Nebulized Isotonic Saline Versus Water Following a Laryngeal Desiccation Challenge in Classically Trained Sopranos

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Purpose: To examine the effects of nebulized isotonic saline (IS) versus sterile water (SW) on self-perceived phonatory effort (PPE) and phonation threshold pressure (PTP) following a surface laryngeal dehydration challenge in classically trained sopranos.
Method: In a double-blind, within-subject crossover design, 34 sopranos breathed dry air (relative humidity < 1%) transorally for 15 min and then nebulized 3 mL of IS or SW, or experienced a no-treatment control condition over 3 consecutive weeks. PPE and PTP were measured every 15 min from baseline through 2 hr postdesiccation.
Results: PPE increased significantly following the laryngeal desiccation challenge in all 3 treatment conditions ($p < .01$). After nebulization, PPE returned to baseline for the IS condition only. For the SW and control conditions, PPE remained above baseline during the 2 hr after desiccation. No statistically significant changes in PTP following laryngeal desiccation were observed, although values for the IS condition remained below baseline for nearly 2 hr after nebulization. PPE and PTP were not significantly correlated.
Conclusions: Following a laryngeal surface dehydration challenge, classically trained sopranos reported increased vocal effort that persisted for at least 2 hr. Compared with SW, nebulized IS showed promise as an effective way to remediate the adverse, self-perceived effects of laryngeal desiccation.

KEY WORDS: isotonic saline, laryngeal desiccation, singers, hydration, voice production

Singers are considered vocal athletes who place rigorous standards and heavy demands on their voices (Broaddus-Lawrence, Treole, McCabe, Allen, & Toppin, 2000; Edwin, 1995; Kitch & Oates, 1994; LeBorgne & Dal Vera, 2009). They rely on optimal vocal health to promote peak performance. Singers believe hydration is essential to efficient voice production (Armbrust, 2001; Behlau & Oliveira, 2009; Fisher, Ligon, Sobecks, & Roxe, 2001; Franca, 2006; Gregg, 1995; Henry, 2009; Timmermans, Vanderwegen, & De Bodt, 2005). Popular remedies to improve the singing voice, at least conceptually, often involve a hydration component (e.g., drinking herbal tea or lemon juice, use of throat sprays, steam inhalation; Verdolini-Marston, Sandage, & Titze, 1994). Similarly, clinical advice is frequently offered to maximize hydration either by increasing fluid consumption or by manipulating ambient humidity levels in the environment. Yet no studies have examined the effects of drinking more water or increasing environmental humidity on classical singing. Singers are often considered to be particularly at risk for vocal health issues—including dehydration—because of a variety of factors, such as frequent airplane travel, erratic schedules, changing performance venues, fluctuating environmental humidity levels, voice demands in the presence of vocal fatigue or illness, and laryngopharyngeal reflux, to name a few (David, 1996; Phyland, Oates, & Greenwood, 1999; Sapir, Mathers-Schmidt, & Larson, 1996; Webb, 2007; Welham & Maclagan, 2004). Thus, singers represent one of the principal populations of interest in studying the effects of dehydration and hydration treatments on the voice.

Two primary biological mechanisms are believed to be responsible for mediating vocal fold hydration and the laryngeal water environment. The first, systemic hydration, involves the maintenance of normal cell volume levels through the body's homeostatic regulation of extracellular fluids and is believed to affect voice production if inadequately managed (Fisher, Ligon, Sobecks, & Roxe, 2001; Fisher, Telser, Phillips, & Yeates, 2001). The second mechanism, surface tissue hydration of the vocal fold mucosa and airway epithelia, is accomplished through ion transport mechanisms that govern transepithelial water fluxes, thereby maintaining the liquid layer required for laryngeal lubrication and efficient vocal fold oscillation (Leydon, Sivasankar, Falciglia, Atkins, & Fisher, 2009). Both of these biological mechanisms are thought to sustain vocal fold vibratory efficiency and voice quality, although the specific processes responsible for their combined influence are not fully understood. Previous research involving hydration and the voice has used aeromechanical, acoustic, auditory–perceptual, and participant-based vocal effort measures to examine the presumed influence of dehydration and hydration treatments on voice production and vocal fold physiology (Chan & Tayama, 2002; Erickson & Sivasankar, 2010; Fisher, Ligon, et al., 2001; Fisher, Telser, et al., 2001; Hemler, Wieneke, & Dejonckere, 1997; Hemler, Wieneke, van Riel, Lebacq, & Dejonckere, 2001; Jiang, Ng, & Hanson, 1999; Jiang, Verdolini, Aquino, Ng, & Hanson, 2000; Sivasankar & Blazer-Yost, 2009; Sivasankar & Erickson, 2009; Sivasankar, Erickson, Schneider, & Hawes, 2008; Sivasankar & Fisher, 2002, 2003, 2007, 2008; Sivasankar, Nofziger, & Blazer-Yost, 2008; Solomon & DiMattia, 2000; Solomon, Glaze, Arnold, & van Mersbergen, 2003; Tanner, Roy, Merrill, & Elstad, 2007; Verdolini et al., 2002; Verdolini, Titze, & Fennell, 1994; Verdolini-Marston et al., 1994; Verdolini-Marston, Titze, & Druker, 1990; Vintturi, Alku, Sala, Sihvo, & Vilkman, 2003; Yiu & Chan, 2003). These studies have explored hydration mechanisms using ex vivo and in vivo methodologies, the latter involving participants with normal voices, vocal attrition, vocal fatigue, structural voice disorders, and asthma, and have collectively documented the influence of both systemic and surface tissue hydration on voice production.

Aeromechanical estimates of phonation threshold pressure (PTP; i.e., the minimum amount of subglottic pressure required to initiate and sustain vocal fold oscillation) have been widely used in vocal fold hydration studies and have been considered the "gold standard" in quasi-objective measures of vocal effort (Titze, 1988). The use of PTP to estimate changes in vocal effort associated with vocal fold hydration has been well described (Chan & Titze, 1999; Finkelhor, Titze, & Durham, 1988; Fisher, Ligon, et al., 2001; Fisher & Swank, 1997; Verdolini et al., 1994; Verdolini-Marston et al., 1990), with changes in vocal fold viscosity, presumably related to variations in systemic or surface tissue hydration, generally resulting in corresponding changes in PTP. However, the changes in PTP associated with dehydration challenges and hydration treatments have been relatively modest. In addition, issues surrounding observer and participant bias, as well as PTP measurement variability, have been identified (Erickson & Sivasankar, 2010; Roy, Tanner, Gray, Blomgren, & Fisher, 2003; Sivasankar & Fisher, 2002, 2003; Tanner et al., 2007). To complicate matters, the relationship between PTP and participant-based ratings of self-perceived phonatory effort (PPE) has also been questioned, and there is an expanding literature to suggest that PTP and PPE are poorly correlated (Erickson & Sivasankar, 2010; Leydon et al., 2009; Sivasankar, Erickson, et al., 2008; Tanner et al., 2007; Verdolini et al., 2002). The interpretation of PPE findings from previous hydration studies has been made difficult by diverse methods related to measurement scales and the vocal tasks sampled (Erickson & Sivasankar, 2010; Leydon et al., 2009; Roy et al., 2003; Sivasankar, Erickson, et al., 2008; Sivasankar & Fisher, 2002, 2003; Solomon & DiMattia, 2000; Solomon et al., 2003; Tanner et al., 2007).

These concerns notwithstanding, several investigators have attempted to elucidate the effects of systemic hydration on voice production, either in isolation or combined with presumed surface tissue hydration manipulation(s). Verdolini-Marston and colleagues (1990) observed differences in PTP for six participants who underwent a combined systemic and surface tissue dehydration challenge versus hydration treatment and placebo control conditions. Two later studies replicated and extended these results in 12 participants with normal voices (Verdolini et al., 1994) and six with vocal nodules or polyps (Verdolini-Marston et al., 1994). These double-blind, placebo-controlled studies reported moderate correlations between PPE and PTP, based on direct magnitude estimation of vocal effort during conversational speech. A later investigation by Verdolini and colleagues (2002) confirmed the influence of systemic hydration on PTP in four nonsingers. However, PPE based on direct magnitude estimation of the PTP task failed to produce similar results. Solomon and colleagues (Solomon & DiMattia, 2000; Solomon et al., 2003) also have reported evidence for the influence of systemic dehydration on PTP, when combined with a vocally fatiguing task, in two studies involving four female and four male nonsingers, respectively. However, no consistent trends in dehydration were observed for PPE based on visual analog scale ratings of connected speech. In summary, evidence from small-group studies exists to support the influence of systemic hydration on PTP, but the relationship with PPE seems less clear.

Although earlier research has demonstrated effects of systemic dehydration and rehydration on the voice, later studies have focused on the mechanisms responsible for maintaining surface tissue hydration of the vocal fold mucosa and airway epithelial tissue. Surface tissue hydration is believed to be regulated by ionic transport mechanisms across the cell membranes of airway and vocal fold epithelial cells; specifically, the movement of sodium (Na^+) and chloride (Cl^-) across the cell membrane results in transepithelial water fluxes that influence the depth and viscosity of the layer of liquid—including a deeper sol (water) layer and an overlaying, more viscous mucus blanket—that lubricates the larynx (Fisher, Ligon, et al., 2001; Leydon et al., 2009). It has been postulated that reductions in environmental relative humidity (RH) result in reduced depth and increased viscosity of the surface liquid that covers the airway (Yeates, 1991), ultimately producing corresponding increases in PTP and PPE (Roy et al., 2003, Sivasankar & Fisher, 2002, 2003; Tanner et al., 2007). Hemler, Wieneke, and Dejonckere (1997) confirmed the potential influence of low-percentage RH exposure on phonatory instability measures in eight participants with healthy voices. Several studies have also demonstrated statistically significant differences in PTP—and, to some extent, PPE—following short-duration exposure to low environmental RH. Sivasankar and Fisher (2002) studied the effects of 15 min of oral breathing on PTP and PPE in 20 females with healthy voices. PTP increased significantly following the oral breathing challenge, but only six of 10 participants demonstrated significant increases in PPE as measured using direct magnitude estimation (based on singing “Happy Birthday”). Similar results have been observed in participants with vocal attrition (Sivasankar & Fisher, 2003). In a later study, Sivasankar, Erickson, et al. (2008) demonstrated the differential effects of exposure to various percentage RH levels based on an oral breathing challenge in eight participants with a history of vocal fatigue and eight control participants with normal voices. Using a repeated measures, within-subject experimental design, the authors reported that exposure to extremely low RH resulted in the greatest increase in PTP following oral breathing, but PTP and PPE were poorly correlated. Collectively these studies substantiate the deleterious effects of surface tissue dehydration on phonation, and these findings warrant further examination in the singing voice.

Only two investigations have studied the potential temporal effects of surface tissue hydration treatments in vivo. In the first, Roy and colleagues (2003) examined the effects of three possible laryngeal lubricants on PTP. Eighteen healthy females nebulized 2 mL of sterile water, mannitol, and Entertainer’s Secret Throat Relief, a glycerin-based product, for 3 consecutive weeks. A short-lived lowering of PTP was observed for mannitol but not for the other two treatments. The authors hypothesized that mannitol, a hyperosmotic agent, might influence the transepithelial water fluxes in the direction of the airway, thereby increasing laryngeal lubrication. In the second, later investigation, Tanner et al. (2007) examined the effects of three nebulized osmotic agents—hypertonic saline (7%), isotonic saline (0.9%), and (hyposmotic) sterile water—on PTP and PPE (measured as the self-perceived vocal effort required to produce the PTP task) in 60 healthy female nonsingers using a double-blind, randomized experimental design. Participants first underwent a laryngeal desiccation challenge involving oral breathing of medical-grade dry air for 15 min and were subsequently observed for 50 min following the administration of each nebulized treatment. The results indicated a reversal of the negative effects of laryngeal desiccation on PTP for the isotonic saline group and, to a lesser extent, for the sterile water group. PPE was poorly correlated with PTP measures. The authors suggested that nebulized isotonic saline into the airway might be potentially advantageous, perhaps facilitating short-term laryngeal lubrication without altering the ionic balance maintained by systemic and surface tissue hydration mechanisms in healthy individuals. However, singers were intentionally excluded from the study, leaving questions surrounding the generalizability of the findings to this group of elite voice users.

To date, only one investigation has attempted to examine the potential influence of hydration on the singing voice. Yiu and Chan (2003) studied the effects of systemic dehydration and a vocally fatiguing task in 20 amateur (untrained) singers. Participants combined extended karaoke singing with either a hydration or dehydration condition. The hydration condition included the ingestion of 100 mL of water and a 1-min rest period between songs. The acoustic measurement results suggested that amateur singers experienced vocal fatigue less quickly with frequent vocal rest and ingestion of water, as compared with withholding rest and water. However, on the basis of auditory–perceptual ratings, no detectable differences in voice quality were observed between conditions. This study did not, however, examine the effects of hydration in classically trained singers, thus limiting the generalizability of the findings. Vocal training presumably increases one’s ability to mitigate or compensate for factors such as dehydration that might otherwise influence voice production; therefore, it is necessary to study the effects of surface tissue dehydration and potential rehydration conditions in populations with voice training to determine the external validity of these treatments.

In summary, previous research has established the potential of both systemic and surface tissue dehydration and rehydration treatments to influence voice production; however, the effects of hydration on the classical singing voice have not been directly examined. Singers represent a unique population who place extreme demands on their voices and are believed to be at risk for vocal health issues, including dehydration. Surface tissue dehydration studies seem particularly useful in terms of measuring the negative effects of dehydration on voice production in individuals with normal voices; however, the variable relationship between PTP and PPE as previously reported in the literature is somewhat troubling, particularly when one considers that the individual's *perception* of the relative effects of treatments in mitigating dehydration will likely influence overarching treatment compliance and success. Thus issues surrounding the poor relationship between PTP and PPE in assessing potential hydration treatment benefits should also be explored. We therefore undertook the present investigation to address the following experimental questions: What are the effects of laryngeal desiccation and subsequent nebulized treatments on PTP and PPE in classically trained sopranos? What is the relationship between PTP and PPE?

Method

Participants

Thirty-four classically trained female sopranos (M = 30.2 years, SD = 11.9, range = 18–56) participated in the study. Singers were identified and recruited from classical vocal studios at The University of Utah School of Music and the greater Salt Lake City area. To maximize the generalizability (i.e., external validity) of this initial attempt to study surface tissue hydration in the classical singing voice, a somewhat heterogeneous group of professional singers, voice teachers, and college students was included in the study. Singers ranged in their experience and years of instruction. However, all participants were at the university level or greater as verified by their voice teachers or, in the case of the professional singers, via self-report. None of the singers were experiencing upper respiratory symptoms or voice problems at the time of the study. All singers were nonsmokers and denied a history of asthma or hearing loss. Participant identification and recruitment methods, as well as data collection procedures, were approved by The University of Utah institutional review board.

Design

In a double-blind, within-subject crossover design, each singer attended three data collection sessions on 3 consecutive weeks. Each session involved a laryngeal desiccation challenge followed by one of three treatment conditions, including nebulized isotonic saline (IS) (0.9% NaCl) or nebulized hypotonic sterile water (SW) or a nontreatment control condition. The order of treatment administration was counterbalanced across weeks. Sessions were scheduled near the same time of day for each participant across the 3 weeks. Singers were also asked to use their voices in a similar manner on the day of each scheduled session prior to data collection. Each data collection session was approximately 2.5 hr.

Procedure

Laryngeal desiccation and treatment administration procedures in this study were identical to previous research involving surface tissue hydration in nonsingers (Tanner et al., 2007). For purposes of laryngeal desiccation, each singer received medical-grade dry air (<1% RH; 78% nitrogen, 21% oxygen, <350 ppm carbon dioxide, and <5 ppm water) transorally via an oral–nasal mask with the nose clipped. Dry air was administered at a rate of 8 L per minute for 15 min. Following the laryngeal desiccation challenge, singers received 3 mL of nebulized IS or SW or underwent a control condition (sitting quietly and breathing naturally without oral or nasal specification) during a 10-min period. Treatments were administered using a Micro Mist Nebulizer ("T" Up-Draft II, No. 1883, Hudson/RCI) at a rate of 8 L per minute, with the nose clipped to prevent nasal inhalation. PPE and PTP were measured at baseline, immediately postdesiccation, and at 5, 20, 35, 50, 65, 80, 95, and 110 min postnebulization (i.e., 10 total observations). We selected the 2-hr postdesiccation observation period to facilitate measurement of recovery from laryngeal desiccation during the control condition on the basis of previous evidence that 60 min was inadequate to measure laryngeal desiccation recovery in nonsingers (Tanner et al., 2007). Participants were instructed to sit quietly and did not eat or drink during the session. Percentage environmental relative humidity was measured for each session using a Mannix wireless thermo-hygrometer (Model EMR963HG). Average environmental humidity levels were 17.9% (SD = 2.8%) and were not significantly different among the three treatment conditions as indicated by a one-way analysis of variance (ANOVA), $F(2, 99) = 0.83$, $p = .439$.

PPE measurement. Because the purpose of this study was to examine the effects of surface tissue dehydration and two nebulized treatments on the classical singing voice, it was important to base self-perceived vocal effort measures on a singing task. Therefore, each soprano sang a five-note ascending-to-descending scale at a self-determined mezzo-forte loudness level, with vibrato, in legato style as follows: C5, D5, E5, F5, G5, F5, E5, D5, C5. Following this task, singers sustained a pianissimo G5, with vibrato, for 5 s. These tasks were selected to sample the upper passaggio, where voice production is often more difficult and presumably might be more susceptible to surface tissue hydration changes (Miller, 1986).

For the purposes of the present investigation, each singer estimated her vocal effort based on the singing voice task for each of the 10 observations. PPE ratings were accomplished using Alvin (Version 1.01), a public domain software used for listening experiments (Hillenbrand & Gayvert, 2005). Participants placed the cursor on a 100-point visual analog scale to indicate self-perceived phonatory effort for the singing task, with the extreme left indicating *no effort* and the extreme right indicating *extreme effort*. PPE values reflect the number of points (0–100) from the left of the scale, for each of the 10 observations. Singers were not permitted to view previous self-rating responses during PPE sampling.

PTP measurement. Consistent with previous research involving PTP acquisition (Erickson & Sivasankar, 2010; Sivisankar & Fisher, 2003, 2007; Solomon & DiMattia, 2000; Solomon et al., 2003; Verdolini et al., 1994), the 80th percentile of each singer's F_0 range during soft phonation was selected for PTP acquisition. To establish each singer's F_0 range, participants glided *softly* on the vowel /i/ from their midrange to the highest and lowest sustainable 3-s tone. Pitch range was established by rounding each pitch to the nearest semitone using a piano keyboard, and the 80th percentile was calculated. Singers were instructed to practice /pi/ repetitions (<5 trials) using soft phonation, just above a whisper, at the 80th percentile target pitch.

We accomplished PTP measurement using the methodology reported in Tanner et al. (2007) and previously described and validated by others (Milbrath & Solomon, 2003; Roy et al., 2003; Solomon & DiMattia, 2000; Solomon et al., 2003). In brief, singers produced three sets of seven-syllable /pi/ strings, just above a whisper, at a rate of 1.5 per second (Holmberg, Perkell, & Hillman, 1987) into the oral–nasal mask of the PERCI Speech-Aeromechanics Research System, Version 3.21 (MicroTronics). A heated pneumotachometer (Hans Rudolph) set at 37 °C was used for PTP measurement. The PERCI system was calibrated at the beginning of each data collection session, and calibration was confirmed every 30 min. Pressure calibration was accomplished using a U-tube manometer. For calibration purposes, pressure variations of less than 5% were considered within acceptable limits.

During PTP acquisition, an 8-Fr catheter (Kendall Co.) was placed just behind the central incisors for purposes of oral pressure measurement. All productions were monitored by the examiner related to mask and catheter placement and loudness and pitch requirements. Resampling was undertaken (fewer than three trials) if productions failed to meet measurement criteria based on auditory–perceptual judgment by the examiner (e.g., supra- or subthreshold productions). For data analysis, the central five pressure peaks of the middle syllable string were identified and pairs of adjacent peaks averaged (Smitheran & Hixon, 1981) to obtain PTP estimates.

Reliability. Although the PTP calculations involved in the present study were fairly automatic, we confirmed each oral pressure peak manually to eliminate the possibility of "peak-skipping." Therefore, 10% of the samples were reanalyzed by the original examiner and a second examiner to assess reliability for the measurement analysis procedure. Pearson correlations of 1.0 (mean difference = 0.000 cmH_20) and .99 (mean difference = 0.009 cmH_20) were obtained as estimates of interjudge and intrajudge reliability, respectively.

Statistical Methods

One-way ANOVAs were conducted to establish baseline equivalence among the three treatment conditions for PPE and PTP. Paired t tests were used to evaluate desiccation and treatment effects using a corrected alpha level of .0125 to control for potential Type I error rate inflation due to multiple comparisons (i.e., four paired t tests for PPE and PTP, respectively). Third-order polynomial modeling of each treatment condition for PPE and PTP was performed to evaluate temporal trends. The Pearson correlation coefficient was used to evaluate the relationship between PPE and PTP. Data were analyzed using SAS for personal computers, Version 9.1.

Results

Baseline Equivalence

Although this study involves a within-subject design, we undertook testing for each of the three treatment conditions for PPE and PTP measures to assess equivalence at baseline. The results from one-way ANOVAs confirmed no differences at baseline among the three conditions for PPE, $F(2, 99) = 0.49$, $p = .613$, or PTP, $F(2, 99) = 0.25$, $p = .780$.

PPE

Mean PPE data for each of the 10 observations are presented in Table 1. PPE increased significantly following laryngeal desiccation, $t(101) = 5.74$, $p = .001$, and in a similar manner across the three treatment conditions, $F(2, 99) = 0.49$, $p = .613$. The IS condition produced PPE values that approximated baseline by 5 min postnebulization and that ultimately returned to baseline by 110 min postnebulization. PPE gradually lowered following SW nebulization but did not return to baseline

Table 1. Self-perceived phonatory effort (in millimeters) means and standard deviations (in parentheses) for the isotonic saline, sterile water, and control conditions at each observation.

Condition	Baseline	Postdesiccation	Postnebulization 5 min	20 min	35 min	50 min	65 min	80 min	95 min	110 min
Isotonic saline	39.4 (23.3)	46.7 (24.0)	40.9 (25.1)	40.0 (22.7)	40.4 (23.1)	42.4 (26.1)	40.6 (25.8)	40.5 (24.5)	41.7 (24.3)	39.1 (27.7)
Sterile water	34.4 (24.0)	43.6 (25.1)	42.9 (25.4)	42.7 (25.4)	40.0 (24.4)	41.2 (25.5)	40.1 (25.9)	40.3 (25.0)	43.9 (25.2)	38.6 (24.6)
Control (no treatment)	34.9 (20.4)	42.5 (22.0)	42.9 (21.3)	43.2 (20.6)	41.5 (20.3)	42.6 (22.6)	45.1 (22.5)	44.3 (23.9)	44.0 (24.0)	44.2 (23.9)

within the 2 hr. For the control condition, PPE continued to worsen gradually over the subsequent 2-hr period, and differences between baseline and 110 min postnebulization remained statistically significant, $t(33) = 2.91$, $p = .006$. Differences between baseline and 110 min postnebulization were not significant for the IS condition, $t(33) = 0.11$, $p = .914$, or the SW condition, $t(33) = 1.07$, $p = .292$.

PTP

Mean PTP data for each of the 10 observations are presented in Table 2. PTP did not change significantly following laryngeal desiccation, $t(101) = 0.68$, $p = .497$, and postdesiccation values were similar across the three treatment conditions, $F(2, 99) = 0.14$, $p = .869$. Although no significant differences were observed immediately following the laryngeal desiccation challenge, PTP progressively increased for the SW and control conditions throughout subsequent observations. At 110 min postnebulization, PTP was significantly above baseline for the SW condition, $t(33) = -3.54$, $p = .001$, but not for the IS condition, $t(33) = -0.58$, $p = .569$, or the control condition, $t(33) = -1.23$, $p = .214$. Only the PTP values for the IS condition remained below baseline through 95 min postnebulization.

Trend Analysis

Although no statistically significant differences were observed among the three conditions at baseline, PPE and PTP raw values were numerically greater for the IS condition as compared with the SW and control conditions. We therefore performed trend analyses to examine the relative changes in PPE and PTP from baseline to each subsequent observation (i.e., mean change) for each of the three conditions. Third-order polynomial models provided the best fit for the temporal effects associated with laryngeal desiccation and subsequent nebulization. For PPE, models accounted for 79.4% of the variance in mean change for the IS condition, 40.8% for the SW condition, and 51.3% for the control condition (see Figure 1). For PTP, models accounted for 48.7% of the variance in mean change for the IS condition, 70.1% for the SW condition, and 67.8% for the control condition (see Figure 2).

Relationship Between PPE and PTP

To evaluate the relationship between PPE and PTP, we calculated a Pearson correlation coefficient based on all PPE and PTP measures. A nonsignificant and slightly negative correlation coefficient was observed, $r = -.20$, $p = .319$, indicating a poor, and slightly inverse, relationship between PPE and PTP.

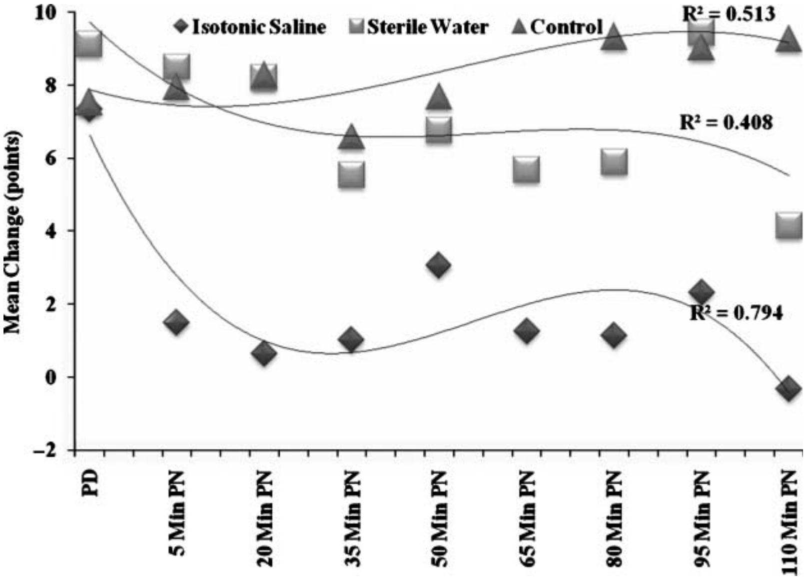
Discussion

This investigation examined the effects of nebulized IS and SW following a laryngeal desiccation challenge and is the first to directly study surface tissue dehydration in classically trained singers. The double-blind,

Table 2. Phonation threshold pressure (in cmH$_2$0) means and standard deviations (in parentheses) for the isotonic saline, sterile water, and control conditions at each observation.

Condition	Baseline	Postdesiccation	Postnebulization 5 min	20 min	35 min	50 min	65 min	80 min	95 min	110 min
Isotonic saline	12.3 (3.9)	11.9 (4.2)	11.6 (4.5)	11.8 (4.6)	12.2 (4.9)	12.2 (4.7)	12.2 (5.0)	11.8 (4.7)	12.1 (4.8)	12.6 (5.4)
Sterile water	11.7 (5.1)	12.0 (5.4)	12.8 (5.2)	12.5 (5.6)	12.9 (5.2)	13.2 (5.2)	12.8 (5.4)	12.6 (5.2)	13.0 (5.6)	13.0 (5.6)
Control (no treatment)	11.6 (4.7)	11.4 (4.5)	11.7 (4.7)	12.1 (5.2)	11.7 (4.7)	11.6 (4.5)	11.7 (4.1)	12.0 (4.8)	12.1 (4.9)	12.2 (4.7)

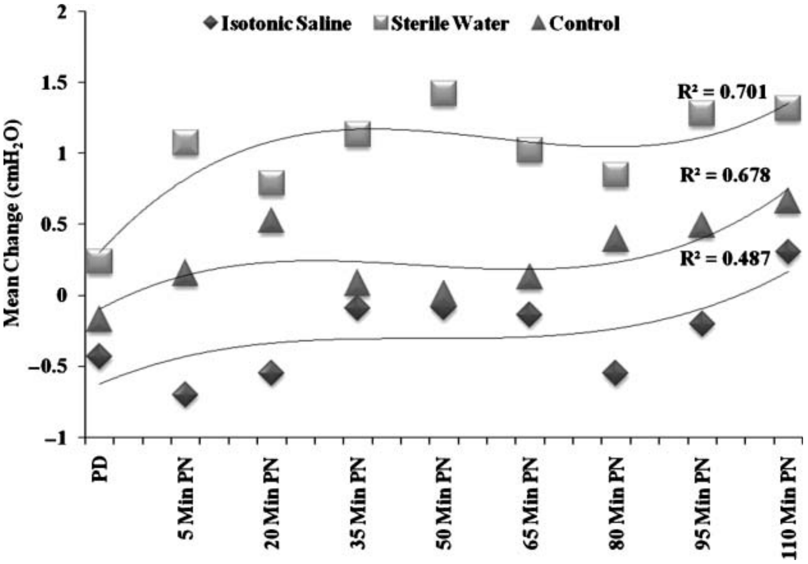
Figure 1. Mean change in self-perceived phonatory effort from baseline to each subsequent observation for the isotonic saline, sterile water, and control conditions. PD = postdesiccation; PN = postnebulization.



within-subject crossover design afforded an optimal examination of the temporal effects associated with laryngeal dryness and subsequent treatment. The results from this study indicate that nebulized IS has the potential to reverse the perceived adverse effects associated with laryngeal dryness in singers, whereas nebulized SW was inadequate in addressing these effects. Following a short-duration laryngeal desiccation challenge, vocal effort continued to worsen progressively without treatment based on PPE and PTP measures. PPE was more sensitive to the temporal effects associated with the laryngeal desiccation and subsequent treatment paradigm in classical singers as compared with PTP.

The results of this study provide confirmatory evidence of the adverse effects associated with short-duration exposure to dry air via mouth breathing in classical

Figure 2. Mean change in phonation threshold pressure from baseline to each subsequent observation for the isotonic saline, sterile water, and control conditions.



singers. The sopranos in this study experienced significant increases in PPE following a 15-min laryngeal desiccation challenge, results that were replicated in this same group of singers across three experimental conditions. In addition, the nebulized IS treatment in this study reversed the observed desiccation effect based on PPE measures. These findings suggest that nebulized IS provides immediate relief from the perceived negative effects associated with brief exposure to dry air in the singing voice. The reduction in PPE associated with nebulized IS in singers was not surprising given previous research supporting the potential efficacy of this treatment based on nonsingers (Ferreira & Fujita, 1999; Tanner et al., 2007) and animal studies (Jiang et al., 1999). Additional evidence for the potential benefits of a nebulized ionic solution on voice production has been offered by more recent studies that have explored the biological mechanisms responsible for regulating transepithelial water fluxes in the airway (Sivasankar & Blazer-Yost, 2009; Sivasankar & Fisher, 2007; Sivasankar, Nofziger, & Blazer-Yost, 2008). Increased viscosity of the surface liquid on the vocal fold mucosa is believed to have deleterious effects on vocal fold vibration and ease of phonation (Ayache, Ouaknine, Dejonckere, Prindere, & Giovanni, 2004); thus, treatments that promote a reduction in the viscosity of the surface liquid in the airway might reverse or offset the negative effects associated with surface tissue dehydration. It has also been posited that ion and water transport mechanisms maintain the homeostatic processes of airway epithelial cells and are responsive to changes in surface liquid ionic imbalance (Jayaraman, Song, & Verkman, 2001; Leydon et al., 2009). Therefore, the introduction of a solution that is isotonic to the fluid within the epithelial cells of the airway and vocal fold mucosa might serve to lubricate the larynx without disrupting the homeostatic processes associated with transepithelial ion and water transport on the airway surface. In essence, IS might serve simply as a laryngeal lubricant, decreasing the viscosity of the surface liquid on the vocal folds and thereby reducing vocal effort and increasing vocal fold vibratory efficiency.

It is interesting, however, that the observed effects of laryngeal desiccation and subsequent treatment administration on PPE in this study were not strongly supported by PTP measures. Although a short-lived decrease in PTP was noted between 5 and 20 min following the administration of nebulized IS, this difference was modest (0.25 cm H_2O) and failed to approach statistical significance. In addition, no immediate, statistically significant desiccation effect was observed for PTP. It should be noted, however, that PTP was observed to increase over time for each of the three treatment conditions, a finding that is consistent with previous research that examined the temporal effects associated with laryngeal desiccation (Tanner et al., 2007). It is possible that these findings indicate a delay in laryngeal response to the desiccation challenge as measured by PTP, or that the greatest influence of laryngeal desiccation occurs at a later point in time. Thus, PTP and PPE may be temporally offset, perhaps explaining the relatively poor correlation between these two measures. Previous research has overwhelmingly relied on PTP as the gold standard for estimating changes in vocal effort associated with hydration manipulation. Many of these studies have attempted to offer corroborating evidence for observed changes in PTP associated with laryngeal dehydration using a variety of PPE measures (e.g., direct magnitude estimation, visual analog scales) and based on a variety of sample tasks (e.g., PTP productions, oral reading, singing "Happy Birthday"), with mixed success (Sivasankar & Fisher, 2002, 2003, 2007; Tanner et al., 2007; Verdolini et al., 2002). Although some studies have reported relatively strong agreement between PTP and PPE (Chang & Karnell, 2004), recent studies have concluded that these measures are generally poorly correlated, at least in laryngeal hydration research. Still, both measures continue to be used, with the majority of emphasis placed on PTP findings. Caution certainly is warranted when interpreting the PPE results alone in the present investigation; however, the differences observed in previous studies of PTP following laryngeal desiccation and the application of surface tissue hydration treatments have traditionally been modest and somewhat variable (Roy et al., 2003; Sivasankar & Fisher, 2002, 2003, 2007; Tanner et al., 2007). It is possible that the PPE measurement task in this study—a psychological measure of vocal effort (Verdolini et al., 1994)—was more sensitive to the adverse effects associated with laryngeal desiccation than the physiological measure (i.e., PTP). One potential explanation for this difference might be the sensory alterations associated with throat dryness during singing. PPE, a presumably multidimensional measure, might be based in part on the sensory changes associated with throat dryness, whereas PTP is likely insensitive to these changes. For purposes of PPE measurement, in this investigation we used a singing task ostensibly aimed at sampling a purportedly vulnerable area of the soprano voice (i.e., the upper passaggio) in a group of singers with a fairly diverse range of vocal abilities and experience. On the basis of the results of this study, this task seems to be more sensitive to changes in PPE, perhaps because it involves the specific vocal task to which it aims to generalize (i.e., classical singing) and is therefore more ecologically valid.

Singers are believed to have a unique set of vocal skills that may be used to influence voice production. It seems intuitive that singers might have greater self-awareness during vocalization. Perhaps singers are able to compensate for the physiological changes associated with laryngeal desiccation. An increase in cognitive resources allocation or other psychological factors might be reflected in PPE measures of the singing voice. Some evidence for the use of compensatory strategies by trained versus untrained singers has been reported based on functional magnetic resonance imaging studies of the sensory-motor feedback loop during singing (Zarate & Zatorre, 2008). Singers demonstrated increased activity in the anterior cingulate cortex, the auditory cortices, and the putamen as compared with nonsingers during singing. The authors hypothesized that these regions of the brain may be increasingly recruited to facilitate auditory feedback and vocal motor integration with singing voice training. Additional evidence for compensatory strategies applied by trained versus untrained singers has been offered from research involving auditory-motor mapping of the singing voice (Jones & Keough, 2008). It has been hypothesized that PPE is likely multidimensional, perhaps involving additional cognitive processes, such as resource allocation and learning effects (Sivasankar & Fisher, 2007; Tanner et al., 2007), which might partially explain the poor correlation between PPE and PTP. Further research involving the nature of PPE measurement and the tasks used to elicit these perceptual ratings is necessary. With respect to the present study, however, it is possible that singers were inherently more aware of all aspects of voice production and thus more sensitive to modest increases in vocal effort associated with surface tissue laryngeal dehydration. In addition, singers may compensate for modest changes in physiological vocal effort (i.e., PTP), perhaps accounting for more significant changes in psychological vocal effort (i.e., PPE) and the apparent disconnect between these two measures.

Nebulized SW produced some benefit in offsetting the adverse effects associated with laryngeal desiccation on the singing voice as compared with the nontreatment control condition. This finding is also consistent with previous research involving the nebulized administration of SW following a laryngeal desiccation challenge in nonsingers (Tanner et al., 2007). Although some studies have used humidifiers to increase percentage RH in combined surface and systemic laryngeal hydration studies (e.g., Verdolini et al., 1994), different delivery approaches for SW treatment administration have not been explored. We presumed in the present study that the nebulization of SW would optimize its effects on voice production, by facilitating the delivery of small water particles below the level of the vocal folds and promoting laryngeal lubrication via mucociliary transport mechanisms. However, it is possible that more traditional delivery mechanisms of SW (e.g., humidifiers, vaporizers, facial steamers) might be more beneficial to voice production. These devices putatively increase the ambient humidity of the environment and likely have some benefit to reducing vocal effort. Future studies should compare these more traditional delivery mechanisms of SW and their effects on voice production, particularly given their widespread use in clinical populations and singing communities.

Qualifications and Caveats

Although this study provides additional evidence to support nebulized IS as a treatment for surface tissue dehydration, it does not clarify the specific biological mechanisms responsible for the effects. Additional research, likely involving both in vitro and in vivo methodologies, is warranted to explore the precise mechanisms that influence surface tissue dehydration and treatment response. Frequency, timing, duration, method of administration (e.g., nebulizers, humidifiers, facial steamers), and dosing effects also should be examined. It is possible that prophylactic administration of nebulized IS might offset or prevent the negative effects associated with laryngeal desiccation. Future research should explore the potential for preventive hydration treatments and their effects on voice production. Because of the lack of an immediate, statistically significant desiccation effect based on PTP measures, some caution is warranted when interpreting the findings based on PPE data alone. More research is needed to examine the long-term effects of dehydration and rehydration treatments, because most have been pre–post designs, although new research in parallel areas is moving toward the examination of temporal effects of topical airway treatments on voice production (e.g., Erickson & Sivasankar, 2010). Admittedly, studies examining the temporal and durational effects of surface tissue dehydration on the voice will require large groups of participants because of multiple repeated measures. However, given the variability of PTP and PPE measures that has been previously observed (Roy et al., 2003; Tanner et al., 2007), it seems that this would be a logical next step to elucidate the effects of rehydration treatments on voice production.

An additional point regarding both PPE and PTP measures should be considered. In the present study, we applied a double-blind, counterbalanced experimental design to prevent any influence of examiner or participant bias on the results. However, one could argue that the singers in this study were not completely blinded to the desiccation challenge, thereby potentially influencing postdesiccation PPE measures. However, any participant awareness related to the desiccation challenge presumably would have affected both PPE and PTP measures similarly. Thus, it is somewhat incongruous that blinding seemingly failed only for PPE but not for PTP. In addition, the differential response to the nebulized agents postdesiccation, with IS outperforming SW, suggests that the postdesiccation improvements observed in PPE for IS are real and not merely related to possible placebo effects. It is also notable that neither PTP nor PPE measures have been explored or validated with respect to their generalizability to the singing voice. Particularly in light of our findings, however, we maintain that double blinding is an essential component to studies involving PTP and PPE measures of vocal effort.

Summary

Singers often practice regimens seemingly intended to promote laryngeal lubrication and minimize the presence of thick mucus, including the avoidance of dairy products, the use of humidifiers, and the drinking of lemon tea, to name a few. Although research involving the regulation of hydration in the singing voice has been extremely limited, singers are considered to be a group at particular risk for developing voice problems, including those related to dehydration. This large, double-blind, within-subject experimental trial provides preliminary evidence that singers experience significant adverse effects associated with mouth breathing combined with dry air exposure. These effects appear to worsen over time without treatment. Nebulized IS shows promise as a potential laryngeal lubricant, possibly facilitating surface tissue hydration and reducing perceived vocal effort during singing. Future research should explore frequency, duration, delivery, and dosing effects associated with laryngeal desiccation and treatment administration as well as the biological mechanisms responsible for regulating surface tissue hydration in the singing voice. In addition, differences based on voice type, gender, singing style (e.g., classical, belt, pop), and the presence of other voice-related problems or complaints should be explored.

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Nebulized Isotonic Saline Versus Water Following a Laryngeal Desiccation Challenge in Classically Trained Sopranos

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