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 1H and 2H spectra, respectively. 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 2H exchange to quantify total BW and differences in T1 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 (3x14 mm, 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% D2O saline for ~48hrs at 50°C. Total BW was calculated by measuring the amount of H2O that had exchanged into the D2O saline with a calibration curve by integration of the water NMR line. Each D2O saturated specimen was blotted dry and placed in a 5mm NMR tube and a 2H 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.

Results and Discussion

Table 1. Summary of Results

| Bone Specimen | Total BW (mg) | Mobile Water Bound (mg) | Mobile Water Free (mg) | Micro-CT Bone Porosity (%) | NMR Bone Porosity (%) | Bone Porosity Percentage
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<tbody>
<tr>
<td>Anterior</td>
<td>37.1</td>
<td>25</td>
<td>9.3</td>
<td>107.7</td>
<td>12.3</td>
<td>11.8</td>
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<tr>
<td>Medial</td>
<td>40.6</td>
<td>20</td>
<td>8.1</td>
<td>102</td>
<td>11.4</td>
<td>11.0</td>
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<tr>
<td>Posterior</td>
<td>29.0</td>
<td>30</td>
<td>7.2</td>
<td>95</td>
<td>10.9</td>
<td>10.4</td>
</tr>
<tr>
<td>Lateral</td>
<td>38.6</td>
<td>25</td>
<td>9.6</td>
<td>104.6</td>
<td>13.2</td>
<td>14.5</td>
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Figure 1. Flow diagram summarizing procedure.

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

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.