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Quantifying the Effects of Altering Ambient Humidity on Ionic Composition of Vocal Fold Surface Fluid

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Abstract

Objective—Vocal fold surface fluid (VFSF) is important in hydration and defense of underlying epithelial cells. The objective of this study was to quantify changes in the ionic composition of VFSF after altering the humidity of inhaled air. We tested the hypothesis that low humidity exposure would increase the concentration of VFSF sodium (Na^+) and chloride (Cl^-) ions but that high humidity exposure would decrease the concentration of VFSF Na^+ and Cl^- ions as compared to the low humidity challenge.

Study Design—Prospective design.

Methods—Eighteen healthy adults participated in this study. VFSF was collected from each subject at baseline, and following exposure to low humidity and high humidity environments. VFSF Na^+ concentration was assessed using inductively-coupled plasma mass spectrometry. VFSF Cl^- concentration was measured with indirect potentiometry. All analyses were completed by personnel blinded to the hypothesis being tested.

Results—The low humidity environment increased Na^+ concentration in the majority of the subjects. Data for changes in Cl^- concentrations were variable. Overall the data did not reach statistical significance ($p > 0.05$). Subjective impressions suggested that VFSF collection was more difficult in low humidity as compared to the high humidity and baseline conditions.

Conclusions—This study is the first attempt to measure the ionic concentration of VFSF. The results from the current study have important implications for future programmatic research quantifying the effects of pollutants and laryngopharyngeal reflux on VFSF composition, epithelial hydration, and vocal fold defense.

Keywords

vocal fold surface fluid; dehydration; humidification; sodium; chloride

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Conflict of Interest: None

Level of Evidence: NA

INTRODUCTION

The vocal fold surface is lined by a thin layer of fluid (vocal fold surface fluid; VFSF) that is continuous with the fluid covering the distal and proximal airway. VFSF consists of mucus, electrolytes, and water^{1, 2} but the exact proportion of each of these constituents is yet to be determined. Quantifying the make-up of VFSF is inherently complex because of difficulties in collecting a fluid of small volume, without damaging underlying epithelial and glandular cells.

The composition of VFSF is thought to change after environmental and systemic challenges such as low humidity, mouth breathing, pollution, tissue inflammation with associated mucus hypersecretion, and laryngopharyngeal reflux of gastric contents, although this hypothesis has not been specifically tested. Altered depth and rheology of VFSF negatively impact vocal fold vibration and voice production.^{3, 4} These adverse changes include greater contact time between the vocal folds⁵, enhanced permeability of the vocal fold epithelium⁶, and increased pulmonary effort for voice production.⁷

In this study we investigated whether we could quantify changes in VFSF after altering the humidity of inhaled air. Specifically we examined whether inhaling low humidity air would increase VFSF ionic composition from baseline and conversely, whether high humidity exposure would restore VFSF ionic composition back to baseline levels. There are clinical implications for studying changes in the ionic balance of VFSF after environmental perturbations. One potential application is in developing hydration treatments that rebalance the ionic composition of VFSF in an attempt to improve surface hydration, increase mucus clearance, and reduce the thick, viscous mucus that characterizes vocal pathologies.

A low humidity environment was used to simulate airway dehydration and a high humidity environment was utilized to simulate a rehydration task. Exposure to low humidities dehydrates the fluid lining the airway.^{8–11} In this study we focused on quantifying the effects of low and high humidity on the concentration of two specific ions (Na^+ , sodium and Cl^- , chloride). These ions were selected because they are the predominant electrolytes in the surface fluid overlying the proximal and distal airway.^{9, 12–16} Further, the concentration of these ions can change with dehydration challenges.

METHODS

Subjects

Eighteen (12 females and 6 males) volunteers (ages: 18–42 years) participated in this study. All participants were evaluated by a laryngologist. Absence of laryngeal pathology was confirmed by videostroboscopy, case history, and auditory-perceptual ratings of voice in conversation speech. No specific perceptual scales were used for ratings. Four subjects reported a history of vocal fatigue after prolonged voice use. Vocal fatigue was accompanied by throat dryness in all four subjects. None of the subjects reported any vocal fatigue at the time of participation. Three subjects had singing training (range: 3–8 years). All subjects reported general good health. Eight subjects reported a history of upper respiratory airway disease including asthma, sinusitis, and allergies. No subject was symptomatic at the time of participation. Two subjects reported that they smoked (range: every other day – 5/day). Subjects ate one meal of their choosing during the study day and the timing of the meal varied among participants. Subjects drank between 50mL to 1.3L of water during the study. Five subjects did not consume any water.

Vocal Fold Surface Fluid Collection

VFSF was collected from each subject using identical procedures. To collect VFSF, each subject was administered 3 mL of topical, nebulized 4% lidocaine. Additionally, approximately 2 mL of 4% lidocaine was dripped directly onto the true vocal folds via a drip catheter passed through the working channel of a flexible laryngoscope. The subject was allowed to swallow and clear their larynx ad lib. Once sufficient anesthesia was achieved, a flexible channel laryngoscope was passed through the more patent nostril and a suction catheter was then passed through the working channel of the laryngoscope. Fluid was aspirated and collected from the superior, medial, and anterior aspects of the true vocal folds using clean, dry suction tubing and a lukens trap. Care was taken to avoid fluid collection from the supraglottic or subglottic areas. Approximately 200uL VFSF was collected from each subject. VFSF was mixed with 0.5mL isotonic saline, transferred to an Eppendorf tube, and placed in a -80°C freezer for further analysis.

Low and High Humidities

VFSF was collected at three different time points from each subject. These time points included baseline, after low humidity challenge, and after high humidity challenge. Following baseline VFSF collection, subjects underwent a low humidity challenge which consisted of 2 hours of obligatory mouth breathing in 20%–40% ambient humidity. This challenge duration has been shown to change the composition of surface fluid in the canine airway.¹¹ To the best of our knowledge, no studies have been completed in awake, human subjects. Next, VFSF was collected using the procedure described above. Subjects then underwent a high humidity challenge which consisted of 2 hours of obligatory oral breathing in 63%–77% ambient humidity. VFSF was collected again at the end of the high humidity exposure. During the mouth breathing challenge, subjects occluded their nostrils with nose plugs or with foam ear plugs held in place with medical tape.

VFSF Analysis

Analysis of ion composition was completed by personnel who were blinded to the type of challenge and hypothesis being tested. VFSF Na^{+} concentration was determined with inductively-coupled plasma mass spectroscopy (ICP-MS). VFSF Cl^{-} concentration was determined using indirect potentiometry. First, VFSF samples were thawed and centrifuged to remove any particulates or bubbles. To determine Na^{+} concentration, 1uL from each VFSF sample was added to 70% BDH Aristar ultra-pure nitric acid (VWR International, Radnor, PA), and left at room temperature overnight to facilitate complete digestion of any organic constituents that could interfere with ICP-MS. Once digested, the nitric acid concentration was reduced to 2% using MilliQ water. Samples were then further diluted 1:5 using 2% BDH Aristar ultra-pure nitric acid solution (VWR International, Radnor, PA). A standard curve was set-up using the standard addition method and a 68 element part A [49 element] standard (Exaxol, Clearwater, FL) that included Na^{+} . ICP-MS was finally run on all prepared samples and standards using a ThermoFinnigan ELEMENT2 inductively coupled argon plasma mass spectrometer system (Thermo Scientific, Waltham, MA). ICP-MS data were initially reported in counts-per-second, converted to ng/mL using the standard curve and finally into mM using the molecular weight of sodium. Indirect potentiometry utilized a solid state Cl^{-} electrode in conjugation with a glass Na^{+} reference electrode. A Beckman Coulter DxC 800 system (Beckman Coulter, Brea, CA) was used to determine Cl^{-} concentration (in mM) with indirect potentiometry. Each sample run used 200uL VFSF, 1.27mL of ion sensitive electrode electrolyte buffer reagent and 3.23mL ion sensitive electrode electrolyte reference reagent. Both reagents were obtained from Beckman Coulter (Brea, CA).

Data and Statistical Analysis

VFSF Na^+ and Cl^- concentrations were measured for each subject at baseline, after exposure to the low humidity environment, and following exposure to the high humidity environment. Due to the addition of a relatively high volume of saline to each sample, absolute ionic concentrations could not be determined at any time point. Since we were testing a hypothesis of change in ionic concentration after challenges, Na^+ and Cl^- concentrations after the low humidity challenge were subtracted from baseline data for that respective subject. Likewise, Na^+ and Cl^- concentrations after high humidity challenge were subtracted from low humidity values for each subject to investigate if the VFSF ionic concentrations decreased after exposure to high humidity. Wilcoxon signed rank tests were used to compare data at low humidity and high humidity ($p < 0.05$).

RESULTS

Figure 1 shows individual subject data after low and high humidity exposure. The low humidity environment increased Na^+ concentration in 11 subjects (Mean $\pm$ SD: 44.26 ± 35.93), reduced Na^+ concentration in 4 subjects (36.59 ± 21.72) and did not change in 4 subjects. Na^+ concentration following high humidity challenge was measured in VFSF from 17 subjects, as there was insufficient VFSF volume in one subject to permit analysis. High humidity decreased Na^+ concentration in 10 subjects (34.96 ± 27.18), increased Na^+ concentration in 6 subjects (38.4 ± 38.23) and did not change in 1 subject. There were no significant differences between Na^+ concentrations in low and high humidity ($p = 0.14$). Cl^- concentration after low humidity challenges could only be measured in 15 subjects due to insufficient VFSF volume. Of these subjects, Cl^- concentration increased in 4 subjects (27 ± 23.57), decreased in 8 subjects (17.38 ± 7.78), and did not change in 3 subjects. Of the VFSF samples from 16 subjects that could be assayed for Cl^- concentration after high humidity exposure, Cl^- concentration decreased in 5 subjects (10 ± 6.63), increased in 10 subjects (27.10 ± 26.80), and did not change in 1 subject. Cl^- concentration in VFSF did not significantly differ between the low and high humidity conditions ($p = 0.31$).

DISCUSSION

The purpose of this study was to provide objective data to support the hypothesis that low humidity environments dehydrate VFSF and increase the concentration of Na^+ and Cl^- while high humidity environments can restore VFSF ionic concentration. Anecdotally, VFSF collection was more difficult after low humidity exposure than at baseline or following high humidity exposure due to less secretions being present in the endolarynx. This suggests that the low humidity challenge dehydrated the vocal fold surface. However, our findings did not reach statistical significance. The reasons for this lack of significance are unclear. Several studies^{16–18} have shown that the fluid lining the airway surface is near isotonic prior to a dehydration challenge. Dehydration evaporates liquid from the airway surface.^{9, 10, 15–17, 19} This fluid loss increases the ionic concentration of surface fluid¹⁰ and generates an osmotic gradient to drive compensatory water movement to restore surface fluid to physiological levels.²⁰

Given the abundance of data demonstrating that dehydration evaporates the fluid lining the airway, the non-significant results obtained here deserve further exploration. Potential reasons for the lack of statistical significance could pertain to low subject size, issues with VFSF collection, or VFSF analysis. In regards to VFSF collection, it is possible that we did not induce sufficient airway dehydration in our subjects. The low humidities were selected as representative of everyday, dry environments where subjects spend a majority of their time. However, this resulted in a wide range of acceptable humidities (20%–40%). It is also possible that some subject's experienced more protracted desiccation effects and that the

high humidity environment was inadequate in exposure duration and/or magnitude to reverse these negative effects. Secondly, the VFSF collection procedure involved topical lidocaine application. This may have irritated the laryngeal afferent system and stimulated epithelial and gland fluid secretion and/or induced changes in epithelial ion transport. At a more elementary level, the topical nebulized lidocaine treatments may have provided direct surface hydration and/or delayed the time from being in the dehydrated environment to having the secretions collected by approximately 10 to 15 minutes. It is also possible that the anesthetic may have interfered with the subject's ability to manage their secretions thereby altering VFSF composition. Finally, we cannot rule out the effects of the sensitivity of the experimental techniques for assaying VFSF ionic concentration. The addition of isotonic saline may have diluted the sample significantly. The volume of VFSF collected precluded rheological measurement of VFSF. It is possible that the measurement of VFSF viscoelastic properties would be more sensitive to changes in humidity, because of evidence that optimal hydration of mucus is necessary for mucociliary clearance from the airway surface and that airway dehydration significantly hinders the clearance of viscous mucus.²¹

Low humidity challenges have negative effects on phonation.^{7, 22} It has been previously suggested that the adverse changes to voice quality in low humidity environments result from dehydration of VFSF.²³ It may appear that the results reported here do not support this hypothesis. Arguably, changes in VFSF ionic composition alone may not be a marker of dehydration in the larynx. Vocal fold epithelial cells are water permeable²⁴ and capable of generating water fluxes to reduce an osmotic gradient on the surface.²⁵ Mucus in VFSF may also serve as a reservoir for water, so potential changes to mucus biological properties may be more indicative of dehydration than alterations in the ionic composition of VFSF. Further studies are needed to quantify the biological mechanisms for changes in voice quality after challenges that superficially dehydrate the vocal folds. It has been hypothesized that females may be more susceptible to dehydration as compared to males.²³ Data from this study did not reveal any differences with regards to gender.

The current investigation is the first to quantify changes in the ionic composition of surface fluid in the laryngeal lumen in response to everyday fluctuations in environmental humidity. Previous studies have assayed surface fluid for the presence of inflammatory mediators.²⁶ Here we demonstrate the feasibility of analyzing the ionic concentration of a small volume of VFSF. The current study lays the foundation for future research exploring the proportion of various constituents of VFSF and how they vary systematically as a function of inflammation, laryngopharyngeal reflux, and exposure to pollution.

CONCLUSIONS

Vocal fold surface fluid (VFSF) was collected from eighteen subjects after exposure to low and high humidities to test the hypothesis that low humidity environments would increase the concentration of Na^+ and Cl^- while high humidity environments would reduce the concentration of these ions. While subjective impressions confirmed greater difficulty in collecting VFSF, and more scant VFSF after the low humidity challenge, the data were variable and no clear statistically significant trends emerged in alterations in ionic concentration, as a function of changing ambient humidity.

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Figure 1.

Change in ionic concentration of VFSF Na⁺ (circles) and VFSF Cl⁻ (squares) after low humidity and high humidity exposure for each subject. A positive Δ value at low humidity shows an increase in ionic concentration from baseline, while a negative Δ value at low humidity shows a decrease in ionic concentration from baseline. A positive Δ value at high humidity shows an increase in ionic concentration as compared to low humidity, while a negative Δ value at high humidity shows a decrease in ionic concentration from low humidity.