Comparison of Relaxation-Based NMR Methods for Quantifying Bound and Pore Bone Water Fractions

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Introduction: Increased cortical porosity is a major cause of the impaired strength of osteoporotic bone. Micro-computed tomography (μCT) is a gold-standard method for quantification of cortical porosity [1], but this method requires long scan times, and segmentation of pores is sensitive to user input. Gravimetry, in which the masses of water, organic matter, and mineral are determined by drying and ashing the bone, is a widely accepted validation method [2], but results in destruction of the specimen. In this work, we compare several NMR methods for quantifying bound and pore water, which are biomarkers for matrix density and porosity, respectively: (1) 1D T_2* [3,4] and (2) 2D T_1-T_2* [5] bi-component fitting at 3T, (3) 1D T_2 [6] and (4) 2D T_1-T_2 bi-component fitting at 9.4T, and (5) ^{2}H inversion-recovery (IR) at 9.4T [7]. These methods have the advantage of being non-destructive, relatively quick, and less dependent on user input.

Methods:

Specimens: 15 cylindrical samples of human cortical bone (8F, 27-97 y; 7M, 37-93 y) were cut from tibial specimens. The long axis of each cylinder was perpendicular to the anatomic axis of the bone, so this axis can be oriented parallel to B_0 in a solenoidal RF coil.

NMR: Bone samples were scanned in a 3T human MRI scanner (Siemens, Erlangen, Germany) using a saturation-recovery (SR)-FID pulse sequence [8] of the form [90°-SPOIL]$_{12}$-T_{SR}-90°-ACQ. Readout bandwidth was 250 kHz, and 12 T_{SR}s were arrayed exponentially from 3 ms to 6 s. Samples were then scanned in a 9.4T NMR spectrometer (Bruker, Billerica, MA) using both a SR-FID sequence and a SR-CPMG sequence of the form [90°-SPOIL]$_{12}$-T_{SR}-90°-[TE/2-180°-TE/2]$_n$-ACQ. Echo spacing was 200 μs with the loop iterator n arrayed logarithmically from 0 to 5000, and all other parameters were identical to those used at lower fields. Finally, labile protons were exchanged with ^{2}H by immersion in 99.9% D_2O-PBS for six days, and the D_2O-exchanged bones were scanned using ^{2}H IR with pulse durations t_{90}/t_{180} = 30/60 μs. A ^{2}H spectrum consists of a narrow central peak with T_1 = 200±40 ms corresponding to free D_2O in pores, flanked by a doublet with T_1 = 11±2 ms arising from D_2O whose motion is anisotropically restricted due to interaction with matrix collagen [7]. The integral of the spectrum with the narrow pore water peak nulled by inversion-recovery divided by the integral of the fully relaxed spectrum yields bound water fraction. Scan times for these five methods were: 1D T_2*, 3 min; 2D T_1-T_2*, 6 min; 1D T_2, 4 min; 2D T_1-T_2, 29 min; and ^{2}H IR, 21 min.

Analysis: Reconstruction and fitting were performed in Matlab (Mathworks, Natick, MA). For $T_2^{(*)}$ bi-exponential fitting, a sum of two decaying exponentials, $f(t) = ae^{\frac{-t}{b}} + ce^{\frac{-t}{d}} + e$, was fitted to the CPMG echo train (or FID) after the longest T_{SR} = 6 s by non-linear least squares. Two-dimensional T_1-$T_2^{(*)}$ bi-component fitting, which should improve accuracy [5], was performed by fitting $f(T_{SR}, t) = g(1 - e^{\frac{-T_{SR}}{h}})e^{\frac{-t}{k}} + m(1 - e^{\frac{-T_{SR}}{n}})e^{\frac{-t}{p}} + q$ to SR-CPMG (or SR-FID) data arrays.

μCT: Bones were scanned on a Scanco μCT35 scanner (Scanco, Brüttisellen, Switzerland) at 18.5-μm isotropic resolution. Bone exteriors were masked by active snakes in ITK-SNAP [10], and pores were segmented by thresholding. Porosity was calculated as pore volume / total volume.

Gravimetry: The fully hydrated samples were then weighed, dried at 105° C for 110 hr to remove all bound and pore water, re-weighed, ashed at 600° C for 30 hr to burn off all organic matrix, and weighed again. Organic matrix density was quantified as the difference between dry and ash masses divided by total volume measured by μCT.

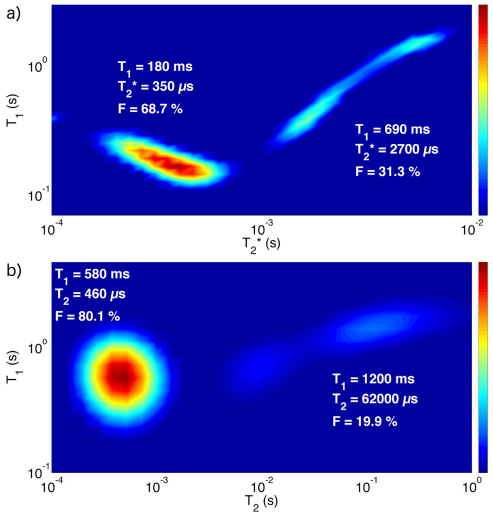

Fig. 1: a) T_1-T_2* 2D relaxation spectrum at 3T and b) T_1-T_2 spectrum at 9.4T (generated using the MERA software package [9]) of a bone specimen taken from a 37 y/o male donor. The two pools, corresponding to bound and pore water, are separated by several orders of magnitude in the T_2 dimension, but by much less in T_2*. Addition of the T_1 dimension slightly improves results relative to 1D T_2 or T_2* fitting alone.

	μCT Porosity	Organic Density	9.4T 2H IR Bound Fraction	3T 1D T2* Short-T2* Fraction	3T 2D T1-T2* Short-T2* Fraction	9.4T 1D T2 Short-T2 Fraction
9.4T 2D T1-T2 Short-T2 Fraction	0.90	0.89	0.66	0.50	0.74	1.00
9.4T 1D T2 Short-T2 Fraction	0.87	0.88	0.67	0.49	0.72	
3T 2D T1-T2* Short-T2* Fraction	0.76	0.61	0.51	0.26		
3T 1D T2* Short-T2* Fraction	0.50	0.44	0.46			
9.4T 2H IR Bound Fraction	0.50	0.46				
Organic Density	0.91					

Table 1: Matrix of R^2 values between parameters. Color indicates the strength of the correlation, from green (strong) to red (weak).

Results: Two example 2D relaxation spectra, generated by non-negative least squares, are shown in Fig. 1. Pore water is distributed across a broad range of relaxation times; this presumably reflects a large distribution of pore sizes and orientations within cortical bone. A T_1-T_2* spectrum (Fig. 1a) shows the two components separated by a factor of 8, while a T_1-T_2 spectrum (Fig. 1b) shows the components separated by over two orders of magnitude (also note the difference in x-axis limits between panels a and b). A correlation matrix (Table 1) provides the strengths of the correlations between the various NMR and validation measurements. T_2 fitting of CPMG data at 9.4T is found to outperform T_2* fitting of FID data at 3T, while ^{2}H IR at 9.4T performs approximately as well as 1D T_2* fitting. Addition of the T_1 dimension yields improved fidelity for both T_2 and T_2* fitting.

Discussion and Conclusions: Success of bi-exponential fitting generally improves as the separation of the time constants of the two pools increases. Because the T_2* of pore water is substantially shortened by dephasing due to internal magnetic field gradients arising from the susceptibility difference between water and bone ($\Delta\chi$ ~ 2.5 ppm SI), T_2* bi-component fitting of FIDs is at a disadvantage compared to T_2 fitting of CPMG echoes. Surprisingly, ^{2}H IR, which relies on the large T_1 difference between bound and free D_2O, was found to perform less well than T_2 bi-component analysis. This may be due to a distribution of T_1 values within the pore D_2O pool, rendering it impossible to fully null this component. These results show that bi-component fitting of CPMG echo amplitudes is a reliable method for quantification of bound and pore water, while other methods should be used with caution.

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