Research Article

The Effects of Hyper- and Hypocapnia on Phonatory Laryngeal Airway Resistance in Women

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Purpose: The larynx has a dual role in the regulation of gas flow into and out of the lungs while also establishing resistance required for vocal fold vibration. This study assessed reciprocal relations between phonatory functions—specifically, phonatory laryngeal airway resistance (R_{law})—and respiratory homeostasis during states of ventilatory gas perturbations.

Method: Twenty-four healthy women performed phonatory tasks while exposed to induced hypercapnia (high CO_2), hypocapnia (low CO_2), and normal breathing (eupnea). Effects of gas perturbations on R_{law} were investigated as were the reciprocal effects of R_{law} modulations on respiratory homeostasis.

Results: R_{law} remained stable despite manipulations of inspired gas concentrations. In contrast, end-tidal CO_2 levels increased significantly during all phonatory tasks. Thus, for the conditions tested, R_{law} did not adjust to accommodate ventilatory needs as predicted. Rather, stable R_{law} was spontaneously accomplished at the cost of those needs.

Conclusions: Findings provide support for a theory of regulation wherein R_{law} may be a control parameter in phonation. Results also provide insight into the influence of phonation on respiration. The work sets the foundation for future studies on laryngeal function during phonation in individuals with lower airway disease and other patient populations.

The larynx, as part of the upper airway, has a challenging dual role in the simultaneous regulation of gas flow into and out of the lungs for respiration as well as in the maintenance of upper airway resistance via vocal fold adduction for phonation. During nonphonatory ventilation (gas exchange, i.e., O_2 intake and CO_2 release; Hixon & Hoit, 2005), the larynx has been shown to alter its resistance to airflow to release or retain CO_2 in a way that leads to O_2/CO_2 homeostasis. In phonation, the larynx plays a different role. It generates varying resistances to modulate pulmonary airflows to produce voice (Titze, 1994). However, details are lacking about the role of the larynx in the simultaneous regulation of ventilatory homeostasis and

phonation. To be specific, information is lacking about how the larynx balances its resistance requirements for phonation when those requirements are at odds with those needed for ventilation. Further, it is unclear how upper airway resistance changes during phonation may affect ventilation itself. The purpose of the present study was to address these questions at a preliminary level.

Role of the Larynx in Respiration

Much is known about the role of the larynx in respiration. The larynx is an important factor in the determination of respiratory resistance, airflow volume, and breathing rate. Specific to respiratory resistance, the larynx is responsible for 25%–60% of overall resistance (England, Bartlett, & Daubenspeck, 1982; Levitzky, 1995; Savard, Cole, Miljeteig, & Haight, 1993). During inspiration for tidal (normal) breathing, glottal opening is wide, offering low resistance. For tidal expiration, the vocal folds move slightly toward midline, and resistance marginally increases compared to inspiration (England, Bartlett, & Daubenspeck, 1982).

In conditions of abnormal variations in levels of arterial CO_2 , such as hypercapnia (CO_2 levels above 45 mmHg) and hypocapnia (CO_2 levels below 35 mmHg), the larynx

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changes its resistance to airflow, facilitating intake of O₂ and release of CO₂ in a way that favors a return to ventilatory homeostasis (see Table 1). In hypercapnic conditions, laryngeal resistance during breathing decreases in both inspiratory and expiratory phases of the cycle, presumably as an adaptation to the hypercapnic perturbation (Bartlett, 1979; Brancatisano, Dodd, & Engel, 1991; England & Bartlett, 1982). In contrast, during hypocapnia an increase in laryngeal resistance has been observed during inspiration and expiration (Bartlett, 1979; Kuna, McCarthy, & Smickley, 1993). This latter mechanism is presumed to assist in CO₂ retention and maintenance of alveolar inflation. However, hyperventilation, with and without hypocapnia, has the opposite effect by decreasing laryngeal resistance (Bartlett & Knuth, 1984; Savard et al., 1993). In that situation, the laryngeal response to hyperventilation is thought to override the laryngeal response to decreasing CO₂. Despite changing resistance as a function of level of CO₂, inspiratory resistance is consistently lower than expiratory resistance across healthy individuals and those with respiratory disease (Bartlett, 1979; Savard et al., 1993).

Laryngeal Airway Resistance in Phonation

The larynx is clearly central to phonation. As noted, during phonation, the vocal folds oscillate to create varying resistance to pulmonary airflow, thereby modulating it into systematic air columns that are the acoustic foundation of voice. In contrast to qualitative visual measures used to estimate laryngeal resistance in the respiration physiology literature, for voice, phonatory laryngeal airway resistance (R_{law}) is quantitatively assessed as the ratio of estimated subglottic pressure in cmH₂O (P_{sub}) to laryngeal airflow in L/s (Smitheran & Hixon, 1981).

Although phonatory laryngeal resistance has been used to reflect laryngeal functions, (Holmberg, Hillman, & Perkell, 1988; Smitheran & Hixon, 1981), careful interpretation is needed because the R_{law} ratio can be affected by numerous extralaryngeal factors, such as pressure changes due to muscular actions of the chest wall and pulmonary recoil as well. R_{law} is also influenced by changes in vocal intensity but is fairly insensitive to fundamental frequency changes (Leeper & Graves, 1984). It has demonstrated within-subject stability over repeated trials even in conditions of auditory masking (Grillo & Verdolini, 2007; Leeper & Graves, 1984). However, it is unknown how this stability in phonatory laryngeal airway resistance is affected by chemical ventilatory perturbations that are known to affect nonphonatory laryngeal resistance during breathing.

Goals of the Current Study

As stated in the preceding paragraphs, the larynx has indisputably critical functions for both respiration and phonation. For respiration, glottal aperture changes mediate retention or expulsion of respiratory gases. For phonation, the glottal aperture narrows to provide resistance to airflow to achieve consistent vocal fold vibration. The reciprocal relations between the laryngeal functions for breathing and phonation have been only minimally investigated. For example, lung volume and laryngeal resistance appear to be inversely related in that phonatory laryngeal airway resistance is reactive to lung volume changes (Iwarsson, Thomasson, & Sundberg, 1998). Further, the laryngeal and respiratory systems have also been shown to issue active responses to oral/upper airway perturbations (Huber & Stathopoulos, 2003). Last, and related to the current investigation, end-tidal carbon dioxide ($P_{et}CO_2$) increases as a function of phonation (Hoit & Lohmeier, 2000). Although it is clear that the phonatory and respiratory systems are intertwined for both voicing and breathing, it is unknown if phonatory resistance goals are sacrificed in conditions of respiratory disruption when the laryngeal response to lower airway perturbations is vital to ventilatory homeostasis.

The current study expands on well-established knowledge about the role of the larynx in homeostatic regulation of blood-gas concentrations. To be specific, decreased laryngeal resistance in response to hypercapnia and increased resistance in hypocapnia have been demonstrated in the respiratory literature (Bartlett, 1979; Brancatisano et al., 1991; England & Bartlett, 1982; Kuna, McCarthy, & Smickley, 1993) but have not yet been explored during phonation. It is unknown if phonation in hyper- and hypocapnia would change these laryngeal resistance patterns observed during nonphonated breathing. The primary theoretical hypothesis pursued in this study was that when challenged to produce voice under conditions of varying CO₂ levels, phonatory laryngeal airway resistance would be sacrificed in favor of respiratory resistance mechanisms to return the respiratory system to physiologic homeostatic baseline. Specific subhypotheses were as follows: First, experimentally induced hyper- and hypocapnia would both result in increased glottal airflow during phonation. However, for hypercapnia this increase would be accompanied by a decrease in estimated subglottal pressure mediated by reduced vocal fold adduction, resulting in expulsion of excess CO₂ and decreased R_{law} . For hypocapnia, the hypothesis was that increased airflow would be accompanied by increased estimated subglottal pressure and vocal fold adduction, favoring retention of CO₂ and thus increased

Table 1. Upper airway response to changing blood-gas concentrations

Condition	Respiratory rate response	Laryngeal/upper airway response
Hypercapnia	Increased ventilation	Decreased laryngeal resistance
Hypocapnia	Decreased ventilation	Increased laryngeal resistance
Homeostasis	Eupnea	Low laryngeal resistance

R_{law} . In other words, ventilatory needs should “win” over phonatory needs when a competition is introduced. The importance of the study was that it examined the effects of respiratory condition on phonatory physiology and attempted to identify the larynx’s varying functions at the intersection of respiration and communication.

Method

Participants

All study procedures were approved by the University of Pittsburgh Institutional Review Board. Healthy women, 18–45 years of age, were recruited from the Pittsburgh metropolitan region. Power analysis determined that, using a repeated-measures design with an α of .05, a sample size of 20 participants would be necessary to achieve 80% statistical power for the primary research question, “Do hyper- and hypocapnia affect phonatory laryngeal resistance?” However, because we utilized counterbalancing across three conditions, the total number of participants required to complete the experiment had to be a multiple of six. Therefore, we targeted a total of 24 participants to complete the experimental procedures.

Inclusion/exclusion criteria were as follows: Requirements that were based on self-report were negative history of voice problems (voice disturbance lasting for greater than 2 weeks or recurring more than three times during the preceding year) or history of any prior voice treatment; negative history of respiratory disorders, including asthma, chronic obstructive pulmonary disease, emphysema, and sleep apnea; negative history of psychological disorders, including depression, anxiety, and 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. Requirements that were based on clinical assessment were English comprehension and hearing sufficient for the participant to provide fully informed consent and follow study instructions and speech production sufficient to produce the target phoneme /pa/. Requirements that were based on instrumental assessment were not pregnant (on the basis of results of a urine pregnancy test), normal vocal quality as judged by Consensus Auditory Perceptual Evaluation of Voice (Kempster, Gerratt, Verdolini Abbott, Barkmeier-Kraemer, & Hillman, 2009) score independently judged by a rater not otherwise involved in the study (a PhD student licensed speech-language pathologist who specialized in voice), normal hearing as determined by hearing screening (30 dB at 500, 1000, 1500, and 2000 Hz bilaterally), no self-perceived voice problem as determined by the patient self-report instrument Voice Handicap Index-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 score < 13 (Belafsky, Postma, & Koufman, 2002), normal larynx as judged independently by the principal investigator (PI; first author) and a fellowship-trained laryngologist on the basis of rigid

or flexible endoscopy, and 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).

Design

The primary focus of the study—whether phonatory laryngeal airway resistance (R_{law}) varies as a function of respiratory condition—involved a within-subject repeated-measures design. The independent variable was experimental respiratory condition (eupnea, hypocapnia, and hypercapnia). The primary dependent variable was R_{law} (estimated P_{sub} in cmH₂O/glottal airflow in L/s). Additional dependent variables were component parameters of R_{law} (estimated P_{sub} and glottal airflow), which were analyzed separately. The secondary focus—whether glottal adduction during phonation significantly alters arterial CO₂ levels (estimated via measurement of gas concentrations in expired breaths, $P_{et}CO_2$)—used data from the same within-subject experiment. For this question, the design was a 3 × 2 within-subject design. Independent variables were experimental condition (eupnea, hypocapnia, and hypercapnia) and phonation (yes/no). The dependent variable was end-tidal CO₂ ($P_{et}CO_2$).

Procedures and Equipment

Screening and Initial Training

After eligibility criteria were satisfied (see Participants section), participants were trained in data collection procedures that would be used for the subsequent experiment, specifically for the collection of R_{law} measures. To limit 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 phonatory task utilized the PAS6600 (KayPENTAX, Montvale, NJ), a clinical system for phonatory aerodynamic assessment, low-pass filtered with a nominal cutoff of 10,025 Hz and a sampling rate of 22,050 samples per second. Participants were trained to take a breath in and produce a string of five consonant–vowel syllables (/pa pa pa pa pa/) on one breath at a rate of 1.5 syllables/s (90 beats per minute, guided by a metronome) at the participant’s comfortable pitch and loudness level (Smitheran & Hixon, 1981). Care was taken to assure the participant fit the face mask snugly over the nose and mouth during task production and that the pressure tube sat lightly on top of the tongue during speech production. During training, the morphology of pressure peaks and airflow plateaus was inspected visually for each syllable. Morphology was considered acceptable if Pressure Peaks 2–4 were not pointy or jagged and flow minima corresponded with pressure maxima on the basis of visual inspection (Helou & Solomon, 2011). Training ceased when the participant produced the consonant–vowel string with Peaks 2–4—which would be used for data analysis—showing acceptable morphology as verified by the PI on the basis of output from the PAS6600

(Helou & Solomon, 2011; Holmberg et al., 1988). The participant was then dismissed and asked to return the following day for the experiment proper.

Equipment and Experimental Setup

Participants were seated comfortably in a standard desk chair. Baseline heart rate and O_2 saturation were measured using a five-lead ECG and transcutaneous O_2 saturation monitor (Model Sirecust 732, Siemens Medical Systems, Inc., Danvers, MA; Model 504-USP, Criticare Systems, Inc., Waukesha, WI), respectively. Each participant's nose and mouth were fit snugly into a standard anesthesia face mask (KayPENTAX), connected to the PAS6600. The face mask was secured around the participant's head with an elastic band. The mask was visually inspected by the PI to verify a leak-free seal on the face. The PAS6600 system was connected to a one-way Hans-Rudolph valve (Hans Rudolph, Inc., Shawnee, KS), which allowed participants to inspire a given concentration and volume of air and expire into the atmosphere to prevent rebreathing 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 sensor lines connected into a Viasys Data Acquisition System (SensorMedics/Viasys Corp., Yorba Linda, CA), which provided real-time breath-by-breath analyses of respiratory data. 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 (21.00% O_2 , 0.05% CO_2 , 78.00% nitrogen) or CO_2 -enriched air (7% CO_2 , 21% O_2 , balanced nitrogen). A rotameter connected to the cylinders allowed the examiner to select the appropriate cylinder and control the flow rate of gases from that cylinder to the participant (see Figure 1). The examiner, blinded to the 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. All three conditions utilized air from one of the two cylinders. During the steady-state portions of the eupneic, hypocapnic, and hypercapnic trials, that is, 30 s prior to the onset of phonation, $P_{et}CO_2$, minute ventilation (V_E), respiratory rate (RR), heart rate (HR), inspiratory time (Ti), expiratory time (Te), and transcutaneous O_2 saturation values were 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 the R_{law} task) was later analyzed for statistically significant differences. Fundamental frequency and vocal intensity were captured by the PAS6600 microphone, 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 face mask. Because the microphone is calibrated internally by the manufacturer (KayPENTAX) to represent 15 cm from the speaker's mouth, it had to be recalibrated to account for any recorded dB SPL changes in its new position. Per the manufacturer's direction (S. Crump, personal communication, June 12, 2012), a 200-Hz pure tone was generated via standard computer speakers against the face mask. 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.

Experimental Procedure

The experimental day took place 1 day following screening and training for the protocol. Participants first completed respiratory baseline measurements and then produced the trained R_{law} voice task in three experimental conditions:

Figure 1. Schematic of experimental setup.

eupnea, hypercapnia, and hypocapnia, which were counter-balanced and randomly presented via a computer-based random number generator to control for order effects. Each experimental exposure lasted approximately 5 min, with a 15-min break between conditions to allow respiratory variables to return to baseline (Gorman et al., 1994; Papp et al., 1997). Total time for completion of the experiment was about 65 min. Table 2 outlines the experimental procedures. The baseline and intervention procedures are described in detail next.

Baseline. A respiratory baseline was obtained for each participant. For baseline data collection, participants sat with the face mask in place as previously described and breathed room air. Baseline tidal volume (V_t), V_E , T_i , T_e , RR , $P_{et}CO_2$, HR , and O_2 data were collected and analyzed on a breath-by-breath basis for 5 min of tidal breathing. Sixty-second averages were calculated by the computer separately for the third, fourth, and fifth minute of baseline breath-by-breath values of $P_{et}CO_2$, RR , and V_t , and those three values were averaged to provide one baseline value for each variable of interest. Baseline $P_{et}CO_2$ and minute volume values were used to determine the target hypocapnic range for each participant, described shortly.

Eupnea. For the eupneic condition, participants breathed room air through the cylinder for 5 min, metered in at participants' baseline minute volume, and produced the five-syllable R_{law} task five times. As for all experimental conditions, a 15-min break followed, during which time participants removed the face mask and breathed normally.

Hypercapnia. For the hypercapnic condition, the 7% CO_2 gas was delivered via a 60-L Douglas bag (Vacumetrics, Inc., Ventura, CA). Participants first breathed room air, and the CO_2 gas mixture was switched to the inspiratory limb of the breathing circuit (Antony, Brown, & Barlow, 1997; Gorman et al., 1994; Hoit, Lansing, & Perona, 2007; Papp et al., 1997). The PI monitored the breath-by-breath analyses to determine when participants had reached the

hypercapnic state ($P_{et}CO_2$ 50 mmHg $\pm$ 2 mmHg). Once participants had achieved that state for 30 s (steady state), they were instructed to perform the five-syllable R_{law} task five times. The hypercapnic gas was delivered continuously during the trial. Another 15-min break of breathing room air followed, with the final 5 min of rest recorded to ensure return to baseline of biologic variables (HR , O_2 , CO_2 , RR).

Hypocapnia. For the hypocapnic condition, the examiner metered room air to the participants at a rate double the participants' resting respiratory minute volume as determined during baseline procedures via the cylinder containing room air. Participants were instructed to visually monitor the rubber balloon collection bag positioned between the face mask 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 5-L increments until $P_{et}CO_2$ reached 50% $\pm$ 2 mmHg of the baseline value. This procedure resulted in spontaneous hyperventilation for each participant as previously verified in the pulmonary literature (Antony et al., 1997; Rapee, Brown, Antony, & Barlow, 1992; Zvolensky & Eifert, 2001). Breath-by-breath analysis was monitored to determine when participants had become hypocapnic. Once the participant's $P_{et}CO_2$ was 50% $\pm$ 2 mmHg of baseline for 30 s (steady state), the participant was instructed to perform the R_{law} task five times as previously trained. Again, a 15-min break followed.

Blinding

All experimental conditions required the participant to breathe gas from a cylinder through a face mask. Participants were not informed which gas concentration they were given during the experiment. However, each condition produced a change in breathing pattern, so participants were aware the conditions were different. All R_{law} analyses were completed post hoc by a PhD candidate speech-language pathologist who specialized in voice with substantial experience analyzing aerodynamic data and who was blinded to participants' condition.

Data Reduction

Aerodynamic data were analyzed to obtain estimated P_{sub} , airflow, and their ratio, R_{law} . For these calculations, first the middle three pressure peaks generated during the /p/ and corresponding flow signal during the voiced /a/ from each five-syllable /pa/ string were manually selected by placing a cursor in the predetermined location in the syllable string and examined for acceptable morphology via methods similar to those well vetted in the phonatory aerodynamic literature (Holmberg et al., 1988; Smitheran & Hixon, 1981). The middle three tokens (Pressure Peaks 2, 3, and 4) were selected as default for analysis unless morphology of the pressure peaks and airflow plateaus were deemed unacceptable, in which case the best three of the five syllables in the string were analyzed. Of note, a buildup of pressure from an increase in dead space due to additional connective tubing beyond the end of the pneumotach prevented

Table 2. Template for experimental procedures

Condition	Activity	Duration (min)
Baseline	Participant performs normal, tidal breathing, no phonation	5
Eupnea	Participant breathes room air Phonation task	5 (approx.)
Rest	Rest	15
Hypocapnia	Participant breathes room air metered from an H-cylinder starting at 2 $\times$ the participant's resting respiratory rate Phonation task	5 (approx.)
Rest	Rest	15
Hypercapnia	Participant breathes CO_2 -enriched air metered from an H-cylinder Phonation task	5 (approx.)
Rest	Rest	15

Note. The order of breathing conditions was counterbalanced and randomized across participants.

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 setup prohibited the PI from monitoring sample requirements during actual collection. Fundamental frequency and intensity data were analyzed by the PAS6600 software for the same three selected segments of each syllable train used for pressure and flow analysis.

As noted (see Equipment and Experimental Setup section), $P_{et}CO_2$ values were recorded via breath-by-breath analysis during the periods of interest, that is, during the 30-s nonphonated 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 by a program in the Viasys software system over the time of interest, and the averaged values were used for analyses. Ten percent of data were reanalyzed by the first author, who was blinded to condition, for evaluation of reliability.

Statistical Analyses

All statistical analyses were conducted using SPSS, Version 19.0. For the first question, a within-subject analysis of variance (ANOVA) was conducted with experimental condition (eupnea, hypocapnia, and hypercapnia) as the independent variables and phonatory laryngeal airway resistance (estimated P_{sub} in cmH_2O /glottal airflow in L/s) as the dependent variable. Secondary analyses of P_{sub} , airflow, and minute ventilation were also performed with a within-subject ANOVA for each independent variable. Phonatory intensity and fundamental frequency were also analyzed with a one-way within-subject ANOVA to determine the effects of respiratory perturbations on these variables. For the second question, a two-way within-subject ANOVA was performed on the data, and the independent variables were experimental condition (eupnea, hypocapnia, and hypercapnia) and phonation (yes/no), and the dependent variable was $P_{et}CO_2$. Interaction effects were tested first. If an interaction was found, the main effect of each experimental condition was investigated in each level of phonation and vice versa. If a significant main effect of condition was found, post hoc comparisons using Bonferroni adjustment were performed if needed. Last, R_{law} reliability was assessed by the interclass correlation coefficient.

Results

Participants

Fifty-seven potential participants were screened. Figure 2 details the participant flowchart from screening through study completion. Twenty-four participants satisfied all of the inclusion/exclusion criteria. All of those individuals completed the study. R_{law} data from two participants were excluded from final R_{law} analyses because of task violations detailed shortly. In addition, phonatory intensity and fundamental frequency data were available for only 36/72 possible tokens due to presumed factors, including microphone

repositioning and noise produced by the experimental gas cylinders. Demographic and baseline inclusion information for participants was as follows: The mean age was 24.6 years with a range of 19–45 years. Sixty-two percent of participants were White, 13% were Black/African American, and 25% were Asian. The mean Consensus Auditory Perceptual Evaluation of Voice score for all participants was 0.75 with a range from 0 to 3. The mean Voice Handicap Index-10 score was 1.08 with a range of 0 to 6, and the mean Reflux Severity Index score was 2.13 with a range of 0 to 10. These results indicate that all participants met inclusion criteria with regard to having normal voice quality, no self-perceived voice problem, and no voice complaints due to laryngopharyngeal reflux.

Phonatory Laryngeal Airway Resistance

The first hypothesis was that the larynx would sacrifice its phonatory resistance (R_{law}) mechanisms in favor of respiratory resistance mechanisms, tending to return the system to physiologic homeostatic baseline under conditions of ventilatory perturbation. R_{law} data for 22/24 participants were available for final analyses. Two participants' data were excluded because of 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, data from other conditions were not usable, and all data for the participants in question were excluded from final analysis.

To be descriptive, R_{law} was greater in the hypocapnic condition than the hypercapnic condition in 12/22 participants (55%). The opposite result, showing greater R_{law} in hyper- as opposed to hypocapnia, occurred in 10/22 participants (45%).

As to the statistics, the main effect of breathing condition was not significant for R_{law} , $F(2, 42) = 0.274$, $p = .762$, partial $\eta^2 = .013$. Means, standard deviations, and ranges for R_{law} in each breathing condition are displayed in Table 3. Interrater reliability of R_{law} data showed excellent correlation between raters ($r = .988$).

The mean R_{law} values observed in these data for each breathing condition were well within 1 SD of R_{law} values for normal speakers under normal breathing conditions (Zraick, Smith-Olinde, & Shotts, 2012). Moreover, the failure to confirm the experimental hypothesis (R_{law} values would be lowest in hypercapnia and greatest in hypocapnia) was not attributable to large variability in the data or poor statistical power: Mean values were not positioned in the anticipated order, even descriptively. Figure 3 displays the relatively stable mean value of R_{law} across conditions throughout the range of $P_{et}CO_2$ values. One extreme outlier was identified in the data set for each breathing condition; however, the results of the statistical analysis did not change with removal of these values. Therefore, to show completeness of the data set, these values remain in the final analysis.

Figure 2. Participant flowchart. VHI = Voice Handicap Index-10, RSI = Reflux Symptom Index, PFT = pulmonary function test, R_{law} = phonatory laryngeal airway resistance, $P_{et}CO_2$ = end-tidal carbon dioxide.

To further explore the results for R_{law} , the component parts of the R_{law} ratio—mean translaryngeal airflow (airflow) and mean estimated subglottal pressure (P_{sub})—were analyzed for differences across the breathing conditions. It is interesting to note results showed that although R_{law} did not change detectably across conditions, its component

parameters clearly did, showing that respiratory perturbations indeed influenced phonatory aerodynamics. Data for airflow values for each breathing condition are displayed in Table 3.

The main effect of breathing condition on airflow values was significant, $F(2, 42) = 5.225, p = .009$, partial $\eta^2 = .199$. Pairwise comparisons revealed that airflow values were significantly higher in the hypocapnic condition than in the hypercapnic condition ($p = .021$). None of the other comparisons achieved significance.

Data for P_{sub} as a function of breathing condition are shown in Table 3. Again, a significant difference 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 ($M = 10.62$ cmH₂O, $SE = .641$) than the eupneic ($M = 7.65$ cmH₂O, $SE = .429$) and the hypercapnic ($M = 7.97$, $SE = .397$) conditions. None of the other comparisons achieved significance.

Minute Ventilation

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. In a similar manner, the hypercapnic condition

Table 3. R_{law} , airflow, P_{sub} , and V_E values for each experimental condition

Measure	Eupnea	Hypocapnia	Hypercapnia
R_{law}			
M	50.27	54.77	52.31
SD	28.43	25.66	30.67
Range	12.17–139.20	24.87–125.42	17.76–168.29
Airflow (L/s)			
M	0.182	0.213	0.176
SD	0.076	0.067	0.058
Range	0.05–0.39	0.11–0.35	0.07–0.26
P_{sub} (cmH ₂ O)			
M	7.65	10.62	7.97
SD	2.01	3	1.86
Range	2.80–10.97	5.72–15.25	4.44–11.78
V_E M (L/min)			
M	12.26	23.92	55.88
SD	9.93	10.77	12.93

Figure 3. $P_{et}CO_2$ (mmHg) as a function of R_{law} (cmH₂O/L/s). Vertical lines separate the three conditions: hypocapnia, eupnea, and hypercapnia.

caused an increase in minute ventilation as a response to elevating levels of CO₂. On average, participants increased their minute ventilation by 12 L/min in the hypercapnic over the eupneic condition.

A within-subjects ANOVA revealed a significant difference in minute ventilation across breathing conditions, $F(2, 46) = 133.34$, $p < .001$, partial $\eta^2 = .853$. The assumption of normality was not met for the eupneic condition; therefore, all conditions were subjected to nonparametric testing with the Friedman test for significance as well as the parametric testing with ANOVA. The Friedman test also detected a significant difference in V_E among breathing conditions: $\chi^2(2) = 40.583$, $p < .001$. Because of the equivalent results between the parametric and nonparametric tests, only results of the parametric tests are discussed here. Pairwise comparisons showed that 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).

End-Tidal CO₂

$P_{et}CO_2$ 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. Means and standard deviations for all conditions are shown in Table 4.

The interaction of breathing condition and phonation groups was significant, $F(2, 46) = 8.165$, $p = .001$, partial $\eta^2 = .262$. In order to find the pattern of differences on $P_{et}CO_2$ values across breathing condition and phonation separately, the simple 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 $\eta^2 = .942$. 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 nonphonated steady state was also significant, $F(2, 46) = 1456.583$, $p < .001$, partial $\eta^2 = .984$. Again, pairwise comparisons using Bonferroni adjustment revealed significantly greater $P_{et}CO_2$ during nonphonated 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.

To assess 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 $\eta^2 = .400$. In a similar manner, the simple main effect of phonation during hypocapnia was also significant, $F(1, 23) = 65.081$, $p < .001$, partial $\eta^2 = .739$. Last, 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 nonphonated steady state in each of the breathing conditions (see Figure 4). The interaction between phonation and

Table 4. Means, standard deviations, and ranges of $P_{et}CO_2$ values (mmHg) for steady-state and phonated portions of each breathing condition

Condition	M	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

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

Fundamental Frequency

A within-subject ANOVA was performed on F_0 values as a function of breathing condition (eupnea, hypocapnia, hypercapnia). A significant difference in F_0 values across breathing conditions was found, $F(2, 22) = 17.365$, $p < .001$, partial $\eta^2 = .612$. 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 ($p = .076$).

Intensity

A one-way within-subject ANOVA on dB SPL in each breathing condition was performed. No significant difference in vocal intensity in dB SPL values among breathing conditions was found, $F(2, 22) = 3.367$, $p = .053$, partial $\eta^2 = .234$.

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 in the

Figure 5. $P_{et}CO_2$ (mmHg) as a function of breathing condition (interaction shown).

breathing conditions were found for R_{law} ($p = .268$), the primary variable of interest.

Discussion

We hypothesized that, when confronted with conflicting phonatory and respiratory needs, phonatory R_{law} would fluctuate in a direction that favors ventilatory regulation, sacrificing its role in phonatory aerodynamics.

Figure 4. $P_{et}CO_2$ values (mmHg) for each observation during steady-state respiration and phonation.

However, counter to expectations, no evidence whatsoever was found for such vulnerability in R_{law} , which remained remarkably stable across substantial perturbations to ventilation. Moreover, average R_{law} remained within the range of normal values for healthy speakers across all conditions. Separate analyses of airflow and estimated subglottic pressure (P_{sub}) data, component parameters of R_{law} , revealed that these parameters were modulated by ventilatory condition. Airflow values changed as a result of experimental condition, and P_{sub} values appeared to respond to the changes in airflow in a way that maintained R_{law} . The potential significance of constant R_{law} values across conditions are discussed shortly. However, first the behavior of its component parts, phonatory airflow and P_{sub} , as well as the findings for acoustic data, are explained.

The increase in airflow in both hyper- and hypocapnic conditions compared to eupnea was not surprising. The hypercapnic condition caused an increase in minute ventilation (respiratory rate $\times$ volume) on average 12 L/min over the eupneic condition in the range of those reported elsewhere for speech breathing in hypercapnia (Bailey & Hoit, 2002). On the other hand, the hypocapnic condition required participants to hyperventilate (increase respiratory rate above metabolic demands), causing an average 43 L/min increase in minute ventilation in order to expire enough CO_2 to achieve and maintain hypocapnia. Therefore, both experimental conditions, hypercapnia and hypocapnia, required or resulted in hyperventilation.

Hyperventilation and increased minute ventilation in hyper- and hypocapnia over eupnea may have affected the R_{law} results. In the broader respiratory literature, non-phonatory laryngeal resistance during breathing is affected by hyperventilation and changes in lung volume in at least three ways. First, as ventilation rate increases, laryngeal resistance during breathing decreases (England & Bartlett, 1982; England, Bartlett, & Daubenspeck, 1982; Kuna, Insalaco, Villeponteaux, Vanoye, & Smickley, 1993; Shindoh, Sekizawa, Hida, Sasaki, & Takishima, 1985). Second, as lung volume increases, laryngeal resistance during breathing decreases (Hoit et al., 2007; Shindoh et al., 1985). Hypercapnia can cause an increase in functional residual capacity over eupnea, thereby increasing lung volume and decreasing laryngeal resistance during breathing (England, Bartlett, & Knuth, 1982; Iwarsson et al., 1998; Lowell, Barkmeier-Kraemer, Hoit, & Story, 2008; Savard et al., 1993). Last, 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). Hyperventilation in both the hyper- and hypocapnic conditions in the present study may have effectively eliminated the hypothesized effects of the CO_2 manipulations and counteracted the expected R_{law} responses to the conditions (Bartlett & Knuth, 1984; England, Ho, & Zamel, 1985; Savard et al., 1993).

Despite stability in R_{law} , an increase in F_0 was observed in the hypocapnic condition compared to the other two conditions. In hypocapnia, both airflow and P_{sub} were

also greater than in eupnea. This F_0 change is relatively straightforward to explain as increased P_{sub} is a secondary mechanism for F_0 increase (Titze, 1994). Increases in airflow have also been shown to increase F_0 , although not systematically (Bielamowicz et al., 1993; Holmberg et al., 1988). R_{law} has been shown to be insensitive to changes in F_0 (Leeper & Graves, 1984) as was the case in the current study.

Unlike F_0 but similar to R_{law} , intensity remained constant in each of the experimental conditions, although subjects received no instructions regarding either intensity or F_0 . 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 face mask 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} without clear auditory feedback during the trials. Increasing lung volume is one mechanism by which vocal intensity increases (Huber, 2007, 2008). However, increasing lung volume in the current study was not met with increasing intensity. These data may provide support for evidence that points toward the phonatory system as one that is regulated to maintain an acoustic goal (Guenther, Hampson, & Johnson, 1998). Further evidence of system regulation in phonation is discussed next.

Phonation as a Regulated System

The results explained thus far can perhaps be interpreted within a framework of regulated systems. A biological system is considered physiologically regulated if it detects perturbations and makes adaptive changes to achieve inherent system goals (Brobeck, 1965; Warren, Dalston, Morr, Hairfield, & Smith, 1989). The respiratory system is one example of a regulated system (Brobeck, 1965; Levitzky, 1995). It detects perturbations in ventilatory homeostasis, such as abnormal blood-gas levels, and issues corrective responses, such as changing respiratory rate, airflow volumes, and upper airway resistance (Levitzky, 1995). Such regulation was demonstrated in the current study with observations of increasing airflow in response to hypercapnia. To date, phonation has not been understood as an aerodynamically regulated system, although a related system, speech, has been.

Research on upper airway perturbations of the speech 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, Rochet, & Dalston, 1989; Warren, Rochet, Dalston, & Mayo, 1992). Such pressures are generally maintained with changes in articulatory strategy (Zajac, 1995), sometimes at the expense of articulatory precision and intelligibility as well as through increased respiratory effort (airflow) or changes in constrictions elsewhere in the upper vocal tract (Hammond, Warren, Mayo, & Zajac, 1999; Kim, Zajac, Warren, Mayo, & Essick, 1997;

Warren, Dalston, et al., 1989; Warren et al., 1992). The suggestion has been that the speech system makes coordinated changes in an attempt to maintain “aerodynamic integrity” in the face of perturbations (Warren, Dalston, et al., 1989, p. 566). Across studies, an increase in airflow has 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 intraoral pressure for consonant production (Hammond et al., 1999; Kim et al., 1997; Warren et al., 1992).

Regarding regulation in phonation, past studies have suggested a pressure-regulation goal for voicing. To be specific, assuming a stable SPL, the lungs are viewed as providing a constant pressure source to the vocal tract, and the larynx adjusts resistance with changes in laryngeally mediated P_{sub} (Nasri et al., 1994; Zhang, Neubauer, & Berry, 2006). Although the lower airway can alter resistance with increasing muscular actions of the chest wall and lung elastic recoil, the larynx, via vocal fold adduction, provides greater resistance than what can be achieved by the lower airways alone (Nasri et al., 1994). In this view, the role of the larynx may be to adjust resistance in order to maintain relatively constant pressure from the lungs through the vocal tract (Finnegan, Luschei, & Hoffman, 2000). Similar evidence exists that the speech mechanism aims to meet mechanosensory goals even at the expense of an acoustic goal, during communication (Tremblay, Shiller, & Ostry, 2003). In the current study, however, airflow—and, by extension, pulmonary pressures—varied in response to the experimental conditions, and P_{sub} appeared reactive to these changes in a way that maintained constant R_{law} .

To be specific, in the present study, R_{law} appeared resistant to respiratory and ventilatory threats. The post hoc hypothesis that emerges is that R_{law} may represent an inherent control parameter in phonation. The constancy in R_{law} may be explained via Ohm’s law, which states that as flow increases, resistance decreases. Perhaps the increase in flow observed as a result of the interventions was such that the resistance drop would have been too great to continue to support phonation without a matched increase in pressure. Therefore, at least temporarily, the phonatory goal was able to “win” over the respiratory goal. As such, phonation would involve a physiologically regulated system as occurs for other biologic systems. Of note, within a motor-equivalence framework, in the present study respiratory kinematic changes may have occurred as an adaptation to the experimental conditions in a way that favored the consistency of phonatory resistance (Guenther et al., 1998; Huber, Stathopoulos, & Sussman, 2004). Although conceptually consistent with prior literature to this effect, the present study was not designed to assess this possibility. Future studies can be planned to pursue it explicitly.

End-Tidal Carbon Dioxide

The second aim of the current study was to examine the effects of phonation on P_{etCO_2} . Significant main effects of phonation and breathing condition were found, revealing

that participants achieved and maintained the P_{etCO_2} target values for each breathing condition regardless of the presence or absence of phonation. Examination of the non-phonated steady-state P_{etCO_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_{etCO_2} for the hypercapnic condition was 50 mmHg, which was the overall mean for the participants. The target P_{etCO_2} for the hypocapnic condition was 50% of the baseline ± 2 mmHg.

In all three breathing conditions, the act of phonation resulted in a significant increase in P_{etCO_2} . This increase makes physiologic sense. During steady state, the vocal folds are abducted to allow the flow of gas into and out of the lungs. During phonation, the vocal folds adduct, causing a momentary slowdown of expiration and retention of gas, leading to an increase in arterial CO_2 , as measured by P_{etCO_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_{etCO_2} values even during phonation. This result supports findings from past research, which have shown P_{etCO_2} values to be greater during speaking than during rest breathing under normal breathing conditions (Russell, Cerny, & Stathopoulos, 1998).

In the current study, a significant interaction between phonation and experimental gas condition was found, such that P_{etCO_2} increased more during phonation for the hypocapnic condition, followed by the eupneic and, finally, the hypercapnic conditions. This interaction lends support for the original hypothesis: that phonation would assist in returning the system to homeostatic baseline for the hypocapnic condition. However, this return to baseline was unsuccessful, and thus, at least temporarily, phonatory goals of the respiratory system appeared to “win” over ventilatory goals. During phonation in hypocapnia, more CO_2 was retained than during phonation in the other two conditions as would be expected in a physiologic system working toward 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 for return to baseline.

Theoretical Discussion of Voice Problems With Presumed Respiratory Origins

The current study did not investigate regulatory phenomena in individuals with voice problems. However, some speculative discussion about the possible relevance of results in these populations is warranted. To be specific, suggestions have been made that one common voice problem, primary muscle tension dysphonia (MTD-1), may be associated with abnormal coordinative patterns not only at the laryngeal level but also across respiratory and laryngeal subsystems of phonation (Hixon & Putnam, 1983; Morrison & Rammage, 1993; Rubin, Macdonald, & Blake, 2010). MTD-1 is defined as a voice disturbance in the absence of

known structural or neurologic abnormalities (Roy, 2003). Aerodynamic studies of people with MTD-1 have revealed a variety of profiles, including greater-than-normal P_{sub} and phonatory airflow rates as well as normal P_{sub} with low airflow (Gillespie, Gartner-Schmidt, Rubinstein, & Verdolini Abbott, 2013; Hillman, Holmberg, Perkell, Walsh, & Vaughan, 1989). The variety of aerodynamic profiles for individuals with MTD-1 indicates that, at least conceptually, multiple mechanisms of respiratory and laryngeal dysfunction may be involved in this condition (Gillespie et al., 2013). On the basis of the results of the current investigation, it could be that some individuals with MTD-1 have a dysregulated voice motor control system, unable to adapt to internal perturbation forces, such as lower airway disease, however temporary these perturbations may be. For example, comorbidity of dysphonia and pulmonary disease is not uncommon (Cohen, 2010; England et al., 1985; Hackenberg, Hacki, Hagen, & Kleinsasser, 2010; Stanton, Sellars, Mackenzie, McConnachie, & Bucknall, 2009), and an estimated 25%–50% of people with pulmonary disease also experience voice problems (Cohen, 2010; Hone et al., 1996; Lavy, Wood, Rubin, & Harries, 2000). MTD-1 commonly occurs following upper respiratory infection (Roy, 2003). It remains unknown whether the phonatory system is able to remain regulated in instances of acute or chronic lower airway compromise.

Limitations and Cautions in the Interpretation of the Data

Some aspects of the method may have inadvertently influenced results. First, participants were trained in the voice task the day before the experiment, and a review of task procedures was conducted immediately prior to the start of the experiment proper (Dastolfo, 2011; Helou & Solomon, 2011). However, this practice may have influenced the results. 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. Thus, the act of practice may have contributed to the stabilization of R_{law} across conditions (Kim et al., 1997; Zandone & Kelso, 1997). In support of this claim, a separate 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). In the present study as well as the study by 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 stability of a learned behavior. If subjects had not been pretrained in the phonation task prior to data collection, learning effects during experimental procedures could have affected the data equally or more.

The second methodological issue regards the time participants spent phonating in each experimental breathing

condition. The phonatory tasks were brief (no more than 3 s 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 time spent in phonation. In this sense, the higher-level phonatory goal (production of a trained syllable string) was able, in the short term, to temporarily “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 the breathing literature, which has shown the glottal response to altered CO_2 to occur immediately upon exposure (England, Bartlett, & Knuth, 1982). In contrast, past research on speech breathing in chemically induced dyspnea has utilized speaking tasks of 7–10 min 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 adjustments to conditions of hypo- and hypercapnia.

Third, as addressed in the first part of the Discussion, both hyper- and hypocapnia caused hyperventilation, which may have an influence on laryngeal airway resistance. As expected, hyperventilation was caused by hypercapnia. In addition, induced hyperventilation was necessary to achieve hypocapnia in the healthy participants; therefore, this method was unavoidable. However, it should be noted that hyperventilation in both conditions is a limitation potentially influencing the results.

Last, the stability of R_{law} in the current study, despite substantial shifts in both P_{sub} and airflow, indicate that it is critical to examine the changes not only in R_{law} but also its component parts— P_{sub} and airflow—to fully appreciate the multiple regulatory mechanisms at play in phonation. Perhaps when the goal is to determine system compensations to perturbations, including pathology affecting voice, it is more appropriate to assess pressure and airflow variations independently than as a ratio as in R_{law} . Changes in R_{law} do not indicate only a laryngeal effect. These changes can be mediated by lower airway resistance changes through mechanisms of chest wall muscular force and lung elastic recoil to a point, after which changes observed must be attributed to laryngeal function. Still, caution is called for when interpreting R_{law} as a measure solely of primary laryngeal function.

Conclusions

The current study demonstrated that within healthy participants, R_{law} is maintained at constant values, despite manipulations of inspired gas concentrations causing substantial increases and decreases in expired CO_2 levels, at least for brief exposures. The study also showed that phonation can cause a significant increase in CO_2 in expired

breaths compared to nonphonated expired breaths. In summary, data from the study are consistent with the proposal that phonation belongs to 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, at least for brief intervals. Unknown are effects of chronic respiratory impairments on laryngeal function. Further, the results of the current study are also consistent with past literature demonstrating that phonation causes a significant increase in P_{aCO_2} . Last, 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.

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References

- Antony, M. M., Brown, T. A., & Barlow, D. H. (1997). Response to hyperventilation and 5.5% CO_2 inhalation of subjects with types of specific phobia, panic disorder, or no mental disorder. *American Journal of Psychiatry*, 154, 1089–1095.
- Arffa, R. E., Krishna, P., Gartner-Schmidt, J., & Rosen, C. A. (2012). Normative values for the Voice Handicap Index-10. *Journal of Voice*, 26, 462–465. doi:10.1016/j.jvoice.2011.04.006
- Bailey, E. F., & Hoit, J. D. (2002). Speaking and breathing in high respiratory drive. *Journal of Speech, Language, and Hearing Research*, 45, 89–99. doi:10.1044/1092-4388(2002/007)
- Bartlett, D., Jr. (1979). Effects of hypercapnia and hypoxia on laryngeal resistance to airflow. *Respiratory Physiology*, 37, 293–302.
- Bartlett, D., Jr., & Knuth, S. L. (1984). Human vocal cord movements during voluntary hyperventilation. *Respiratory Physiology*, 58, 289–294.
- Belafsky, P. C., Postma, G. N., & Koufman, J. A. (2002). Validity and reliability of the Reflux Symptom Index (RSI). *Journal of Voice*, 16, 274–277.
- Bielamowicz, S., Berke, G. S., Kreiman, J., Sercarz, J. A., Green, D. C., & Gerratt, B. R. (1993). Effect of tension, stiffness, and airflow on laryngeal resistance in the in vivo canine model. *Annals of Otology, Rhinology & Laryngology*, 102, 761–768.
- Brancatisano, A., Dodd, D. S., & Engel, L. A. (1991). Posterior cricoarytenoid activity and glottic size during hyperpnea in humans. *Journal of Applied Physiology*, 71, 977–982.
- Brobeck, J. R. (1965). Exchange, control, and regulation. In W. S. Yamamoto & J. R. Brobeck (Eds.), *Physiological controls and regulations* (pp. 1–13). Philadelphia, PA: Saunders.
- Cohen, S. M. (2010). Self-reported impact of dysphonia in a primary care population: An epidemiological study. *The Laryngoscope*, 120, 2022–2032. doi:10.1002/lary.21058
- Dastolfo, C. A. (2011). *Effects of repetition on phonation threshold pressure task performance* (Unpublished master's thesis). University of Pittsburgh, Pittsburgh, PA.
- England, S. J., & Bartlett, D., Jr. (1982). Changes in respiratory movements of the human vocal cords during hyperpnea. *Journal of Applied Physiology*, 52, 780–785.
- England, S. J., Bartlett, D., Jr., & Daubenspeck, J. A. (1982). Influence of human vocal cord movements on airflow and resistance during eupnea. *Journal of Applied Physiology*, 52, 773–779.
- England, S. J., Bartlett, D., Jr., & Knuth, S. L. (1982). Comparison of human vocal cord movements during isocapnic hypoxia and hypercapnia. *Journal of Applied Physiology*, 53, 81–86.
- England, S. J., Ho, V., & Zamel, N. (1985). Laryngeal constriction in normal humans during experimentally induced bronchoconstriction. *Journal of Applied Physiology*, 58, 352–356.
- Finnegan, E. M., Luschei, E. S., & Hoffman, H. T. (2000). Modulations in respiratory and laryngeal activity associated with changes in vocal intensity during speech. *Journal of Speech, Language, and Hearing Research*, 43, 934–950.
- Gillespie, A. I., Gartner-Schmidt, J. L., Rubinstein, E., & Verdolini Abbott, K. (2013). Aerodynamic profiles of females with muscle tension dysphonia. *Journal of Speech, Language, and Hearing Research*, 56, 481–488.
- Gorman, J. M., Papp, L. A., Coplan, J. D., Martinez, J. M., Lennon, S., Goetz, R. R., ... Klein, D. F. (1994). Anxiogenic effects of CO_2 and hyperventilation in patients with panic disorder. *American Journal of Psychiatry*, 151, 547–553.
- Grillo, E. U., & Verdolini, K. (2007). Evidence for distinguishing pressed, normal, resonant, and breathy voice qualities by laryngeal resistance and vocal efficiency in vocally trained subjects. *Journal of Voice*. Advance online publication. doi:10.1016/j.jvoice.2006.12.008
- Guenther, F. H., Hampson, M., & Johnson, D. (1998). A theoretical investigation of reference frames for the planning of speech movements. *Psychology Review*, 105, 611–633.
- Hackenberg, S., Hacki, T., Hagen, R., & Kleinsasser, N. H. (2010). Voice disorders in asthma. *Laryngorhinootologie*, 89, 460–464. doi:10.1055/s-0030-1249694
- Hammond, C. S., Warren, D. W., Mayo, R., & Zajac, D. (1999). Respiratory responses to sudden pressure venting during stop consonant production. *Folia Phoniatrica et Logopaedica*, 51(6), 250–260.
- Helou, L. B., & Solomon, N. P. (2011). *Workshop: Data done right: Avoiding common mistakes in aerodynamics*. Paper presented at the Voice Foundation Symposium, Philadelphia, PA.
- Hillman, E., Holmberg, E. B., Perkell, J. S., Walsh, M., & Vaughan, C. (1989). Objective assessment of vocal hyperfunction: An experimental framework and initial results. *Journal of Speech and Hearing Disorders*, 32, 373–392.
- Hixon, T. J., & Hoit, J. D. (2005). *Evaluation and management of speech breathing disorders: Principles and methods*. Tucson, AZ: Reddington Brown.
- Hixon, T. J., & Putnam, A. (1983). Voice disorders in relation to respiratory kinematics. *Seminars in Speech and Language*, 4, 217–231.
- Hoit, J. D., Lansing, R. W., & Perona, K. E. (2007). Speaking-related dyspnea in healthy adults. *Journal of Speech, Language, and Hearing Research*, 50, 361–374. doi:10.1044/1092-4388(2007/026)
- Hoit, J. D., & Lohmeier, H. L. (2000). Influence of continuous speaking on ventilation. *Journal of Speech, Language, and Hearing Research*, 43, 1240–1251.

- Holmberg, E. B., Hillman, R. E., & Perkell, J. S. (1988). Glottal airflow and transglottal air pressure measurements for male and female speakers in soft, normal, and loud voice. *The Journal of the Acoustical Society of America*, 84, 511–529.
- Hone, S. W., Donnelly, M. J., Robertson, J., Coakley, R., O'Neill, S., & Walsh, M. J. (1996). Dysphonia and inhalation of corticoids: A prospective study. *Revue de Laryngologie, Otologie, Rhinologie*, 117, 331–333.
- Huber, J. E. (2007). Effect of cues to increase sound pressure level on respiratory kinematic patterns during connected speech. *Journal of Speech, Language, and Hearing Research*, 50, 621–634. doi:10.1044/1092-4388(2007/044)
- Huber, J. E. (2008). Effects of utterance length and vocal loudness on speech breathing in older adults. *Respiratory Physiology & Neurobiology*, 164, 323–330.
- Huber, J. E., & Stathopoulos, E. T. (2003). Respiratory and laryngeal responses to an oral air pressure bleed during speech. *Journal of Speech, Language, and Hearing Research*, 46, 1207–1220.
- Huber, J. E., Stathopoulos, E. T., & Sussman, J. E. (2004). The control of aerodynamics, acoustics, and perceptual characteristics during speech production. *The Journal of the Acoustical Society of America*, 116(4, Pt. 1), 2345–2353.
- Iwarsson, J., Thomasson, M., & Sundberg, J. (1998). Effects of lung volume on the glottal voice source. *Journal of Voice*, 12, 424–433.
- Kempster, G. B., Gerratt, B. R., Verdolini Abbott, K., Barkmeier-Kraemer, J., & Hillman, R. E. (2009). Consensus auditory-perceptual evaluation of voice: Development of a standardized clinical protocol. *American Journal of Speech-Language Pathology*, 18, 124–132. doi:10.1044/1058-0360(2008/08-0017)
- Kim, J. R., Zajac, D. J., Warren, D. W., Mayo, R., & Essick, G. K. (1997). The response to sudden change in vocal tract resistance during stop consonant production. *Journal of Speech, Language, and Hearing Research*, 40, 848–857.
- Kuna, S. T., Insalaco, G., Villeponteaux, D. R., Vanoye, C. R., & Smickley, J. S. (1993). Effect of hypercapnia and hypoxia on arytenoid muscle activity in normal adult humans. *Journal of Applied Physiology*, 75, 1781–1789.
- Kuna, S. T., McCarthy, M. P., & Smickley, J. S. (1993). Laryngeal response to passively induced hypocapnia during NREM sleep in normal adult humans. *Journal of Applied Physiology*, 75, 1088–1096.
- Lavy, J. A., Wood, G., Rubin, J. S., & Harries, M. (2000). Dysphonia associated with inhaled steroids. *Journal of Voice*, 14, 581–588.
- Leeper, H. A., Jr., & Graves, D. K. (1984). Consistency of laryngeal airway resistance in adult women. *Journal of Communication Disorders*, 17, 153–163.
- Levitzky, M. G. (1995). *Pulmonary physiology* (4th ed.). McGraw-Hill.
- Lowell, S. Y., Barkmeier-Kraemer, J. M., Hoit, J. D., & Story, B. H. (2008). Respiratory and laryngeal function during spontaneous speaking in teachers with voice disorders. *Journal of Speech, Language, and Hearing Research*, 51, 333–349. doi:10.1044/1092-4388(2008/025)
- Miller, M. R., Hankinson, J., Brusasco, V., Burgos, F., Casaburi, R., Coates, A., ... Wanger, J. (2005). Standardisation of spirometry. *European Respiratory Journal*, 26, 319–338. doi:10.1044/1092-4388(2008/025)
- Morrison, M., & Rammage, L. (1993). Muscle misuse voice disorders: Description and classification. *Acta Oto-Laryngologica*, 113, 428–434.
- Nasri, S., Namazie, A., Kreiman, J., Sercarz, J. A., Gerratt, B. R., & Berke, G. S. (1994). A pressure-regulated model of normal and pathologic phonation. *Otolaryngology—Head & Neck Surgery*, 111, 807–815.
- Papp, L. A., Martinez, J. M., Klein, D. F., Coplan, J. D., Norman, R. G., Cole, R., ... Gorman, J. M. (1997). Respiratory psychophysiology of panic disorder: Three respiratory challenges in 98 subjects. *American Journal of Psychiatry*, 154, 1557–1565.
- Rapee, R. M., Brown, T. A., Antony, M. M., & Barlow, D. H. (1992). Response to hyperventilation and inhalation of 5.5% carbon dioxide-enriched air across the DSM-III-R anxiety disorders. *Journal of Abnormal Psychology*, 101, 538–552.
- Rosen, C. A., Lee, A. S., Osborne, J., Zullo, T., & Murry, T. (2004). Development and validation of the Voice Handicap Index-10. *The Laryngoscope*, 114, 1549–1556.
- Roy, N. (2003). Functional dysphonia. *Current Opinion in Otolaryngology & Head and Neck Surgery*, 11, 144–148.
- Rubin, J. S., Macdonald, I., & Blake, E. (2010). The putative involvement of the transabdominal muscles in dysphonia: A preliminary study and thoughts. *Journal of Voice*, 25, 218–222. doi:10.1016/j.jvoice.2009.09.001
- Russell, B. A., Cerny, F. J., & Stathopoulos, E. T. (1998). Effects of varied vocal intensity on ventilation and energy expenditure in women and men. *Journal of Speech, Language, and Hearing Research*, 41, 239–248.
- Savard, P., Cole, P., Miljeteig, H., & Haight, J. S. (1993). Laryngeal resistance to respiratory airflow in humans. *The Laryngoscope*, 103, 785–792. doi:10.1288/00005537-199307000-00012
- Shindoh, C., Sekizawa, K., Hida, W., Sasaki, H., & Takishima, T. (1985). Upper airway response during bronchoprovocation and asthma attack. *American Review of Respiratory Disorders*, 132, 671–678.
- Smitheran, J. R., & Hixon, T. J. (1981). A clinical method for estimating laryngeal airway resistance during vowel production. *Journal of Speech and Hearing Disorders*, 46, 138–146.
- Stanton, A. E., Sellars, C., Mackenzie, K., McConnachie, A., & Bucknall, C. E. (2009). Perceived vocal morbidity in a problem asthma clinic. *The Journal of Laryngology & Otolaryngology*, 123, 96–102. doi:10.1017/s002221510800323x
- Titze, I. R. (1994). *Principles of voice production*. Englewood Cliffs, NJ: Prentice Hall.
- Tremblay, S., Shiller, D. M., & Ostry, D. J. (2003, June 19). Somatosensory basis of speech production. *Nature*, 423, 866–869. doi:10.1038/nature01710
- Warren, D. W., Dalston, R. M., Morr, K. E., Hairfield, W. M., & Smith, L. R. (1989). The speech regulating system: Temporal and aerodynamic responses to velopharyngeal inadequacy. *Journal of Speech and Hearing Disorders*, 32, 566–575.
- Warren, D. W., Morr, K. E., Rochet, A. P., & Dalston, R. M. (1989). Respiratory response to a decrease in velopharyngeal resistance. *The Journal of the Acoustical Society of America*, 86, 917–924.
- Warren, D. W., Rochet, A. P., Dalston, R. M., & Mayo, R. (1992). Controlling changes in vocal tract resistance. *The Journal of the Acoustical Society of America*, 91, 2947–2953.
- Zajac, D. J. (1995). Laryngeal airway resistance in children with cleft palate and adequate velopharyngeal function. *Cleft Palate Craniofacial Journal*, 32, 138–144. doi:10.1597/1545-1569(1995)032<0138:lariw>2.3.co;2
- Zanone, P. G., & Kelso, J. A. (1997). Coordination dynamics of learning and transfer: Collective and component levels. *Journal*

---

of *Experimental Psychology: Human Perception and Performance*, 23, 1454–1480.

Zhang, Z., Neubauer, J., & Berry, D. A. (2006). The influence of subglottal acoustics on laboratory models of phonation. *The Journal of the Acoustical Society of America*, 120, 1558–1569.

Zraick, R. I., Smith-Olinde, L., & Shotts, L. L. (2012). Adult normative data for the KayPENTAX Phonatory Aerodynamic

System Model 6600. *Journal of Voice*, 26, 164–176. doi:10.1016/j.jvoice.2011.01.006

Zvolensky, M. J., & Eifert, G. H. (2001). A review of psychological factors/processes affecting anxious responding during voluntary hyperventilation and inhalations of carbon dioxide-enriched air. *Clinical Psychological Review*, 21, 375–400. doi:10.1016/S0272-7358(99)00053-7