Is Bound Water a Surrogate for Collagen Matrix Density? Insights from 1H Zero Echo-Time MRI

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Introduction: Bone is composed of two main constituents: an apatite-like mineral deposited within a type I collagen matrix. Even though osteoporosis and osteomalacia, the two most common disorders of bone mineral homeostasis, are indistinguishable on in vivo x-ray-based bone mineral density examinations, they differ in their effects on the density of the collagen matrix. Osteoporosis, which involves structural degradation and bone tissue loss, results in a proportionate loss of both collagen and mineral, while osteomalacia involves impaired bone mineralization only. Recent advances in solid-state ^{1}H MRI have shown promise for imaging and quantification of bound, pore, or total bone water [1-4] (a schematic T_2 spectrum is shown in Fig. 1). The shortest-T_2 (~20-50 μs) component of bone ^{1}H signal arises from protons in the collagen backbone of bone matrix; however, due to their very short lifetime, these protons have thus far eluded spatial encoding and have been detected only spectroscopically [5,9]. In this work, we explore the feasibility of detecting bone matrix collagen in fully D_2O-exchanged bone on a 9.4T micro-imaging scanner with zero echo-time (ZTE) imaging, and establish the validity of the bound water signal as a surrogate for bone matrix density.

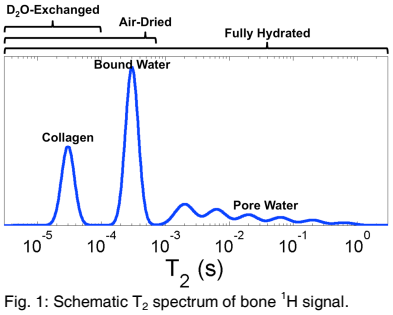

Fig. 1: Schematic T_2 spectrum of bone ^{1}H signal.

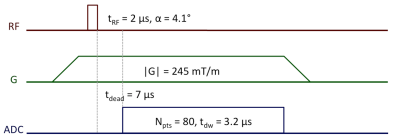

Fig. 2: 3D radial zero echo time (ZTE) pulse sequence with parameters used.

Methods:

Specimens: Five cylindrical pieces of cortical bone (4 mm diameter, 10 mm length) were cut from the midshaft of a lamb tibia and stored in phosphate-buffered saline (PBS). Prior to imaging and spectroscopy, one specimen was air-dried at room temperature for 18 hours to remove pore water, leaving only bound water and collagen ^{1}H signal [6] ('air-dried' specimen). All bound and pore ^1H_2O in the second specimen was exchanged by immersion in 2 mL of 99.9% D_2O-saline for 2 weeks (solution was changed for fresh D_2O-saline at 1 week), leaving only collagen ^{1}H signal ('D_2O-exchanged' specimen). Imaging and spectroscopy were then performed on these two specimens and one 'fully hydrated' specimen. Finally, all specimens were re-exchanged to ^1H_2O-saline, air-dried, imaged, D_2O-exchanged, and re-imaged. Bone signal intensity was normalized to the intensity of the RF coil plastic in all images, and the ratio of D_2O-exchanged to air-dried normalized intensities was calculated to semi-quantitatively assess the relationship between bound water and collagen densities. D_2O-exchange and air-drying are reversible manipulations.

MRI: Bones were imaged using a commercial ZTE pulse sequence (Bruker, Billerica, MA). The pulse sequence and parameters are given in Fig. 2. During the 7 μs transmit/receive dead time, several k-space points in the center of k-space are lost. The image is reconstructed algebraically by 8-fold readout oversampling and the assumption of finite image support [7]. Other parameters: BW=312.5 kHz, FOV=30x30x60 mm, matrix=160^3, resolution=188x188x375μm^3, 80892 projections, 8 signal averages, 21m34s scan time.

Spectroscopy: D_2O-exchanged, air-dried, and fully hydrated bones were scanned using simple pulse-acquire spectroscopy (16k points, BW=200kHz, TR=2s, 128 signal averages, pulse duration=2μs, flip angle=20deg) and in-phase double-quantum filtered NMR [8] (IP-DQF, 16k points, BW=200kHz, TR=2s, 512 averages, 256-step phase cycle, creation time=25μs, 90deg pulse duration=9.7μs) at 9.4T as a means to isolate the dipolar splittings of the collagen ^{1}H signal optimized to detect the 40 kHz splittings resulting from dipolar coupling between the geminal backbone protons in the collagen matrix [9].

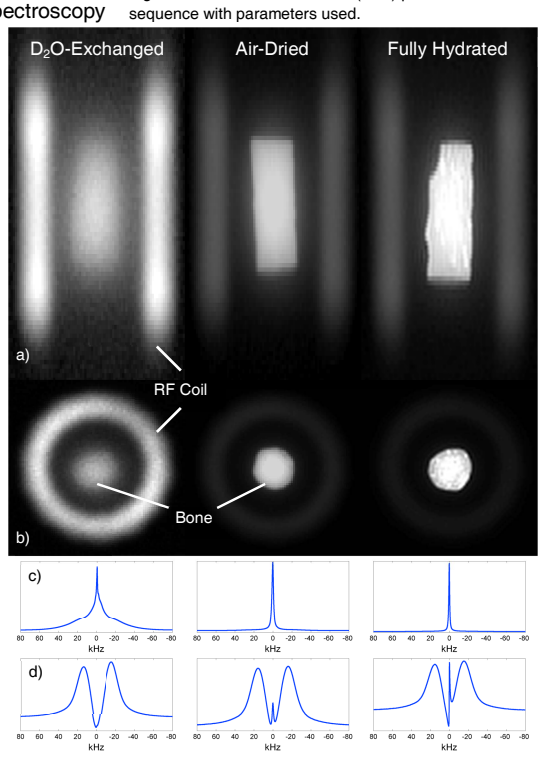

Fig. 3: Sagittal (a) and axial (b) views of D_2O-exchanged, air-dried, and fully H_2O-hydrated bone specimens, with corresponding pulse-acquire (c) and IP-DQF (d) spectra. Signal from the cylindrical plastic RF coil support is visible.

Results: ZTE images of the three bone specimens are shown in Fig. 3a and b. The fully hydrated and air-dried bone images are composed mostly of longer-T_2 bone water signal, and appear much sharper than the D_2O-exchanged bone, which contains only signal from non-labile and highly motionally restricted collagen ^{1}H. The corresponding pulse-acquire and IP-DQF spectra (Fig. 3c and d, respectively) are shown below. D_2O-exchanged spectra are consistent with previously published results [9]. As pore and bound water are removed, the NMR line broadens, consistent with the removal of longer-T_2 proton constituents. In all bones, a similar 40-kHz Pake doublet is visible at a creation time of 25 μs, confirming that collagen protons are not exchanged or removed. The ratio of D_2O-exchanged to air-dried image intensity across five bones was 31.9%±1.4% (μ±σ).

Discussion and Conclusions: Horch et al. had assigned a 60-μs T_2 component of bone ^{1}H signal at 4.7T to collagen [5]. Using a 245 mT/m gradient, $T_2^* \approx T_2 = 60$ μs would yield a point spread function (PSF) full width at half maximum (FWHM) of approximately 500 μm in the x- and y-dimensions and 1000 μm in the z-dimension, roughly consistent with the observed blurring in the D_2O-exchanged bone. The broad line of the D_2O-exchanged bone in Fig. 3c is the superposition of many dipolar splittings due to the highly restricted motion of collagen protons. The sharp central peak likely corresponds to proton pairs at or near the magic angle. The relatively constant ratio of air-dried to D_2O-exchanged bone intensity (corresponding to bound water + collagen density, and collagen density, respectively) across multiple specimens supports the hypothesis that bound water density is proportional to and is a surrogate for bone matrix density.

References: [1] Horch RA, et al. MRM 2012;68(6):1774-84. [2] Du J, et al. JMR 2010;208:304-11. [3] Wu Y, et al. MRM 2003;50:59-68. [4] Techawiboonwong, et al. NMR Biomed 2008;21:59-70. [5] Horch RA, et al. MRM 2010;64:680-7. [6] Biswas R, et al. Bone 2012;50:749-55. [7] Kuethe DO, et al. JMR 1999;139:18-25. [8] Eliav U, et al. JMR 1999;137:295-310. [9] Ong HH, et al. JBMR 2012;27(12):2573-81.

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