Research Note

In Vivo Magnetic Resonance Imaging of the Rat Vocal Folds After Systemic Dehydration and Rehydration

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Objective: Consuming less water (systemic dehydration) has long been thought to dehydrate the vocal folds. An *in vivo*, repeated measures study tested the assumption that systemic dehydration causes vocal fold dehydration. Proton density (PD)-weighted magnetic resonance imaging (MRI) of rat vocal folds was employed to investigate (a) whether varying magnitudes of systemic dehydration would dehydrate the vocal folds and (b) whether systemic rehydration would rehydrate the vocal folds.

Method: Male ($n = 25$) and female ($n = 14$) Sprague Dawley rats were imaged with 7T MRI, and normalized PD-weighted signal intensities were obtained at predehydration, following dehydration, and following rehydration. Animals were dehydrated to 1 of 3 levels by water withholding to induce body weight loss: mild ($< 6\%$ body weight loss), moderate ($6\%–10\%$ body weight loss), and marked ($> 10\%$ body weight loss).

Results: There was a significant decrease in vocal fold signal intensities after moderate and marked dehydration ($p < .0167$). Rehydration increased the normalized signal intensity to predehydration levels for only the moderate group ($p < .0167$). Normalized signal intensity did not significantly change after mild dehydration or when the mildly dehydrated animals were rehydrated. Additionally, there were no significant differences in PD-weighted MRI normalized signal intensity between male and female rats ($p > .05$).

Conclusion: This study provides evidence supporting clinical voice recommendations for rehydration by increasing water intake after an acute, moderate systemic dehydration event. However, acute systemic dehydration of mild levels did not dehydrate the vocal folds as observed by PD-weighted MRI. Future programmatic research will focus on chronic, recurring systemic dehydration.

Systemic dehydration is considered detrimental to voice production. Therefore, increasing water intake to reduce systemic dehydration remains a cornerstone of clinical voice care. Systemic dehydration is the reduction in fluid content within the body (Hartley & Thibeault, 2014; Sivasankar & Leydon, 2010; Verdolini-Marston, Sandage, & Titze, 1994; Verdolini-Marston, Titze, & Druker, 1990; Verdolini, Titze, & Fennell, 1994).

However, there is no direct evidence that low levels of systemic dehydration actually dehydrate the vocal folds.

There is an *ex vivo* research investigating the detrimental effects of vocal fold dehydration. Direct dehydration of excised vocal fold tissues has been shown to change the tissue's biomechanical properties (Chan & Tayama, 2002; King, Steed, Rivera, Wisco, & Thibeault, 2018). Although *ex vivo* studies have linked vocal fold dehydration to altered oscillation, the dehydration levels induced were of large magnitude and not physiologically realistic. Furthermore, excised vocal folds lack homeostatic mechanisms for water regulation and water balance. Therefore, an *in vivo* study is needed to test the hypothesis that physiologically relevant, whole body dehydration (systemic dehydration) can induce vocal fold dehydration.

Reduction of body weight (percent body weight loss [BWL]) is one physiological marker for demonstrating that systemic dehydration has occurred *in vivo* (Cheuvront, Ely, Kenefick, & Sawka, 2010; Goulet, 2013; Shirreffs, 2000). Lecompte, Dumont, Hill, Souich, and Leloir (1981) showed that after rats were dehydrated for 4 days (23% BWL),

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hematocrit levels increased by 25% and the urinary output decreased by 70% compared to rehydrated animals. Physiologically relevant levels of systemic dehydration have been reported as BWL percentages ranging between 1% and 5% (Gorelick, Shaw, & Murphy, 1997; Goulet, 2013). Here, we use magnetic resonance imaging (MRI) to investigate whether similar low magnitudes of BWL directly induce vocal fold dehydration. Additionally, we investigate whether *in vivo* systemic rehydration can revert any vocal fold changes that are observed following *in vivo* systemic dehydration.

MRI is a noninvasive technique that can be used to create high-resolution images of organs and tissues within the body. Recently, this method was used to resolve key anatomical features of the rat vocal folds *in vivo* and *ex vivo* (Kishimoto et al., 2016). MRI was used to visualize substructures within the undamaged vocal fold mucosa and the presence of acute injury and chronic scarring. A specific type of MRI technique, called *proton density (PD)-weighted MRI*, is used to image the local PD within tissue structures. The PD-weighted signal is proportional to water content and can, therefore, be used to assess hydration state of the tissue being imaged. Tissues with more protons (greater hydration) have higher signal intensity, and those with fewer protons have lower signal intensity (Bitar et al., 2006). PD-weighted MRI is useful for studying the hydration state of tissues over time, hydration states between tissues, and hydration levels within a tissue structure. Past studies have utilized PD-weighted MRI to analyze the water concentration gradients within small structures such as the ocular lens (Vaghefi, Pontre, Jacobs, & Donaldson, 2011). PD-weighted MRI has even been used to noninvasively assess hydration states within the brain (Abbas, Gras, Möllenhoff, Oros-Peusquens, & Shah, 2015; Mackay et al., 1994; Meyers, Kolind, & MacKay, 2017; Meyers et al., 2016; Mezer, Rokem, Berman, Hastie, & Wandell, 2016; Whittall et al., 1997). To the best of our knowledge, the sensitivity of PD-weighted MRI to small perturbations in hydration levels has not been investigated to date.

PD-weighted MRI has been employed to study hydration changes in vocal fold tissue. Our group utilized PD-weighted MRI to assess vocal fold changes related to marked levels of systemic dehydration (> 10% BWL) in rats *in vivo* (Oleson, Lu, Liu, Durkes, & Sivasankar, 2018). We demonstrated that systemic dehydration of 10.89% due to water withholding resulted in an average statistically significant decrease in vocal fold normalized signal intensity of 11.38%. King et al. (2018) also used PD-weighted MRI to quantify changes in water content in *ex vivo* porcine vocal fold layers. They used hypertonic solutions to dehydrate porcine vocal fold mucosa by over 30% and showed rehydration restoration of intensity close to pre-dehydration levels (King et al., 2018).

Here, we utilized a water withholding protocol to induce system dehydration. Water withholding is a common method to induce systemic dehydration *in vivo*. Solomon, Glaze, Arnold, and van Mersbergen (2003) had subjects reduce noncaffeinated fluid consumption to induce dehydration.

Franca and Simpson induced systemic dehydration by having their subjects abstain from all fluids and food intake before testing (Franca & Simpson, 2012). Water withholding is also ecologically valid as many cultures follow water deprivation protocols for 12 hr or greater. Other methods to induce systemic dehydration include diuretics such as furosemide (Armstrong, Costill, & Fink, 1985; Butudom et al., 2004; Naylor, Bayly, Schott, Gollnick, & Hodgson, 1993). Dialysis in individuals presenting with renal disease has also served as a means of investigating voice changes after fluid removal (Fisher, Ligon, Sobecks, & Roxe, 2001).

The objective of this study was to investigate if systemic dehydration, defined as a reduction in body weight due to water withholding, would dehydrate the vocal folds. Our study design allowed us to assess within-subject comparisons for the rodent animal model. Average normalized signal intensity from PD-weighted MRI was compared in the same rat model at three time points: prior to dehydration, following systemic dehydration, and following rehydration. Three levels of systemic dehydration were investigated. The levels of systemic dehydration were selected to span realistic, physiological levels (< 6% BWL) up to marked levels (> 10% BWL). The marked level of systemic dehydration was intentionally chosen to demonstrate that PD-weighted MRI can successfully quantify PD levels in a given anatomical area. We also examined whether systemic dehydration effects on vocal fold tissue would be similar across male and female rats and whether vocal fold hydration state was restored to pre-dehydration levels after rehydration.

Materials and Method

Experimental Animals

Twenty-five male (predehydration 355–460 g, < 6 months, Envigo Inc.) and 14 female (predehydration 212–287 g, < 6 months, Envigo Inc.), Sprague Dawley rats were used in this study in accordance with approved protocols by the Purdue Animal Care and Use Committee (1703001551; approval May 2017). The Purdue Animal Care and Use Committee recommended 1 week between consecutive MRI sessions.

MRI

Imaging was conducted with a 7T MRI system (BioSpec 70/30, Bruker Instruments). A transmit-only volume ¹H RF coil (86-mm inner diameter) and a receive-only surface ring coil were used for imaging areas of interest. PD- and T₂-weighted anatomical images were acquired with a multislice multi-echo acquisition sequence (20 slices with 0.5-mm thickness and 0.5 × 0.5 mm² in-plane resolution, repetition time = 5,000 ms, averages = 1, repetitions = 1, echo spacing = 10 ms, echo images = 2, echo time = (15 ms, 75 ms), flip angle = 90°, field of view = 40 × 40 mm²). The first echo image was taken as the PD-weighted image.

Rats were weighed before anesthetization and MRI. Rats were anesthetized with isoflurane (500 ml/min,

4% anesthesia level, 5 min), and anesthetization was maintained throughout magnetic resonance scanning (500 ml/min, anesthesia level dependent on respiratory rate). Respiratory rate was monitored using a magnetic resonance-compatible small animal monitoring and gating system (SA Instruments, Inc.). The temperature was controlled using a water heating system (Thermo Scientific). A custom rat head holder that was designed in-house was used in conjunction with a body holder that was warmed with embedded water tubes (temperature dependent to keep the rat temperature around 37 °C).

Rats were placed supine in the body holder with their noses positioned in a nose cone within the custom head holder. A 3D-printed fixture was placed across the surface of the neck. The fixture was customized to fit the receive-only surface coil on the top surface. Additionally, this piece was designed to hold an external cylindrical phantom ($\text{CuSO}_4 \times \text{H}_2\text{O}$ solution, Bruker Instruments) that fits into a slot below the surface coil. The phantom was used for intensity normalization. The custom fixture ensured a constant distance between the surface coil and the phantom across all experiments. The same experimental procedure was used for all imaging sessions and conditions: pre-dehydration, dehydration, and rehydration.

Vocal folds of each animal were imaged. The location of the vocal fold was determined based on a previously published method (Oleson et al., 2018). In brief, the vocal fold slice was consistently one slice cranial to the sub-glottis slice (see Figure 1).

Figure 1. Proton density-weighted magnetic resonance imaging images showing the vocal fold slice. (A) Image of the slice before a manual mask had been applied. (B) Image of the slice after a manual mask had been applied to the external phantom and vocal folds.

MRI Analysis

MRI data were masked manually using FslView (Smith et al., 2004) for the vocal fold, salivary gland, and phantom region (MATLAB, MathWorks; see Figure 1). A custom MATLAB code that was built in-house was used to extract the signal intensities within the three mask regions (see Figure 1). The signal intensities of every voxel within each anatomic region were then averaged. The average vocal fold signal intensities were normalized by dividing by the average phantom signal intensity. This calculation represents the local hydration level in the vocal fold.

Dehydration and Rehydration

All animals received ad libitum access to food throughout the study. All animals underwent a predehydration MRI session and were returned to their housing environment. Dehydration was induced by water withholding. Animals were weighed before dehydration, which was termed their *predehydration weight*. Percent BWL was calculated by subtracting the animal weight following dehydration from the predehydration weight and normalizing to the predehydration weight. Animals were dehydrated to < 6% BWL (mild; eight males, five females), 6%–10% BWL (moderate; 10 males, five females), and > 10% BWL (marked; seven males, four females). The time range to achieve mild dehydration was 18–24 hr, moderate dehydration was 36–48 hr, and marked dehydration was 66–72 hr. Water withholding did not exceed 72 hr. Rehydration was induced by providing dehydrated animals ad-lib access to water. Once animals were rehydrated to their predehydration weight, the final MRI was completed. Preliminary experiments indicated that, after marked dehydration, it was necessary to allow a rehydration period of 1 week. Therefore, all animals were given 1 week of ad-lib access to water. Animals were not continuously weighed throughout the rehydration period in an attempt to minimize induced stress and maximize rehydration recovery.

Statistical Analysis

Statistical analyses were completed with Minitab 18 statistical software (Minitab) and SPSS (Version 26). Significance was set to $p = .05$ for all statistical analysis. All data were assessed for normality prior to implementing parametric testing. Mauchly's test was employed to test for the assumption of sphericity. Male and female vocal fold normalized signal intensities were compared with t tests. A repeated measures analysis of variance (ANOVA) test was used for comparison between predehydration, dehydration, and rehydration conditions. Depending on the statistical outcome of the repeated measures ANOVA, a paired t test (corrected for multiple comparisons, $p = .0167$) was used as the post hoc test. Partial eta squared values were calculated for effect size of the within-subject comparison of the repeated measures ANOVA. In addition, Cohen's d effect size was computed only for those post

hoc comparisons that were statistically significant. Cohen's d was calculated by using the average standard deviation of both repeated measures as a standardizer, which ignored the correlation between the measures (Lakens, 2013).

Results

Male and Female Comparisons

The normalized signal intensity did not differ significantly between male and female vocal folds following mild dehydration ($t = 1.093$, $df = 11$, $p = .298$), moderate dehydration ($t = 0.590$, $df = 13$, $p = .565$), or marked dehydration ($t = 1.267$, $df = 9$, $p = .237$). All comparisons passed Levene's test for equality of variances. Therefore, all ensuing statistics merged male and female data at each level of dehydration.

Body Weight Dehydration and Rehydration

There were significant differences in body weight after dehydration and rehydration for all three groups (see Figure 2). For the mild dehydration group ($n = 13$), Mauchly's test of sphericity was not violated, $\chi^2(2) = 1.484$, $p = .476$. For this group, the body weights of the rats decreased following dehydration and increased following rehydration, $F(2, 24) = 79.829$, $p < .001$, $\eta_p^2 = .869$ (see Figure 2). The average predehydration weight for the rats was 357.46 ± 22.73 g (mean $\pm$ standard error of the mean [SEM], range between the highest and lowest values = 239 g). The average dehydration weight for the rats was 339.54 ± 21.41 g (mean $\pm$ SEM, range: 226 g). Compared to the predehydration

weights, this resulted in an average body weight percent loss of $4.98\% \pm 0.15\%$ across all rats (mean $\pm$ SEM, $t = 12.778$, $df = 12$, $p < .001$, Cohen's $d = 0.23$). After experiencing the mild dehydration, the average rehydration weight after having free access to water was 359.54 ± 22.29 g (mean $\pm$ SEM, range: 247 g). Compared to the predehydration weights, this resulted in an average body weight percent gain of $0.73\% \pm 0.63\%$ across all rats (mean $\pm$ SEM, $t = -1.078$, $df = 12$, $p = .3022$).

For the moderate dehydration group ($n = 15$), Mauchly's test of sphericity was violated, $\chi^2(2) = 14.924$, $p = .001$. Therefore, Greenhouse–Geisser was used for correction. For this group, the body weights of the rats decreased following dehydration and increased following rehydration, $F(1.189, 16.640) = 83.573$, $p < .001$, $\eta_p^2 = .857$ (see Figure 2). The average predehydration weight for the rats was 340.80 ± 20.87 g (mean $\pm$ SEM, range: 232 g). The average dehydration weight for the rats was 315.20 ± 21.01 g (mean $\pm$ SEM, range: 219 g). Compared to the predehydration weights, this resulted in an average body weight percent loss of $7.43\% \pm 0.37\%$ across all rats (mean $\pm$ SEM, $t = 11.757$, $df = 14$, $p < .001$, Cohen's $d = 0.34$). After experiencing the moderate dehydration, the average rehydration weight after having free access to water was 346.40 ± 21.75 g (mean $\pm$ SEM, range: 238 g). Compared to the predehydration weights, this resulted in an average body weight percent gain of $1.49\% \pm 0.49\%$ across all rats (mean $\pm$ SEM, $t = -3.174$, $df = 14$, $p = .0068$).

For the marked dehydration group ($n = 11$), Mauchly's test of sphericity was not violated, $\chi^2(2) = 5.091$, $p = .078$.

Figure 2. A box and whisker chart of the average body weight loss changes for each dehydration group at each magnetic resonance imaging session. Systemic dehydration (water withholding) and rehydration (free access to water) significantly changed the average body weight for the mild ($n = 13$), moderate ($n = 15$), and marked ($n = 11$) groups. Standard error of the mean bars are shown. $*p < .05$.

For this group, the body weights of the rats decreased following dehydration and increased following rehydration, $F(2, 20) = 174.357$, $p < .001$, $\eta_p^2 = .946$ (see Figure 2). The average predehydration weight for the rats was 345.27 ± 19.74 g (mean $\pm$ SEM, range: 141 g). The average dehydration weight for the rats was 305.91 ± 19.18 g (mean $\pm$ SEM, range: 128 g). Compared to the predehydration weight, this resulted in an average body weight percent loss of $11.46\% \pm 0.21\%$ across all rats (mean $\pm$ SEM, $t = 19.407$, $df = 10$, $p < .001$, Cohen's $d = 0.66$). After experiencing the marked dehydration, the average rehydration weight after having free access to water was 352.64 ± 21.75 g (mean $\pm$ SEM, range: 167 g). Compared to the predehydration weights, this resulted in an average body weight percent gain of $2.12\% \pm 0.63\%$ across all rats (mean $\pm$ SEM, $t = -3.085$, $df = 10$, $p = .0115$).

Vocal Fold Dehydration and Rehydration

For the mild dehydration group ($n = 13$), Mauchly's test of sphericity was not violated, $\chi^2(2) = 1.681$, $p = .432$. The normalized signal intensities within the vocal fold did not significantly change across the hydration states, $F(2, 24) = 1.342$, $p = .280$ (see Figure 3). The average normalized signal intensity for the predehydration rats was 0.2154 ± 0.0086 (mean $\pm$ SEM, range: 0.1256). The average normalized signal intensity for the dehydration rats was 0.2040 ± 0.0116 (mean $\pm$ SEM, range: 0.1332). The average normalized signal intensity after rehydration was 0.1997 ± 0.0089 (mean $\pm$ SEM, range: 0.1227).

For the moderate dehydration group ($n = 15$), Mauchly's test of sphericity was violated, $\chi^2(2) = 6.860$,

$p = .032$. Therefore, Greenhouse–Geisser was used for correction. The normalized signal intensities within the vocal fold decreased following dehydration and increased following rehydration, $F(1.418, 19.858) = 5.287$, $p = .023$, $\eta_p^2 = .274$ (see Figure 3). The average normalized signal intensity for the dehydration rats was 0.1689 ± 0.0111 (mean $\pm$ SEM, range: 0.1518). The average normalized signal intensity after rehydration was 0.2157 ± 0.0189 (mean $\pm$ SEM, range: 0.2578). Compared to the predehydration group, normalized signal intensities within the vocal fold decreased in the moderate dehydration group by an average of $14.47\% \pm 4.72\%$ (mean $\pm$ SEM, $t = 2.824$, $df = 14$, $p = .014$, Cohen's $d = 0.84$) with a range of 77.16%. Compared to the predehydration group, normalized signal intensities within the vocal fold increased in the rehydration group by an average of $9.72\% \pm 8.91\%$ (mean $\pm$ SEM, $t = -0.927$, $df = 14$, $p = .369$) with a range of 113.76%. Compared to the dehydration group, normalized signal intensities within the vocal fold increased in the rehydration group by an average of $28.24\% \pm 7.56\%$ (mean $\pm$ SEM, $t = -3.442$, $df = 14$, $p = .004$) with a range of 96.05%.

For the marked dehydration group ($n = 11$), Mauchly's test of sphericity was not violated, $\chi^2(2) = 4.195$, $p = .123$. The normalized signal intensities within the vocal fold significantly decreased after dehydration but not rehydration, $F(2, 20) = 23.406$, $p < .001$, $\eta_p^2 = .701$ (see Figure 3). The average normalized signal intensity for the predehydration rats was 0.2237 ± 0.0138 (mean $\pm$ SEM, range: 0.1245). The average normalized signal intensity for the dehydration rats was 0.1392 ± 0.0122 (mean $\pm$ SEM, range: 0.1315). The average normalized signal intensity after rehydration was 0.1542 ± 0.0119 (mean $\pm$ SEM, range: 0.1164). Compared

Figure 3. A box and whisker chart of the average normalized signal intensity for each dehydration group (mild [$n = 13$], moderate [$n = 15$], and marked [$n = 11$]) at each magnetic resonance imaging session. Systemic dehydration significantly decreased the average normalized signal intensity for the moderate and marked groups. Rehydration significantly increased the average normalized signal intensity for the moderate group but not for the marked group. Standard error of the mean bars are shown. * $p < .05$.

to the predehydration group, normalized signal intensities within the vocal fold decreased in the dehydration group by an average of $36.72\% \pm 4.91\%$ (mean $\pm$ SEM, $t = 6.221$, $df = 10$, $p < .001$, Cohen's $d = 2.05$) with a range of 51.41%. Compared to the predehydration group, normalized signal intensities within the vocal fold increased in the rehydration group by an average of $-29.08\% \pm 6.01\%$ (mean $\pm$ SEM, $t = 4.312$, $df = 10$, $p = .002$) with a range of 58.09%. Compared to the dehydration group, normalized signal intensities within the vocal fold increased in the rehydration group by an average of $12.63\% \pm 5.75\%$ (mean $\pm$ SEM, $t = -1.715$, $df = 10$, $p = .117$) with a range of 63.17%.

Discussion

This study used PD-weighted MRI to test the hypothesis that systemic dehydration would result in vocal fold dehydration and that systemic rehydration would result in vocal fold rehydration. The dependent measure of hydration status was the normalized signal intensity. At moderate systemic dehydration (6%–10% BWL), the normalized signal intensity significantly decreased. Following rehydration, the normalized signal intensity increased. This finding supports clinical recommendations to increase systemic rehydration through water intake only after an acute dehydrating event of moderate magnitude.

An interesting finding was observed with marked systemic dehydration ($> 10\%$ BWL). Following marked systemic dehydration, the normalized signal intensity significantly decreased, but did not significantly increase after rehydration. Namely, the local PD of the vocal folds, which is proportional to water content, did not increase after 1 week of having free access to water. The decrease in normalized signal intensity after marked systemic dehydration agrees with past results that showed the same reproducible trend after a 10% BWL systemic dehydration (Oleson et al., 2018). However, the 2017 paper did not evaluate normalized signal intensity in the vocal folds after rehydration. Comparisons of the data following moderate and marked dehydration suggest that the vocal folds were able to regain vocal fold hydration after recovering from an average of 7.43% BWL, but they were unable to recover from an average of 11.46% BWL, respectively. These findings agree with data quantifying the recovery in normalized signal intensity back to near predehydration levels after an induced dehydration and rehydration (King et al., 2018). In that study, the authors noted that dehydration may have induced some irreversible changes in vocal fold mucosa, typically at more extreme levels. Here, we also demonstrate this “priming effect” at dehydration levels exceeding 10% BWL. It is also noteworthy that while other studies show this irreversible phenomenon, they were *ex vivo* research (Hanson, Zhang, & Jiang, 2011; Karamzadeh et al., 2005), and *ex vivo* models lack homeostatic mechanisms to respond to dehydration. Following marked dehydration, the vocal folds may need a longer recovery time for rehydration. The effects of a phonotraumatic event during this prolonged recovery might be particularly harmful, and the

interaction of dehydration and phonotraumatic events should be studied further.

Mild systemic dehydration ($< 6\%$ BWL) did not significantly affect the normalized signal intensity of the vocal folds. One possible interpretation is that vocal fold tissue may be protected from low, everyday levels of systemic dehydration, but it is also possible that vocal folds did not dehydrate to detectable levels for PD-weighted MRI analysis. The sensitivity of PD-weighted MRI in detecting hydration changes needs further study.

All three groups were given 1 week of free access to water. Research suggests that dehydration state can be reversed in less than a week. Lecompte et al. (1981) reported that after rats were dehydrated for 4 days and allowed to rehydrate for 24 hr, hematocrit levels returned to predehydration levels. These findings suggest that the rehydration time period of 24 hr was sufficient. Logan-Sprenger and Spriet showed that after athletes ingested 600 ml of water when dehydrated, urine specific gravity was significantly decreased to within the hydrated range within 45 min (Logan-Sprenger & Spriet, 2013).

PD-weighted MRI is a useful technique to determine hydration status of tissue. The multislice multi-echo sequence used within this study gave us the ability to gather high-resolution anatomical images of the vocal folds, while also allowing us to quantify PD-weighted images for detection of hydration change across biological sex. Our data demonstrate that male and female rats did not show a statistically significant difference in PD-weighted MRI signal intensity. Additionally, this technique gives us the ability to assess hydration changes within the vocal fold *in vivo*. The absence of within-subject comparisons is the major pitfall of most laryngeal dehydration studies.

Limitations and Future Research

There are limitations that should be noted. It is possible that the surface coil during imaging may have caused a higher intensity at locations closer to the coil, but we minimized this bias throughout the protocol by controlling the placements of the surface coil, phantom, and the positioning of the rat neck during the experimental setup. Secondly, the predehydration MRI and predehydration weights were obtained on different days. The predehydration weight was computed right before beginning the water withholding protocol in order to ensure that all animals only lost body weight due to water deprivation. This decision ensured that any change in weight did not reflect stress from the imaging, stress of handling, and response to anesthetic.

The dehydration and rehydration findings from this study point to interesting paths for future research. Future extensions of this work will explore prehydrating all animals, using additional biomarkers of dehydration such as renin (Cox, Cannes do Nascimento, Pires dos Santos, & Sivasankar, 2019), and examining the interaction of estrus staging and hydration state using PD-weighted MRI. It will be important to study human subjects since data from

animal models cannot be directly translated to human speakers. Incorporating cellular and biomechanical methodologies such as gene expression and histopathology to quantify functional and structural changes will be needed to fully elucidate the pathobiology of vocal fold hydration state.

Conclusions

We utilized PD-weighted MRI to test the prevailing hypothesis that systemic dehydration induces vocal fold dehydration. Only systemic dehydration exceeding 6% BWL caused vocal fold dehydration. Male and female rats showed similar vocal fold changes due to systemic dehydration. Only the moderate dehydration (6%–10% BWL) and the marked dehydration (> 10% BWL) groups demonstrated vocal fold dehydration as assessed by PD-weighted MRI. After rehydration, the moderate dehydration group displayed an increase in normalized signal intensity, but the marked dehydration group did not.

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