressure is one component, a close look at the literature on voice may reveal further support for the tactile goal hypothesis. Reports indicate phonatory laryngeal resistance (R_{law}) is maintained in healthy speakers across multiple time points and, more importantly for present purposes, also under varying auditory masking conditions (Grillo & Verdolini, 2007; Leeper & Graves, 1984). Specifically, with and without auditory masking, which effectively removes acoustic feedback for voice, R_{law} is maintained as normal. Results from the current study demonstrating relatively stable R_{law} values during respiratory perturbations – moreover under conditions of substantial ambient noise -- may lend support to the idea that not only pressure, but also the combination of pressure and flow – R_{law} – represent fairly immutable, tactile-based control parameters in voice production.

Auditory goal hypothesis. Of course, evidence exists that acoustic feedback is necessary for *normal* voice and speech production. The literature demonstrating inarticulate speech and abnormal vocal quality in congenitally deaf individuals supports the need for auditory input to acquire normal, acceptable speech and voice communication in the long term. Clearly, an acoustic goal for communication exists. However, the foregoing argument posits that an acoustic goal is not the only – or most salient - goal (Warren, et al., 1992). Furthermore, most regulating

systems attempt to preserve more than one component part of system functioning in order to achieve that system's goal (Kim, et al., 1997). In contrast to arguments for a tactile goal in speech and voice, multiple authors have hypothesized that the goal of the speech motor control system is an invariant acoustic one (Guenther & Gjaja, 1996; Guenther, et al., 1998). One example of an acoustic goal theory lies with vowel production, in which it is argued that tactile feedback references are not available, yet vowels are easily produced normally in connected speech. Supporters agree that tactile feedback explains adequate, consistent *consonant* but not *vowel* production (Guenther, et al., 1998; Villacorta, et al., 2007). In addition, many speech sounds can be produced with a variety of oral-pharyngeal configurations (such as the /r/ phoneme) and still be perceived auditorily as normal (Guenther, et al., 1998). In order for the nervous system to respond to a production error, an efferent signal detecting the error must be received so that a motor correction can be made. This sequence must occur rapidly online for normal speech to continue. Proponents of the acoustic goal theory argue that auditory perceptual feedback is constantly available for all speech sounds, whereas information on location of oral-pharyngeal-laryngeal constriction, including pressure and airflow information, is not equally available for consonants and vowels and therefore cannot be the primary means by which speech is regulated (Guenther, et al., 1998).

Although these arguments are appealing, their relevance for present study is unclear. As noted, ambient noise was substantial in the study, and yet not only R_{law}, but also output intensity appeared invariant across conditions. The conclusion is that at least in this study, R_{law} was not somehow acoustically regulated, and must have been regulated instead by some other sense domain – most logically the tactile sense.

9.1.1.1 Respiratory kinematics

Additional possibilities to explain the consistent R_{law} results may lie with respiratory kinematics. Respiratory effort, that is, ventilation, increased in both hypo- and hypercapnic conditions in the current study. Specifically, the hypocapnic condition required participants to hyperventilate in order to expire enough CO_2 to achieve and maintain hypocapnia. The hypercapnic condition caused an increase in minute ventilation on average 12 L/minute over the eupneic condition. This observation is similar to minute ventilation increases of 18 L/minute reported in the literature on speech breathing in hypercapnia (Bailey & Hoit, 2002). Therefore, both experimental conditions- hypocapnia and hypercapnia- required or resulted in hyperventilation.

Upper airway resistance during breathing is affected by respiratory frequency and lung volume. First, as ventilation increases, upper airway resistance during breathing decreases (England & Bartlett, 1982; England, Bartlett, & Daubenspeck, 1982; Kuna, Insalaco, et al., 1993; Shindoh, et al., 1985). Hyperventilation has shown to increase laryngeal abductor muscle activity, thereby increasing glottal area (and decreasing resistance) during breathing (Savard, et al., 1993). Relevant to the present study, an increase in ventilation is also observed during speech breathing as compared to quiet breathing (Hoit & Lohmeier, 2000).

Second, as lung volumes increase, laryngeal resistance during breathing also decreases (Hoit, et al., 2007; Shindoh, et al., 1985). Hypercapnia can cause an increase in functional residual capacity, thereby increasing lung volume, and, decreasing laryngeal resistance during

breathing, and, by extension, phonation (England, Bartlett, & Knuth, 1982; Iwarsson, et al., 1998; Lowell, Barkmeier-Kraemer, Hoit, & Story, 2008; Savard, et al., 1993).

Finally, hyperventilation can override the increase in laryngeal adductor activity expected in hypocapnia, therefore causing a decrease, not increase, in laryngeal resistance during breathing as expected (Bartlett & Knuth, 1984). The rapid breathing in both the hyper- and hypocapnic conditions may have counteracted the expected R_{law} responses to the conditions, effectively eliminating the hypothesized effects of the CO_2 manipulations (England, et al., 1985). In other words, the effects of hyperventilation on R_{law} in conditions of hypercapnia and hypocapnia in the current study may have been strong enough to overcome expected effects of the CO_2 changes, resulting in statistically equivalent R_{law} results (Bartlett & Knuth, 1984; Savard, et al., 1993).

Perturbation studies have established that the speech production system exhibits motor equivalence by using new articulatory configurations to meet a communication goal when the default configuration is precluded by a perturbation (Guenther, et al., 1998). In this view, respiratory kinematic changes may have occurred as an adaptation to the experimental condition in order to keep phonatory resistance consistent. Past studies on speech breathing in healthy participants subjected to conditions of increased CO_2 have demonstrated respiratory kinematic changes in the experimental condition. Specifically, lung volumes were larger and chest wall movements bigger and faster in the high CO_2 condition than in room air breathing (Bailey & Hoit, 2002). However, these volume and kinematic changes were somewhat attenuated by the act of speaking. The authors concluded that "the average ventilatory response of our subjects to high CO_2 was substantially smaller during speaking than during breathing. This…supports the idea

that speaking ‘overrides’ to some extent the metabolic control of breathing.” (Bailey & Hoit, 2002, p. 97).

Other authors have also found that respiratory strategies, such as lung volumes utilized for phonation, differ in individuals with and without voice problems, while direct laryngeal factors, such as vocal fold adduction, remain constant between those with and without voice problems (Lowell, et al., 2008). The current study lends support to this theory of a communication “override,” by demonstrating stable R_{law} despite substantial respiratory perturbation. It should also be noted that in both the current study and the one by Lowell and colleagues, healthy participants were involved. It may be that laryngeal and respiratory variables remain constant only up to a certain level of perturbation, as in the case of respiratory disease, which these experimental manipulations did not fully replicate. Future studies will measure lung volume and respiratory kinematic variables that may have been affected by the experimental conditions.

9.1.2 End-tidal Carbon Dioxide

The second aim of the current study was to examine the effects of phonation on $P_{et}CO_2$. Significant main effects of phonation and breathing condition were found, revealing that participants achieved and maintained the $P_{et}CO_2$ target values for each breathing condition, regardless of the presence or absence of phonation. Examination of the non-phonated steady-state $P_{et}CO_2$ means confirmed that participants achieved the desired breathing condition before the initiation of phonation (eupnea *M* = 34.05 mmHg, hypocapnia *M* = 21.05 mmHg, hypercapnia *M* = 50.99 mmHg). The target $P_{et}CO_2$ for the hypercapnic condition was 50mmHg,

which was the overall mean for the participants. The target $P_{et}CO_2$ for the hypocapnic condition was 50% of the baseline +/- 2 mmHg. All but three participants met their individual hypocapnic target $P_{et}CO_2$. These three participants all had lower than average baseline $P_{et}CO_2$ values (33.9 mmHg, 27.5 mmHg, 29.8 mmHg respectively) and lower than average baseline minute volumes (8 L/min, 5.25 L/min, and 7.8 L/min respectively). These values indicated that at baseline these participants were *hypo*ventilating, making extreme experimental hypocapnia less possible for them than for the other 21 participants. However, their hypocapnic $P_{et}CO_2$ values were still within the typical range for hypocapnia and were therefore retained in the final data set.

In all three breathing conditions, the act of phonation resulted in a significant increase in $P_{et}CO_2$. This increase makes physiologic sense. During steady state, the vocal folds are abducted to allow flow of gas into and out of the lungs. During phonation, the vocal folds adduct, causing a momentary slow-down of expiration and retention of gas, leading to an increase in arterial CO_2, as measured by $P_{et}CO_2$. However, the act of phonation was not strong enough to offset effects of the experimental conditions (eupnea, hypocapnia, and hypercapnia), which persisted with distinctly different $P_{et}CO_2$ values even during phonation. This result supports findings from past research, which have shown $P_{et}CO_2$ values to be greater during speaking than during rest breathing under normal breathing conditions (Russell, et al., 1998).

In the current study, a significant interaction between the two variables was found, so that $P_{et}CO_2$ increased more during phonation for the hypocapnic condition, followed by eupneic, and finally, hypercapnic condition. This interaction lends support for the original hypothesis- that phonation would assist in returning the system to homeostatic baseline for the hypocapnic condition. During phonation in hypocapnia, more CO_2 was retained than during phonation during the other two conditions, as would be expected in a physiologic system working towards the goal

of return to normocapnic levels. Contrary to the original hypothesis was the maintenance of R_{law} even in hypercapnia, which prevented the expulsion of CO_2 to return to baseline.

9.1.3 Intensity and fundamental frequency

Intensity, like R_{law} in the current study, remained constant in each of the experimental conditions. This finding lends further support to the critical relevance of tactile feedback for control of phonation in a goal-oriented system. In the present experiment, the ambient noise in the experimental room changed with each condition due to sound generated by delivery of the experimental gases. In addition, participants wore a facemask for all trials, therefore distorting the acoustic output perceived through air conduction. Despite these changes in acoustic environment, no significant difference in intensity was found across the conditions, indicating that participants maintained intensity as they did R_{law}.

Interpretation of the fundamental frequency results is less clear. Fundamental frequency (F_0) was greatest in the hypocapnic condition than the other two conditions. In that condition, both airflow and P_{sub} were increased. The increase in P_{sub} that occurs with vocal fold lengthening makes P_{sub} a secondary mechanism by which F_0 increases (Titze, 1994). Increases in airflow have also been shown to increase F_0, though not systematically (Holmberg, et al., 1988). Phonatory laryngeal airway resistance has also shown to be insensitive to changes in F_0, as was the case in the current study (Leeper & Graves, 1984). The significant increase in P_{sub} and airflow in the hypocapnic condition may be responsible for the increase in F_0 in that condition above eupnea and hypercapnia.

9.1.3.1 Comment on methods

Two aspects of the methods may have influenced results and deserve mention here. First, participants were trained in the voice task the day before the experiment, and a review was conducted immediately prior to the start of the experiment in order to guarantee proper production of the task and to avoid variations in R_{law} due to unfamiliarity (Dastolfo, 2011; Helou & Solomon, 2011). However, this practice may have caused unwanted learning effects. In other words, if participants were too well-trained in the task, they may have worked to produce it as closely to the learned task as possible, not allowing for compensations that might have been naturally triggered as a result of the experimental exposures. That is, the act of practice may have caused the coordination of R_{law} in the specific experimental voice task to stabilize (Kim, et al., 1997; Zanone & Kelso, 1997). In support of this claim, a study of speech breathing changes induced by hypercapnia found that participants maintained "natural" speech despite substantial dyspnea and respiratory kinematic changes caused by the hypercapnia (Hoit, et al., 2007). Participants adhered to these speech goals *despite* their having received no specific instructions to do so. However, in the present study, there was no good option to minimize the potential learning effect. If subjects had not been pre-trained in the phonation task prior to data collection, learning factors could have introduced unwanted effects in the data.

The second methodologic issue that could have influenced results involved the time participants spent phonating in each experimental breathing condition. The phonatory tasks were quite short (no more than 3 seconds per phonated segment of five /pa/ syllables, with breaths allowed between segments). Therefore, the upper airway could have delayed altering laryngeal resistance to satisfy the physiologic goal to return to respiratory homeostasis for the short period of experimental time in phonation. In this sense, the higher level phonatory goal (production of a

trained syllable string) was able, in the short term, to "win" over the basic physiologic goal of maintaining ventilatory homeostasis. However, one argument against time as a limiting factor can be found in the laryngeal resistance in *breathing* literature, which has shown the glottal response to altered CO_2 to occur immediately (England, Bartlett, & Knuth, 1982). However, past research on speech breathing in chemically-induced dyspnea utilized speaking tasks of 7-10 minutes in duration (Hoit, et al., 2007; Hoit & Lohmeier, 2000; Russell, et al., 1998). Participants in those studies, as in the current study, reported subjective complaints of dyspnea. Although the prior studies did not examine laryngeal effects of dyspnea, it remains possible that the time spent in the vocal task in the current study was not sufficiently long to observe the laryngeal effects of hypo- and hypercapnia.

10.0 FUTURE DIRECTIONS

The current study demonstrated that in healthy participants, phonatory laryngeal resistance is maintained despite manipulations of inspired gas concentrations causing significant increases and decreases in expired carbon dioxide (CO_2) levels. The study also showed that phonation causes a consistent and significant increase in CO_2 in expired breaths compared to non-phonated expired breaths. In sum, data from the study are consistent with the proposal that the vocal control system is a regulated system, capable of maintaining normal phonatory laryngeal resistance values despite significant respiratory perturbations – moreover suggesting that such resistance may be a critical control parameter in voice production. Results of the current study also support past literature demonstrating that phonation causes a significant increase in $P_{et}CO_2$. The study validated the safety and efficacy of a 7% CO_2 inhalation challenge for achieving hypercapnia and inducing dyspnea, as well as guided hyperventilation for achieving hypocapnia, in healthy participants.

The apparent stability of phonatory laryngeal resistance despite changes in minute ventilation, and end-tidal PCO_2 invites questions about the perturbability of the phonatory system. Obviously, voice problems occur. They are, in fact, not uncommon. Estimates show that 3-9% of the population experience voice problems at any given point in time (Verdolini & Ramig, 2001). Furthermore, up to 70% of voice clinic caseloads are comprised of voice

problems without obvious structural or neurologic cause (Angsuwarangsee & Morrison, 2002; Roy, 2003, 2010). These voice problems, called Primary Muscle Tension Dysphonia (MTD-1), are often thought to be due to a dyscoordination between breathing and voicing (Hixon & Hoit, 2005; Hixon & Putnam, 1983; Iwarsson, et al., 1998; Morrison, 1997; Morrison & Rammage, 1993). In addition, people with MTD-1 often complain of dyspnea while speaking (Nguyen, et al., 2009). The current study hypothesized that one cause of MTD-1 may be a laryngeal response to respiratory gas fluctuations. Specifically, the hypothesis stated that phonatory laryngeal airway resistance would change in a similar way as non-phonatory (e.g. respiratory) upper airway resistance as a result of hyper- and hypocapnia. This hypothesis was not supported by the results in healthy participants. The question remains then as to what mechanisms facilitate the dyscoordinated breathing in individuals with voice problems (namely, MTD-1), and why individuals with lower airway disease complain of more voice problems than individuals without lower airway disease (Carding, et al., 2006; Cohen, 2010; Schalen, et al., 1992). Some possible options for future research aimed at exploring these issues are examined next.

First, the original hypothesis of the current study ought to be re-tested with some methodologic changes. Phonatory laryngeal airway resistance (R_{law}) may in fact be susceptible to changes in blood gas concentrations *over time*. To that end, one future study would investigate the effects of hyper- and hypocapnia on R_{law} using longer reading passages prior to calculation of resistance, in order to challenge the phonatory mechanism further. Such a study would use methods already vetted in the speech breathing literature (Hoit, et al., 2007; Russell, et al., 1998) coupled with the methods used for achieving hyper- and hypocapnia in the current study.

Second, the experimental conditions in the current study caused changes in minute ventilation (a product of tidal volume and respiratory rate). Namely, the hypocapnic condition

resulted in an average increase in 43 L/min, and the hypercapnic condition caused an increase of 12 L/min compared to the eupneic condition. Lung volume, although not explicitly measured in the current study, and respiratory rate affect vocal fold behavior both during phonation and rest breathing (Bailey & Hoit, 2002; Brancatisano, et al., 1991; Brancatisano, et al., 1983; Hoit, Solomon, & Hixon, 1993; Insalaco, Kuna, Cibella, & Villeponteaux, 1990; Iwarsson, et al., 1998). In addition, laryngeal behavior has shown to impact lower airway function, especially with regard to lung volumes (Hixon & Hoit, 2005; Lowell, et al., 2008; Sapienza & Stathopoulos, 1994). *Despite* the changes to minute ventilation in the current study, R_{law} was unchanged in the experimental conditions compared to baseline. Future studies will examine the changes in specific lung volumes and capacities (e.g., tidal volume, functional residual capacity) during phonation in hypo- and hypercapnic challenges. Studies will also measure respiratory kinematic adaptations to the challenges- namely the contributions of the rib cage and abdomen to the overall breathing pattern. Measurement of these values will further elucidate the adaptations the entire phonatory system makes in response to respiratory perturbations. This knowledge may also shed further light on how respiratory kinematic changes influence voice production and participate in regulation of the phonatory system.

Third, in addition to hypothesized chest wall kinematic pattern changes, laryngeal muscle activation may have changed as a result of the experimental conditions. In accordance with both acoustic and tactile goals of vocal motor control, phonatory stability may have been maintained through adjustments in not only the respiratory system, but the glottal voice source as well (Guenther, 2012). It was hypothesized that participant hyperventilation overrode the expected increase in adductor muscle activity in hypocapnia and abductor activity in hypercapnia in the current study (Bartlett & Knuth, 1984; Savard, et al., 1993). Laryngeal electromyography of the

major ab- and adductor muscles may reveal different muscle activation patterns among the experimental conditions.

Fourth, one major difference between the experimental conditions in the current study and “real life” is that healthy individuals without lung disease were enrolled as participants. It is realistic to hypothesize that presence of lung disease, and the compensations that occur to the respiratory system over time as a result of lung disease, may cause changes to respiratory mechanics, which influence voice, laryngeal function during phonation; or both. For example, in lung disease, an increased reliance on accessory muscles of respiration is observed. This pattern of breathing has shown to impact phonation by contributing to a harsh and strained voice quality, subjective reports of increased vocal effort and phonatory discomfort, elevated laryngeal position in the neck, and alterations in length and depth of inspiration that can impact, along with vocal quality, utterance length, and vocal loudness (Hixon & Hoit, 2005; Hixon & Putnam, 1983; Hoit, et al., 1993; Iwarsson, 2001; Mathieson et al., 2009; Stone, 1993). Future studies will investigate the effects of chronic lung disease on phonation.

Finally, future studies might be specifically designed to test hypotheses about the voice motor control system in terms of tactile or auditory goals. Those studies could provide further depth to the experimental framework for studies on interactions between voice and respiratory functions.

APPENDIX A.

VHI-10 AND RSI QUESTIONNAIRES

Name_____ Date_____

1- TALK	____
2- VHI-10	____
3- RSI	____
4- SVHI-10	____
5- DI	____
6- CSI	____

University of Pittsburgh Voice Center Questionnaire

1. I would rate my degree of talkativeness as the following: (circle number response)

1	2	3	4	5	6	7
Quiet Listener			Average Talker			Extremely Talkative

2. **VHI-10 Instructions:** These are statements that many people have used to describe their voices and the effects of their voices on their lives. Circle the response that indicates how frequently you have the same experience.

My voice makes it difficult for people to hear me.	0	1	2	3	4
People have difficulty understanding me in a noisy room.	0	1	2	3	4
My voice difficulties restrict personal and social life.	0	1	2	3	4
I feel left out of conversations because of my voice.	0	1	2	3	4
My voice problem causes me to lose income.	0	1	2	3	4
I feel as though I have to strain to produce voice.	0	1	2	3	4
The clarity of my voice is unpredictable.	0	1	2	3	4
My voice problem upsets me.	0	1	2	3	4
My voice makes me feel handicapped.	0	1	2	3	4
People ask "What's wrong with your voice?"	0	1	2	3	4

0 = Never
1 = Almost never
2 = Sometimes
3 = Almost always
4 = Always

3. **RSI Instructions:** These are statements that many people have used to describe their voices and the effects of their voices on their lives. Circle the response that indicates how frequently you have the same experience.

Within the last MONTH, how did the following problems affect you?

	0 = No problem					5 = Severe problem
Hoarseness or a problem with your voice	0	1	2	3	4	5
Clearing your throat	0	1	2	3	4	5
Excess throat mucous	0	1	2	3	4	5
Difficulty swallowing food, liquids or pills	0	1	2	3	4	5
Coughing after eating or after lying down	0	1	2	3	4	5
Breathing difficulties or choking episodes	0	1	2	3	4	5
Troublesome or annoying cough	0	1	2	3	4	5
Sensations of something sticking in your throat or a lump in your throat	0	1	2	3	4	5
Heartburn, chest pain, indigestion, or stomach acid coming up	0	1	2	3	4	5

Please check that you have answered all the questions.

Thank you for your time!

APPENDIX B.

CONSENT FORM

CONSENT TO ACT AS A PARTICIPANT IN A RESEARCH STUDY

TITLE: Effects of Hyper- and Hypocapnia on Phonatory Laryngeal Resistance

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Why is this research being done?

The vocal folds are located in the throat and are responsible for the control of gas flow into and out of the lungs for breathing and for voice production. The vocal folds allow more or less air to enter and leave the

lungs to help rid the body of too much carbon dioxide, or allow more oxygen to come into the body. Abnormal levels of carbon dioxide occur in a wide-range of respiratory disorders, psychological disorders, and neuromuscular disorders. The air we exhale also vibrates the vocal folds to produce sound for speech. Breathing abnormalities are blamed for many voice problems, specifically voice problems that affect women more than men. It is unknown how breathing disorders may affect how the vocal folds produce sound, and possibly lead to the development of voice problems. This research is investigating how changes in the levels of carbon dioxide in the body impact how the vocal folds (cords) function during speech in females. Specifically, we are investigating how the vocal folds (cords) move and produce sound when speaking after breathing air containing changing levels of carbon dioxide.

Who is being asked to take part in this research study?

English speaking females between the ages of 18-45, without a history of voice problems (voice disturbance lasting for greater than 2 weeks, or recurring greater than 3 times per year) including any voice treatment; no history of respiratory disorders including asthma, chronic obstructive pulmonary disease (including chronic bronchitis and emphysema), obstructive sleep apnea (OSA); no history of psychological disorders including depression, anxiety, panic disorder; no history of vocal training ("training" is defined as any private study in vocal performance); no history of any medication use that might affect voice or breathing for a 2-week period before the protocol; and not currently pregnant (as confirmed by a urine pregnancy test at the time of screening), non-menopausal, and not a smoker. If you are eligible to participate, you will then have a qualifying exam to see if you can continue to take part in the study. Approximately 24 people will participate in this study.

What procedures will be performed for research purposes?
If you decide to take part in this research study, you will undergo the following procedures.

Screening Procedures:
Procedures to determine if you are eligible to take part in a research study are called "screening procedures." For this research study, screening procedures include answering demographic (name, age, contact information), medical, and voice history questions. All women of child-bearing potential will be given a urine pregnancy test. You will complete two questionnaires about your voice. A recording of your voice will be done and a speech-language pathologist who specializes in voice will judge if your voice sounds normal or not based on the recording. Your hearing will also be tested. A doctor that specializes in ears, noses, and throats will examine your nasal passage and throat with either a flexible scope, which is a lighted optical instrument passed through the nose (flexible scope) to get a deep look inside the body and examine areas such as the throat. Before the flexible scope is passed, about three cc (approximately ½ teaspoon) of a FDA (Food and Drug Administration) approved topical aesthetic (numbing medicine) (4% atomized Cetacaine) will be sprayed into your nose and throat, to increase your comfort. The pictures of your throat will be videotaped and saved. A test of your breathing will also be completed by a speech-language pathologist. For this test, you will breathe into a mouthpiece at your normal rate of breathing, and then you will take two big, deep breaths. These screening procedures will take about 30-45 minutes of your time and will take place at the University of Pittsburgh Voice Center at UPMC Mercy Hospital.

Pre-experimental training:
If you are eligible to continue in the study based on the results of the screening, you will be trained in the voice procedures. You will be trained to make specific sounds while your voice is being recorded. You will produce these sounds during the experiment on the following day.

Experimental Procedures:
The experiment will take place the day following the screening procedures at UPMC Kauffman Building in Oakland.
For all procedures, you will wear a facemask over your nose and mouth. First, we will take breathing measurements as you breathe normally into the mask. This will last approximately 15 minutes. Then, you will breathe in three different air mixtures- one mixture will have greater than usual amounts of carbon dioxide, another mixture has less than usual carbon dioxide, and the third mixture will be normal air. These mixtures may make you breathe faster. You may also feel light-headed or panicky. For each air mixture, you will produce the sounds that you were trained to make during the pre-experimental training. Each breathing trial will last approximately 5 minutes. You will have a minimum of a 15 minute break between each breathing trial.

For experimental testing purposes we will be collecting data on your breathing rate, levels of carbon dioxide and oxygen in the air you exhale, heart rate, pitch of your voice, loudness of your voice, and the air pressure and airflow you generate to create voice.

The entire study will occur on 2 consecutive days and last approximately 3 hours total time.

What are the possible risks, side effects, and discomforts of this research study?
Risks associated with the examination of your throat include discomfort to the nasal passage with the flexible scope and nosebleed (rare). These risks will be minimized with the use of local anesthesia (numbing medicine) as described above. The risks of the numbing medicine include (rare) hypersensitivity, including contact dermatitis characterized by redness and itching. However this occurs most commonly in patients following prolonged self-medication (rare). The risks associated with the breathing test are shortness of breath and lightheadedness (mild-moderate <1%). These risks will be minimized with training prior to the testing procedure, as well as allowing you as much time as needed to rest after the test.

The risks associated with the experimental breathing conditions (breathing in more or less carbon dioxide than usual) are dizziness, headaches, chest tightness, tingling in your fingers and toes, and, panic attack (rare). There is also a risk of vaso-vagal reaction. This reaction is when an individual experiences symptoms such as lightheadedness, nausea, the feeling of being extremely hot (accompanied by sweating), ringing in the ears (tinnitus), uncomfortable feeling in the heart, fuzzy thoughts, a slight inability to speak/form words (sometimes combined with mild stuttering), weakness and visual disturbances such as lights seeming too bright, fuzzy or tunnel vision, and sometimes a feeling of nervousness can occur as well. In rare instances, fainting or loss of consciousness can occur. This risk is rare.
The risks are the same for breathing in more carbon dioxide (hypercapnia) and less carbon dioxide (hypocapnia) but may be more severe for the hypercapnia condition. These risks will be minimized by monitoring your vital signs (heart rate, oxygen saturation). In addition, all procedures are conducted in a medical setting with a physician in the vicinity and access to a medical crash cart. You are also allowed to stop the procedure at any time without any negative consequences. An additional risk of breach of confidentiality also exists. That is, in very rare cases, people not associated with this research study may inadvertently see your identifiable research results. We will do everything in our power to prevent this from happening by keeping all research records in locked files, and identify all videotaping information by a research record number, rather than by your name or social security number. The codebook containing your name and number will be kept secure by the Study Coordinator.

As with any experimental procedure, there may be adverse events or side effects that are currently unknown, and certain of these unknown risks could be permanent, severe or life-threatening.

What are the possible benefits from taking part in this study?
You will not gain any personal benefit from participation in the study. The risks of the screening procedures are no different than those experienced during routine medical examinations by an ear, nose, and throat doctor and lung doctor.

Will my insurance provider or I be charged for the costs of any procedures performed as part of this research study?
You will not be charged for the costs of any of the procedures performed for the purpose of this research study. The costs of these research procedures will be paid for by the study. You will be compensated for parking.

Will I be paid if I take part in this research study?
You will be paid $50 compensation for completing this research study. If you complete the screening procedures and are deemed ineligible, or choose not to participate, you will be compensated $10.

Who will pay if I am injured as a result of taking part in this study?
University of Pittsburgh researchers and their associates who provide services at the University of Pittsburgh Medical Center (UPMC) recognize the importance of your voluntary participation in their research studies. These individuals and their staffs will make reasonable efforts to minimize, control, and treat any injuries that may arise as a result of this research. If you believe that you are injured as a result of the research procedures being performed, please contact immediately the Principal Investigator or one of the co-investigators listed on the first page of this form.

Emergency medical treatment for injuries solely and directly related to your participation in this research study will be provided to you by the hospitals of the UPMC. It is possible that the UPMC may bill your insurance provider for the costs of this emergency treatment, but none of these costs will be charged directly to you. If your research-related injury requires medical care beyond this emergency treatment, you will be responsible for the costs of this follow-up care unless otherwise specifically stated below. There is no plan for monetary compensation. You do not, however, waive any legal rights by signing this form.

Who will know about my participation in this research study?
Any information about you obtained from this research will be kept as confidential (private) as possible. All records related to your involvement in this research study will be stored in a locked file cabinet. Your identity on these records will be indicated by a code rather than by your name, and the information linking these codes with your identity will be kept separate from the research records. You will not be identified by name in any publication of the research results unless you sign a separate consent form giving your permission (release).

Who will have access to identifiable information related to my participation in this research study?
In addition to the investigators listed on the first page of this authorization (consent) form and their research staff, the following individuals will or may have access to identifiable information related to your participation in this research study:

Authorized representatives of the University of Pittsburgh Research Conduct and Compliance Office may review your identifiable research information for the purpose of monitoring the appropriate conduct of this research study.

In unusual cases, the investigators may be required to release identifiable information related to your participation in this research study in response to an order from a court of law. If the investigators learn that you or someone with whom you are involved is in serious danger or potential harm, they will need to inform, as required by Pennsylvania law, the appropriate agencies.

For how long will the investigators be permitted to use and disclose identifiable information related to my participation in this research study?
It is a University of Pittsburgh policy that all research records be maintained following final reporting or publication of a project, although records can be kept indefinitely. The investigators may continue to use and disclose, for the purposes described above, identifiable information related to your participation in this research study for a minimum of 7 years and for as long (indefinite) as it may take to complete this research study.

Is my participation in this research study voluntary?
Your participation in this research study is completely voluntary. Whether or not you provide your consent for participation in this research study will have no effect on your current or future relationship with the University of Pittsburgh. Whether or not you provide your consent for participation in this research study will have no effect on your current or future medical care at a UPMC hospital or affiliated health care provider or your current or future relationship with a health care insurance provider.

May I withdraw, at a future date, my consent for participation in this research study?
You may withdraw, at any time, your consent for participation in this research study, to include the use and disclosure of your identifiable information for the purposes described above. Any identifiable research information recorded for, or resulting from, your participation in this research study prior to the date that you formally withdrew your consent may continue to be used and disclosed by the investigators for the purposes described above.

To formally withdraw your consent for participation in this research study you should provide a written and dated notice of this decision to the principal investigator of this research study at the address listed on the first page of this form.

Your decision to withdraw your consent for participation in this research study will have no effect on your current or future relationship with the University of Pittsburgh. Your decision to withdraw your consent for participation in this research study will have no effect on your current or future medical care at a UPMC hospital or affiliated health care provider or your current or future relationship with a health care insurance provider.

May I be withdrawn from the study for any reason?
If you are unable to complete any of the procedures required for participation in the study, you may be withdrawn. If you experience an adverse event to the experimental procedures, you will be withdrawn from study participation.

VOLUNTARY CONSENT

All of the above has been explained to me and all of my current questions have been answered. I understand that I am encouraged to ask questions about any aspect of this research study during the course of this study, and that such future questions will be answered by the researchers listed on the first page of this form.

Any questions, which I have about my rights as a research participant, will be answered by the Human Subject Protection Advocate of the IRB Office, University of Pittsburgh (1-866-212-2668).

By signing this form, I agree to participate in this research study. A copy of this consent form will be given to me.

_____ _____

Printed Name of Participant Date

_____

Participant's Signature

CERTIFICATION of INFORMED CONSENT

I certify that I have explained the nature and purpose of this research study to the above-named individual(s), and I have discussed the potential benefits and possible risks of study participation. Any questions the individual(s) have about this study have been answered, and we will always be available to address future questions as they arise. I further certify that no research component of this protocol was begun until after this consent form was signed.

_____ _____

Printed Name of Person Obtaining Consent Role in Research Study

_____ _____

Signature of Person Obtaining Consent Date

APPENDIX C.

EMAIL/PHONE SCRIPT FOR ELIGIBILITY

Hello!
Thank you for your interest in participating in our study, *The Effects of Hyper- and Hypocapnia on Phonatory Laryngeal Resistance*. In this study we will investigate the effects of different types of gas concentrations on voice. In the study, participants will be asked to breathe different gas mixtures, and then produce different sounds. The study will take place on 2 consecutive days at 2 locations- UPMC Mercy Hospital in downtown Pittsburgh, and UPMC Kauffman Building in Oakland. The study will take approximately 3 hours of total time. You may be compensated up to $50 for your participation.

Let me tell you a bit about the study procedures.

First, as part of the study, participants will undergo a screening. This screening will first involve you answering a series of questions here today. If you are still eligible to participate based on your answers, we will schedule an in-clinic screening. That screening will take place at the UPMC Voice Center located in UPMC Mercy Hospital in downtown Pittsburgh. At that screening, a specialized ear, nose, and throat physician will assess if you tolerate passage of a flexible endoscope through your nose in order to visualize your vocal folds. You will also undergo a breathing test to make sure your lungs work normally.

If you are still interested in participating, let's begin with the eligibility questions.

1. Are you female between the ages of 18-45?
2. Have you ever had a voice problem that lasted for longer than 2 weeks, or recurred more than 3 times in one year?
3. Have you ever had any voice therapy?
4. Have you ever had a respiratory disorder including asthma, COPD, emphysema, or obstructive sleep apnea?
5. Have you ever had any vocal training consisting of greater than one year of private study in vocal performance?
6. Do you take any medications which may affect voice?
7. Are you pregnant?

If ineligible: Thank you very much for your time in answering our questions. Unfortunately, you are not eligible to participate in this study.

If eligible: Thank you very much for your time in answering our questions. Based on your answers, you are eligible to participate in this study! Now we need to schedule your in-clinic screening at UPMC Mercy Hospital. If you pass that screening, you will go on to complete the experiment at the UPMC Kauffman Building in Oakland. May we also schedule that appointment now?

APPENDIX D

SCREENING FORM

Subject ID:_____ Date:_____
Consent:_____

Self-Report Questions:
Age 18-45?_____ History of Respiratory Disorder?_____
Smoker?:_____ History of voice training?_____
History of Psychological Disorder?_____ History of a voice problem?_____
English Speaking?_____

Pregnancy test: _____

Hearing Screen: 30dB @: 500 Hz:_____ 1000 Hz:_____
1500 Hz:_____ 1500 Hz:_____

Cape-V score <20:_____

Laryngeal Exam: _____

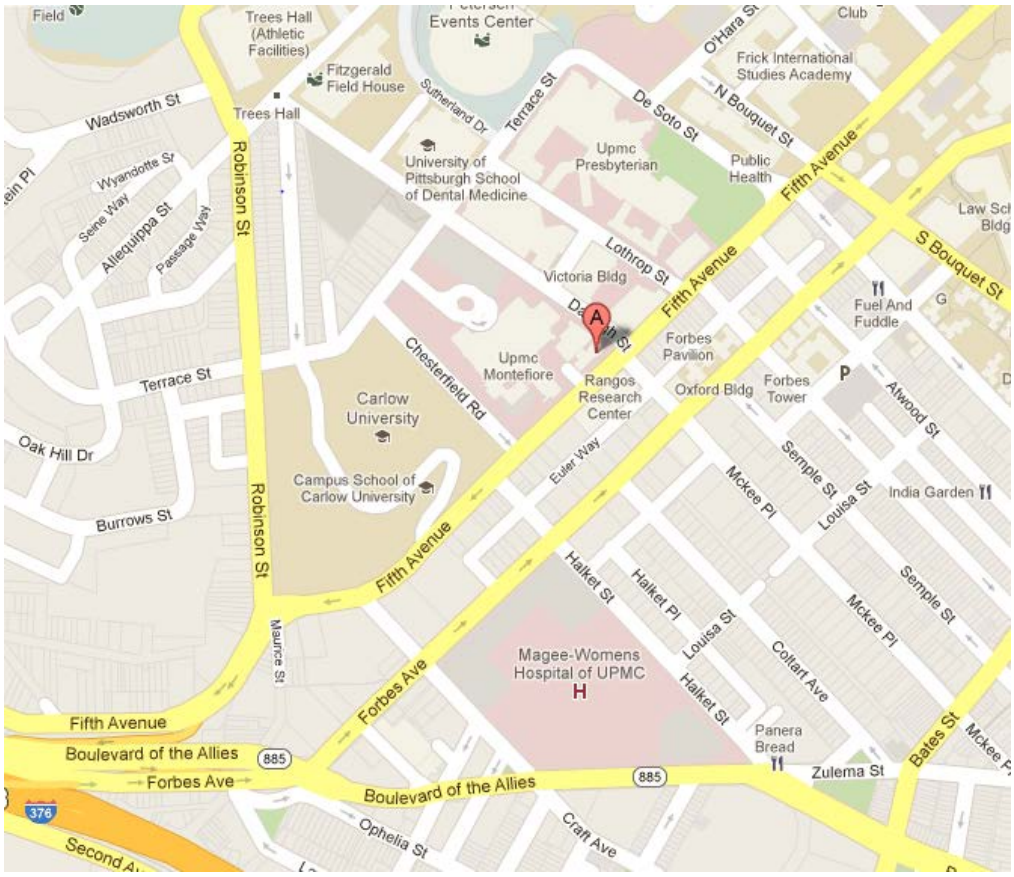
Pulmonary function test: _____

Eligible? _____

Experimental Date: _____

APPENDIX E

SUBJECT INFORMATION FORM



Map of Oakland indicating the Kauffman Building- 3471 5th Ave.
Please go to room 1211 (COPD/Emphysema Research Lab)
Please wear a loose fitting t-shirt so we may attach the heart-rate monitor to your sides.
Please do not wear earrings or fingernail polish.
Time:_____ Date:_____

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