Quantification of bound and mobile water in human cortical bone by 1H and 2H Magnetic Resonance

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Introduction

Magnetic resonance is a powerful tool for non-destructive study of bone water. Bone water (BW) occupies an elaborate network of channels that establish communication between the blood supply and osteocytes covering a size scale ranging from 0.1 to 100μm. Another significant fraction of BW is associated with the collagen matrix, which has anisotropic rotational motion and thus is expected to give rise to residual dipolar and quadrupolar splittings in ^{1}H and ^{2}H spectra, respectively[1]. A quantitative assessment of cortical bone water, and thus porosity, requires an understanding of the various contributions to the overall MR signal. Here, we hypothesize that water in pores is predominantly free (i.e. 'mobile') and water in the bone matrix is predominantly associated with collagen (i.e. 'bound'). Therefore, mobile water content may be an estimate for bone porosity. Using ^{2}H exchange to quantify total BW[2] and differences in T_1 relaxation times to estimate relative fractions of bound and mobile water, we compared porosity estimates from NMR and micro-CT in human cortical bone specimens.

Materials and Methods

The left tibia from a 65 year-old Caucasian female donor was purchased from the Musculoskeletal Transplant Foundation. A 3cm slab centered at 38% of the tibial length was cut and cylindrical specimens (3x14mm, dia. x length) were collected from the posterior, medial, lateral, and anterior sides. Fig. 1 summarizes the following procedure. Each specimen was immersed in 99.8% D_2O saline for ~48hrs at 50C. Total BW was calculated by measuring the amount of H_2O that had exchanged into the D_2O saline with a calibration curve by integration of the water NMR line[2]. Each D_2O saturated specimen was blotted dry and placed in a 5mm NMR tube and a ^{2}H inversion recovery (IR) experiment was performed (inversion time (TI): 50μs to 4s in 24 steps) on a 9.4T spectrometer (DMX-400, Bruker Instruments) and the following processing was done using Xwin-NMR software. T_1 of bound water is much shorter than mobile water because of restricted motion and is identified based on its doublet splitting due to non-averaged quadrupole interaction (T_1 = 4-5ms vs 150-200ms) (Fig. 2). As a result, the spectrum at TI=$T_{null,\ bound\ water}$ will consist predominantly of mobile water and can be subtracted, after correction for T_1 relaxation, from a spectrum at TI>2s (i.e. after full T_1 relaxation) to isolate the bound water spectrum. This process can be repeated with the bound water spectrum to isolate the mobile water spectrum. The relative fractions of bound and mobile water can then be computed by the integral areas of each spectra normalized to the integral area of a spectrum at TI>2s. Fig. 2 shows sample ^{2}H spectra. Bound and mobile water content, the latter being used to estimate pore volume, can be calculated using the relative fractions and total BW values (see Results). 3D μ-CT images (reconstructed at 16 μm^3) were then acquired of the specimens (eXplore Locus SP, GE Healthcare) and the images processed with ImageJ (NIH) and OsiriX (www.osirix-viewer.com) to calculated total bone volume and bone porosity. NMR bone porosity is the pore volume calculated above divided by the total bone volume as measured with micro-CT.

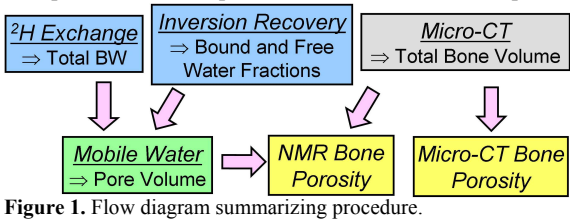

Figure 1. Flow diagram summarizing procedure.

Results and Discussion

Table 1 summarizes the results from the ^{2}H exchange, IR, and micro-CT experiments. The BW concentrations (30-40%) calculated from total BW are in good agreement with *in vivo* UTE quantification[3]. The decrease in total BW on the posterior side also matches our observations with UTE. $T_{null,\ bound}$ was consistent for all specimens (3-3.5ms). Due to the low SNR of ^{2}H, the bound and mobile water fractions were rounded to the nearest multiple of 5. Mobile water content was computed by multiplying the total BW with the mobile water fraction. Using the hypothesis that mobile water mainly occupies pore space, mobile water content is equal to pore volume based on the density of water. However, pore space also contains cellular components and the water concentration cannot be 100%. A water concentration of 70%, a value in the typical range for biological tissues, was used to correct the pore volume estimates. NMR bone porosity was then calculated by dividing the pore volume by the total bone volume as measured with μ-CT. The μ-CT bone porosity was also calculated by dividing the pore volume measured from the 3D images, by the total bone volume. There was excellent correlation between the NMR and micro-CT bone porosities (R=0.96, p=0.04).

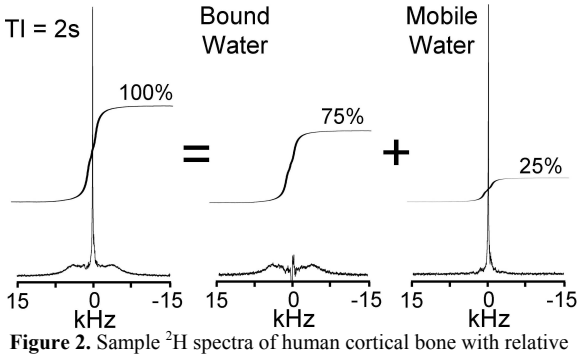

Figure 2. Sample ^{2}H spectra of human cortical bone with relative integral areas. Splitting is ~8 kHz.

While measuring BW by ^{2}H exchange is as accurate as gravimetric methods, the IR experiment may underestimate mobile water fraction due to evaporation from handling the specimens as experiments have shown that only mobile water evaporates during the first hour of drying (not shown). Further, it should also be possible to determine relative water fractions by nulling the mobile water peak. However, it was not possible to null the mobile water at a single TI – variation in pore sizes leads to a range of mobile water T_1 values and so there is no single T_{null}. Empirical estimates of the relative bound and mobile water fractions could provide validation of NMR relaxometry methods of measuring bound water that rely on the ill-posed Laplace inversion[4]. Finally, NMR and micro-CT bone porosity measurements fall within the expected range for a 65-year-old female[5].

Table 1. Summary of Results

Bone Specimen	Total BW (mg)	Mobile Water Fraction (%)	Mobile Water Content (mg)	Micro-CT Bone Volume (μl)	NMR Bone Porosity (%)	Micro-CT Bone Porosity (%)
Anterior	37.1	25	9.3	107.7	12.3	11.8
Medial	40.6	20	8.1	102	11.4	11.0
Posterior	29.0	30	7.2	95	10.9	10.4
Lateral	38.6	25	9.6	104.6	13.2	14.5

Conclusion

BW and bone porosity measurements reported here agree with literature values and suggest that a significant portion of BW is associated with collagen. The strong correlation between NMR and micro-CT bone porosity supports our hypothesis that mobile water is principally found in the pore space.

References: 1. Ong et al, *Proc of 17th ISMRM Meeting*, 2009. 2. Fernandez-Seara et al, *Biophys J*, **82**:522 (2002). 3. Techawiboonwong et al, *Radiology*, **248**:824 (2008). 4. Nyman et al, *Bone*, **42**:193 (2008). 5. Bousson et al, *Radiology*, **217**:179 (2000). **Acknowledgements:** NIH R01 AR50068