Published in final edited form as:

Laryngoscope. 2018 June ; 128(6): E222–E227. doi:10.1002/lary.26978.

Proton Density Weighted Laryngeal MRI in systemically dehydrated rats

Steven Oleson, B.S.¹, Kun-Han Lu, M.S.⁴, Zhongming Liu, Ph.D.^{1,4}, Abigail C. Durkes, DVM, Ph.D.², and M. Preeti Sivasankar, Ph.D., CCC-SLP^{1,3}

¹Weldon School of Biomedical Engineering, Purdue University, West Lafayette, IN

²Department of Comparative Pathobiology, Purdue University, West Lafayette, IN

³Department of Speech, Language, and Hearing Sciences, Purdue University, West Lafayette, IN

⁴School of Electrical and Computer Engineering, Purdue University, West Lafayette, IN

Abstract

Objective—Dehydrated vocal folds are inefficient sound generators. While systemic dehydration of the body is believed to induce vocal fold dehydration, this causative relationship has not been demonstrated *in vivo*. Here we investigate the feasibility of using *in vivo* proton density (PD) weighted magnetic resonance imaging (MRI) to demonstrate hydration changes in vocal fold tissue following systemic dehydration in rats.

Method—Sprague Dawley rats (n=10) were imaged at baseline and following a 10% reduction in body weight secondary to withholding water. *In vivo*, high-field (7T), PD-weighted MRI was used to successfully resolve vocal fold and salivary gland tissue structures.

Results—Normalized signal intensities within the vocal fold decreased post-dehydration by an average of $11.38 \pm 3.95\%$ (mean $\pm$ S.E.M, $p=0.0098$) as compared to pre-dehydration levels. The salivary glands experienced a similar decrease in normalized signal intensity by an average of $10.74 \pm 4.14\%$ (mean $\pm$ S.E.M, $p=0.0195$) following dehydration. The correlation coefficient (percent change from dehydration) between vocal folds and salivary glands was 0.7145 ($p=0.0202$).

Conclusion—10% systemic dehydration induced vocal fold dehydration as assessed by PD-weighted MRI. Changes in the hydration state of vocal fold tissue were highly correlated with that of the salivary glands in dehydrated rats *in vivo*. These preliminary findings demonstrate the feasibility of using PD-weighted MRI to quantify hydration states of the vocal folds and lay the foundation for further studies that explore more routine and realistic magnitudes of systemic dehydration and rehydration.

Level of Evidence—NA

Correspondence may be addressed to: M. Preeti Sivasankar, Ph.D., CCC-SLP, Department of Speech, Language, and Hearing Sciences, Purdue University, West Lafayette, IN 47907, msivasan@purdue.edu, Phone: (765) 496 6628, Fax: (765) 494 0771.

Conflict of Interest: None

Keywords

vocal folds; proton density; magnetic resonance imaging; systemic dehydration

Introduction

Systemic hydration includes extracellular fluid in the body. Systemic dehydration is a reduction of the extracellular fluid content, and this deficiency can be induced by reducing water intake¹. Systemic dehydration of body tissue can have negative health effects. Specific to the larynx, systemic dehydration of the vocal folds is thought to have detrimental effects on vocal fold vibration and voice quality². Prolonged systemic dehydration may increase the risk for phonotraumatic lesions³. Accordingly, increasing systemic hydration by increasing fluid intake has been the mainstay of vocal hygiene programs²⁴. However, there is no direct *in vivo* evidence that systemic dehydration of the body induces dehydration of the vocal fold tissue. In other words, there are no studies that have quantified changes to the same vocal fold tissue in a living animal before and after systemic dehydration.

Current knowledge on the ill-effects of vocal fold dehydration arise largely from studies that have dehydrated the tissue *ex vivo*⁵⁶⁷⁸⁹¹⁰. Examples of these *ex vivo* dehydration methods include submerging tissue samples in hypertonic solutions like sucrose⁶, drying tissues with vacuum ovens⁷, or using air compressors to blow dry air over tissue samples for extended periods of time⁸. The ensuing changes in the material and mechanical properties of vocal fold tissue then lead to increased phonation threshold pressure (PTP; the minimum lung pressure required to initiate and sustain vocal fold vibration), and an overall decreased vocal efficiency⁵¹¹ indicating that dehydration of the vocal folds will degrade laryngeal performance¹¹. Some *in vivo* human subject studies support this hypothesis with data showing increased PTP after water removal with diuretics or dialysis¹²¹³. However, PTP is an indirect measure of vocal fold function. Direct assessment of vocal fold lamina propria (the layer that is critical to optimal vibration and likely to be affected by dehydration due to diuretics and dialysis) was not conducted in these *in vivo* studies.

An alternative methodology is needed to investigate the effects of systemic dehydration on vocal fold tissue *in vivo* because homeostatic mechanisms that regulate water distribution are not available in *ex vivo* tissue. In this study, we explored the utility of proton density (PD)-weighted magnetic resonance imaging (MRI) to assess the effects of systemic dehydration *in vivo*. This technique allows for pre-dehydration and post-dehydration quantification in the same animal.

The purpose of this study was to investigate whether systemic dehydration would dehydrate the vocal folds and salivary glands when utilizing PD-weighted MRI as the observational tool. To the best of our knowledge, PD-weighted MRI has never been used to assess hydration states within the vocal folds of rats, although it has been used to assess water content in other tissue¹⁴¹⁵¹⁶¹⁷¹⁸. Systemic dehydration was defined as 10% loss in body weight resulting from withholding water. A loss of 1% body weight due to diuretic ingestion in young, healthy adults can negatively affect voice¹³. In athletes, body weight loss >2% may impair performance and compromise function¹⁹²⁰. But in a recent meta-analysis,

Goulet²¹ reported that reductions of up to 4% in body weight may not hinder endurance performance. A 10% decrease in body weight is a significant challenge and while unlikely in routine situations was intentionally selected to provide direct evidence that systemic dehydration can change vocal fold tissue. As such we will continue to examine more realistic changes in body weight in future studies. In the current study, the relative hydration state of the regions of interest (vocal folds and salivary glands) was quantified as normalized signal intensities calculated from each region. We hypothesized that systemic dehydration would dehydrate the vocal folds and salivary glands, and that this dehydration would be quantifiable with PD-weighted MRI. Salivary glands were chosen as an additional region of interest because they are easily and repeatedly identified in rat, and can be visualized in the same transverse anatomical plane of section as the vocal folds, and could serve as a control tissue in future human subject studies.

Materials and Methods

Experimental Animals

Ten male, Sprague Dawley (SD) rats (pre-dehydration 400–460g, < 6 months, Envigo Inc, Indianapolis, IN) were used in this study in accordance with approved protocols by the Purdue Animal Care and Use Committee (PACUC). Rats were acclimated and imaged at two time-points (pre-dehydration and post-dehydration). The two-imaging time-points were 1 week apart per recommendations by the PACUC.

Magnetic Resonance Imaging (MRI)

Imaging was conducted with a 7T MRI system (BioSpec 70/30, Bruker Instruments, Billerica, MA, USA). 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-weighted and T₂-weighted anatomical images were acquired with a Multi Slice Multi Echo (MSME) acquisition sequence (20 slices with 0.5 mm thickness and 0.5 × 0.5 mm² in-plane resolution, repetition time (TR) = 5000ms, averages = 1, repetitions = 1, echo spacing = 10ms, echo images = 2, echo time (TE) = (15ms, 75ms), flip angle = 90°, field of view = 40 × 40 mm²). The first echo image is taken as the PD-weighted image. An external cylindrical phantom (CuSO₄ × 2H₂O solution) was placed within the field of view for intensity normalization.

Rats were voided of urine and then weighed before anesthetization and MR imaging. Rats were anesthetized with isoflurane (500 ml/min, 4% anesthesia level) and anesthetization was maintained throughout MR scanning (500 ml/min, 2.2% anesthesia level). Respiratory rate was monitored using a MR-compatible small animal monitoring and gating system (SA Instruments, Inc., Stony Brook, NY, USA). The temperature was controlled using a water heating system (Thermo Scientific, Waltham, MA, USA). 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 kept at 37°C.

Rats were placed supine in the body holder with their noses positioned in a nose cone within the custom head holder. Masking tape was taped horizontally across the neck region. The

receive-only surface ring coil was placed horizontally on the masking tape to ensure the surface ring coil was level and as close to the neck as possible. Additionally, an external cylindrical phantom was placed alongside the rat neck directly under the masking tape. This ensured that the position of the phantom was kept static between rats. The same procedure was used for imaging pre-dehydration and post-dehydration.

Vocal folds and salivary glands of each animal were imaged. In consultation with a laryngologist, it was determined that the most successful method to locate the vocal fold was to first locate the subglottis which was reproducibly indicated by a bright ring in the PD weighted images. After the subglottis was located, the vocal fold slice was consistently one slice cranial to the subglottis slice (Figure 1). After the vocal fold slice was located, the salivary glands were found within the same slice. Both salivary glands were reproducibly pinpointed by two large, discrete regions directly above the vocal folds.

Dehydration Procedure

Animals were voided of urine and a baseline (pre-dehydration) weight was measured. To achieve 10% loss in body weight, animals were deprived of water and allowed *ad lib* food. Animals were weighed at 4hr, 8hr, 12hr, 24hr, 48hr, 60hr, and 72hr following water withholding. Anesthetization and MR imaging began once 10% weight loss was reached, or 72 hours was reached. The average weight loss was 10% at this time point (see results). Reduced food intake may accompany water deprivation in a potential effort to stabilize hydration status. To acknowledge this confound, the data are presented as an outcome of body weight loss (significantly influenced by water withholding and ensuing dehydration) rather than as an outcome of water deficit.

MRI Analysis

MRI data were masked manually using FslView²² for the vocal fold, salivary gland, and phantom region (MATLAB, MathWorks, Natick, MA, Figure 2). A custom MATLAB code that was built in-house was used to extract the signal intensities within the three mask regions. The signal intensities within all mask regions were then averaged. The average vocal fold and salivary gland signal intensities were finally normalized by dividing by the average phantom signal intensity. This calculation represents the local hydration level in each region.

Statistical Analysis

Body weights for pre-dehydration and post-dehydration of all rats were also assessed for normality by using visual inspection of raw data plots and Shapiro-Wilk's test. All body weight data was normally distributed. Therefore, a paired t-test was used for comparison between pre-dehydration and post-dehydration body weights to confirm dehydration. The significance level was 0.05.

The signal intensities were assessed for normality by using visual inspection of raw data plots and Shapiro-Wilk's test. The data were not normally distributed, hence Wilcoxon Signed-Rank nonparametric test was used for comparison between pre-dehydration and post-dehydration conditions. The significance level was 0.05.

The correlation between the change in vocal fold normalized signal intensity and the change in salivary gland normalized signal intensity was calculated using Pearson correlation coefficient test. The p-value between these groups was also calculated for testing the hypothesis that there is no relationship between the observed phenomena. The significance level was 0.05.

Results

Dehydration

Dehydration was defined as a 10% reduction in body weight due to water withholding. The average pre-dehydration weight for the rats was 430.5 ± 3.74 g (mean $\pm$ S.E.M, range between highest and lowest values = 38 g). The average post-dehydration weights for the rats was 383.6 ± 3.25 g (mean $\pm$ S.E.M, range = 35 g). Compared to the pre-dehydration weights, this resulted in an average body weight percent loss of $10.89 \pm 0.27\%$ across all rats (mean $\pm$ S.E.M, $p=0.0001$).

Vocal Fold Dehydration

The average normalized signal intensity of the vocal fold for the pre-dehydration rats was 0.2968 ± 0.0132 (mean $\pm$ S.E.M, range = 0.1433). The average normalized signal intensity of the vocal fold for the post-dehydration rats was 0.2529 ± 0.0105 (mean $\pm$ S.E.M, range = 0.1149). Compared to the pre-dehydration group, normalized signal intensities within the vocal fold decreased in the post-dehydration group by an average of $11.38 \pm 3.95\%$ (mean $\pm$ S.E.M, $p=0.0098$) with a range of 31.96% (Figure 3).

Salivary Gland Dehydration

The average normalized signal intensity of the salivary gland for the pre-dehydration rats was 0.8380 ± 0.0450 (mean $\pm$ S.E.M, range = 0.4950). The average normalized signal intensity of the salivary gland for the post-dehydration rats was 0.7150 ± 0.0534 (mean $\pm$ S.E.M, range = 0.5262). Compared to the control group, normalized signal intensities decreased in the post-dehydration group by an average of $10.74 \pm 4.14\%$ (mean $\pm$ S.E.M, $p=0.0195$) with a range of 37.63% (Figure 4).

Dehydration Correlation of Vocal Fold and Salivary Gland

The correlation coefficient (percent change from dehydration) between vocal folds and salivary glands was 0.7145 ($p=0.0202$).

Discussion

This work establishes an MR imaging-based method to assess the level of water content within the vocal folds and the salivary glands in systemically hydrated and dehydrated states. The Multi Slice Multi Echo (MSME) sequence provides not only a high-resolution anatomical definition of the vocal folds, but also quantification of hydration with PD-weighted MR images in the same animal. As aforementioned, a pre-dehydration and post-dehydration of 10% body weight loss from withholding water showed a statistically significant difference in PD-weighted signal intensity. To the best of our knowledge, there

are no past studies on rat vocal folds that have utilized PD-weighted MRI to characterize hydration states, and only a very recent study has attempted to use MRI to image rat vocal folds *in vivo*²³.

The vocal fold showed a reproducible trend of a decrease in normalized signal intensity after systemic dehydration. This was the first study to use PD-weighted MRI for quantification of dehydration levels within the vocal fold, so we were unable to compare our findings directly to past results. However, we confirmed that dehydration was present in all rats through a primary marker of hydration status, which was the reduction in body mass. Body mass measurement, blood indices, urine indices, and bioelectrical impedance analysis are all considered markers of body hydration²⁴. Additionally, our procedure of *in vivo* systemic dehydration and imaging overcame limitations of past dehydration studies by quantifying water loss within the same animal thus eliminating confounding variables. *In vivo* imaging accounts for homeostatic mechanisms that regulate water content and is a more accurate representation of vocal fold hydration. Wu and Zhang used a computational model to link increased dehydration with increased vibration amplitude, increased epithelial permeability, and reduced water resupply, but there were simplifications and assumptions made within that study as acknowledged by the authors²⁵. Hanson et al. used a tissue dehydration method of *ex vivo* systemic dehydration with a vacuum oven to simulate dehydration of the tissue beyond the epithelium. They noted this procedure as a limitation of having to conduct dehydration as an *ex vivo* process. Furthermore, dehydrating tissues with a 40°C vacuum oven temperature could affect protein folding within the region of interest⁷. Dehydrating *in vivo* is an ecologically valid and physiologically relevant method that is similar to what speakers experience in everyday life.

An interesting trend observed from this experiment was how the vocal folds and the salivary glands were similarly affected from dehydration. The percent change of the vocal fold after dehydration yields a correlation value of 0.7145 with the percent change of the salivary gland after dehydration. This high correlation value shows that the salivary gland may be used as a positive control when detecting for dehydration with PD-weighted MRI, given the larger tissue size of the glands and thus better signal-to-noise ratio. This finding therefore warrants investigation in future human subject studies.

It is important to note that a surface coil was used in the current study. The use of a surface coil provided high signal to noise ratio in PD-weighted MRI to greatly benefit the quantification of vocal fold hydration. However, the inhomogeneous coil sensitivity profile (or B_1) caused spatial bias in intensity: higher intensity at locations closer to the coil, whereas lower intensity at more distant locations. This intensity bias was largely mitigated by using a bar-phantom as the external reference for each experiment. Additionally, the relative positions of the phantom, the rat neck, and the surface coil were kept consistent across experiments with a customized holder. Moreover, the focus of this study was on the longitudinal changes of proton density at a specific region, rather than comparing the intensity difference between regions. Spatial variation in image intensity due to a fixed coil sensitivity profile was unlikely to explain the intensity variation over time from dehydration. As a B_1 mapping sequence was not available in our MRI system, future studies will benefit from a customized sequence for mapping and correcting B_1 inhomogeneity.

This experiment does not directly link the hydration changes of the vocal fold to changes in functionality. Additionally, the technique did not allow for resolution of vocal fold layers to determine where the hydration changes were occurring. Therefore, future work is needed that will include mechanical testing of the post-dehydration vocal folds. Yang et al. quantified the effects of dehydration on the viscoelastic parameters of the vocal fold mucosa. They show that the linear portion of the stress-strain curve and Young's modulus increases with dehydration¹⁰. Quantifying mechanical properties in future studies will allow us to validate PD-weighted MRI dehydration results with structural tissue changes. It will also be necessary to use this imaging modality to investigate the effects across different species. Finally, we acknowledge that a decrease of 10% body weight is moderately extreme, since even changes in body weight of 5% induce fatigue, lethargy, and other CNS changes²⁶. However, individuals may lose up to 10% of body weight in sweat loss during challenging athletic events¹. Hence, although our magnitude of body weight loss is significantly greater than what individuals may experience in a daily basis, it has been reported in human subjects. The purpose of this preliminary study was to show the sensitivity of PD-weighted MRI to changes in water content of the vocal fold tissue. This study will lead to follow up studies assessing the sensitivity of MRI to smaller and more realistic magnitudes of dehydration (1% and 5%) and rehydration levels.

Conclusion

Here we demonstrate the feasibility of using PD-weighted MRI to quantify changes in hydration level of the vocal folds as a result of water withholding. The 10% reduction in body weight induced changes in vocal fold and salivary gland tissue that were detected by MRI. Further technical development and validation of functional differences, within the vocal folds due to dehydration, will be the goal of future studies.

Acknowledgments

We greatly acknowledge the assistance of Stacey Halum, MD, for identifying the vocal fold slice and region of interest (ROI). The study was funded by R01DC011759 (National Institutes of Health/National Institute on Deafness and other Communication Disorders).

References

1. Popkin BM, Rosenberg IH. NIH Public Access. NIH Public Access. 2011; 68(8):439–458. DOI: 10.1111/j.1753-4887.2010.00304.x. Water
2. Sivasankar M, Leydon C. The role of hydration in vocal fold physiology. *Curr Opin Otolaryngol Head Neck Surg*. 2010; 18(3):171–175. DOI: 10.1097/MOO.0b013e3283393784 [PubMed: 20386449]
3. Verdolini-Marston K, Sandage M, Titze IR. Effect of hydration treatments on laryngeal nodules and polyps and related voice measures. *J Voice*. 1994; 8(1):30–47. DOI: 10.1016/S0892-1997(05)80317-0 [PubMed: 8167785]
4. Hartley NA, Thibeault SL. Systemic hydration: Relating science to clinical practice in vocal health. *J Voice*. 2014; 28(5):652.e1–652.e20. DOI: 10.1016/j.jvoice.2014.01.007
5. Finkelhor BK, Titze IR, Durham PL. The effect of viscosity changes in the vocal folds on the range of oscillation. *J Voice*. 1988; 1(4):320–325. DOI: 10.1016/S0892-1997(88)80005-5
6. Chan RW, Tayama N. Biomechanical effects of hydration in vocal fold tissues. *Otolaryngol - Head Neck Surg*. 2002; 126(5):528–537. DOI: 10.1067/mhn.2002.124936 [PubMed: 12075228]

7. Hanson KP, Zhang Y, Jiang JJ. Ex vivo canine vocal fold lamina propria rehydration after varying dehydration levels. *J Voice*. 2011; 25(6):657–662. DOI: 10.1016/j.jvoice.2010.06.005 [PubMed: 20951551]
8. Li L, Zhang Y, Maytag AL, Jiang JJ. Quantitative Study for the Surface Dehydration of Vocal Folds Based on High-Speed Imaging. *J Voice*. 2015; doi: 10.1016/j.jvoice.2014.09.025
9. Li L, Zhang Y, Calawerts W, Jiang JJ. Vibratory Dynamics of Four Types of Excised Larynx Phonations. *J Voice*. 2016; 30(6):649–655. DOI: 10.1016/j.jvoice.2015.08.012 [PubMed: 26476848]
10. Yang S, Zhang Y, Mills RD, Jiang JJ. Quantitative Study of the Effects of Dehydration on the Viscoelastic Parameters in the Vocal Fold Mucosa. *J Voice*. 2016; doi: 10.1016/j.jvoice.2016.05.002
11. Jiang JJ, Verdolini K, NG J, Aquino B, Hanson D. Effects of dehydration on phonation in excised canine larynges. *Ann Otol Rhinol Laryngol*. 2000; (2)
12. Fisher KV, Ligon J, Sobecks JL, Roxe DM. Phonatory effects of body fluid removal. *J Speech Lang Hear Res*. 2001; 44(2):354–367. DOI: 10.1044/1092-4388(2001/029) [PubMed: 11324657]
13. Verdolini K, Min Y, Titze IR, et al. Biological mechanisms underlying voice changes due to dehydration. *J Speech Lang Hear Res*. 2002; 45(2):268–281. doi:10.1044/1092-4388(2002/021). [PubMed: 12003510]
14. Meyers SM, Tam R, Lee JS, et al. Does hydration status affect MRI measures of brain volume or water content? *J Magn Reson Imaging*. 2016; 44(2):296–304. DOI: 10.1002/jmri.25168 [PubMed: 26825048]
15. Whittall KP, Mackay AL, Graeb DA, Nugent RA, Li DKB, Paty DW. In vivo measurement of T2 distributions and water contents in normal human brain. *Magn Reson Med*. 1997; 37(1):34–43. DOI: 10.1002/mrm.1910370107 [PubMed: 8978630]
16. Meyers SM, Kolind SH, MacKay AL. Simultaneous measurement of total water content and myelin water fraction in brain at 3T using a T2 relaxation based method. *Magn Reson Imaging*. 2017; 37:187–194. DOI: 10.1016/j.mri.2016.12.001 [PubMed: 27923744]
17. Mackay A, Whittall K, Adler J, Li D, Paty D, Graeb D. In vivo visualization of myelin water in brain by magnetic resonance. *Magn Reson Med*. 1994; 31(6):673–677. DOI: 10.1002/mrm.1910310614 [PubMed: 8057820]
18. Meyers SM, Kolind SH, Laule C, MacKay AL. Measuring water content using T2 relaxation at 3 T: Phantom validations and simulations. *Magn Reson Imaging*. 2016; 34(3):246–251. DOI: 10.1016/j.mri.2015.11.008 [PubMed: 26657977]
19. Magee PJ, Gallagher AM, McCormack JM. High prevalence of dehydration and inadequate nutritional knowledge among university and club level athletes. *Int J Sport Nutr Exerc Metab*. 2017; 27(2):158–168. DOI: 10.1123/ijnsnem.2016-0053 [PubMed: 27710146]
20. Casa DJ, Clarkson PM, Roberts WO. American College of Sports Medicine roundtable on hydration and physical activity: consensus statements. *Curr Sports Med Rep*. 2005; 4(3):115–127. DOI: 10.1097/01.CSMR.0000306194.67241.76 [PubMed: 15907263]
21. Goulet EDB. Effect of exercise-induced dehydration on time-trial exercise performance: a meta-analysis. *Br J Sports Med*. 2011; 45(14):1149–1156. DOI: 10.1136/bjsm.2010.077966 [PubMed: 21454440]
22. Smith SM, Jenkinson M, Woolrich MW, et al. Advances in functional and structural MR image analysis and implementation as FSL. *Neuroimage*. 2004; 23(SUPPL. 1):208–219. DOI: 10.1016/j.neuroimage.2004.07.051
23. Kishimoto AO, Kishimoto Y, Young DL, Zhang J, Rowland IJ, Welham NV. High- and ultrahigh-field magnetic resonance imaging of naïve, injured and scarred vocal fold mucosae in rats. *Dis Model Mech*. 2016; 9(11):1397–1403. DOI: 10.1242/dmm.026526 [PubMed: 27638667]
24. Shirreffs SM. Markers of hydration status. *J Sports Med Phys Fitness*. 2000; 40(1):80–84. DOI: 10.1038/sj.ejcn.1601895 [PubMed: 10822913]
25. Wu L, Zhang Z. A computational study of vocal fold dehydration during phonation. *IEEE Trans Biomed Eng*. 2017; 9294(c):1-1. doi: 10.1109/TBME.2017.2691399
26. Lieberman HR. Hydration and cognition: a critical review and recommendations for future research. *J Am Coll Nutr*. 2007; 26(5 Suppl):555S–561S. doi:10.1080/07391630701411111 [PubMed: 17921465]

Figure 1.

Proton density weighted images showing the location of the larynx. Images in column 'A' show the subglottis slice. Images in column 'B' show the vocal fold slice, located one slice cranial to the subglottis slice. S1 and S2 rows indicate two different animals to show reproducibility of the MR imaging procedure.

Figure 2. Proton density weighted images showing the vocal fold slice. Image 'A' shows the slice before a manual mask has been applied. Image 'B' shows the slice after a manual mask has been applied.

Figure 3.

Scatter plot showing the pre-dehydration and post-dehydration normalized signal intensities of the vocal fold. The black circles show the pre-dehydration normalized signal intensities.

The white circles show the post-dehydration normalized signal intensities.

Figure 4. Scatter plot showing the pre-dehydration and post-dehydration normalized signal intensities of the salivary gland. The black circles show the pre-dehydration normalized signal intensities. The white circles show the post-dehydration normalized signal intensities.