Correlation of $^1$H NMR Characteristics and Mechanical Properties in Human Cortical Bone

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Introduction: Modern Magnetic Resonance Imaging methods such as ultra-short echo time (uTE) imaging are capable of imaging signals from human cortical bone [1], which shows much promise for non-invasively assessing bone health in ways that current X-ray based methods cannot provide. Since human cortical bone consists of numerous proton and/or water-bearing physiological sites such as collagen, lipids, minerals, and nanoscale-to-macroscale porosity, it is expected that the bone proton signal exhibits a distribution of transverse relaxation ($T_2$) components [2]. In a previous NMR characterization of human cortical bone specimens [3], it was determined that $T_2$ components ranging from 50µs to 1s can be attributed to collagen, collagen-bound water, lipids, and mobile water in porous spaces. Herein, we employ this characterization in conjunction with mechanical testing to probe the ability of $T_2$ relaxometry to predict cortical bone mechanical properties. Sensitivity of $T_2$ features to mechanical properties in bone would provide a contrast mechanism that many MRI protocols could exploit as a new means for assessing bone health.

Methods: Human cortical bone specimens were harvested from the midshaft of seventeen healthy male and female donor femurs (5 young donors, 26.2 ± 5.4 Y.O.; 8 middle-age donors, 52.8 ± 4.2 Y.O.; and 4 old donors, 88.8 ± 7.1 Y.O.). Specimens were machined into 5x2x60mm beams to remove periosteum and endosteum layers, yielding uniform cortical bone. The beams were sectioned into a central 40mm piece for destructive mechanical testing and two flanking 10mm end pieces for NMR and µCT analysis. Mechanical testing was performed under 3-point bending with a 35mm span to determine flexural modulus, yield stress (0.2% linear offset), ultimate stress, and toughness. NMR measurements were performed at 4.7T in a low-proton loop-gap coil with negligible background signal. For each specimen, a CPMG sequence was collected with 100 µs echo spacing, 10000 total echoes, and 90°/180° hard pulses of approximately 7.5/15 µs. CPMG echo magnitudes were fitted with a broad range of decaying exponential functions in a constrained non-negative least-squares sense, producing a so-called $T_2$ spectrum [4]. A 20µL water marker ($T_2 \approx 3$s) was included with each bone specimen so $T_2$ spectral components could be quantified in terms of the volume fraction (VF) of bone that an equivalent amount of water would occupy. Finally, µCT images were collected at 6µm resolution, from which apparent bone mineral density (aBMD) was derived. All measurements were compared with a Pearson’s linear correlation.

Results and Discussion: All human cortical bone specimens exhibited two discrete, sub-millisecond $T_2$ components and a broad range of $T_2$ values spanning 1ms-1s. For analysis, these components were grouped into three pools (Fig. 1): a short $T_2$ pool ($T_{2A} \approx 67µs$) of volume fraction $V_{FA}$, representing collagen macromolecules; an intermediate $T_2$ pool ($T_{2B} \approx 420µs$, $V_{FB}$) consisting of collagen-bound water; and a long $T_2$ pool ($T_{2C} > 1ms$, $V_{FC}$) containing a mixture of lipids and free water [3]. The pools' $T_2$s and volume fractions were compared to $\mu$CT and mechanical properties (Table 1); data from a strong correlation set are shown in Figure 2. Interestingly, the pool $T_2$s had poorer correlations to mechanical data than pool volume fractions. $V_{FB}$ had the strongest correlation to mechanical properties, indicating that collagen-bound water is beneficial to bone integrity. Surprisingly, $V_{FB}$ was a better predictor of all bone mechanical properties than aBMD (sensitive only to mineralization), which shows the importance of non-mineralized components to bone strength. $V_{FC}$ was negatively-correlated to mechanical properties, indicating that stronger bones possess less lipids/mobile (pore-space) water than weaker bones. Importantly, the opposing mechanical correlations of $V_{FB}$ and $V_{FC}$ represent competing phenomena which would confound an MRI-based bone health diagnostic that could not distinguish short- from long-lived $T_2$ signals.


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![Figure 1. Human Cortical Bone $T_2$ Spectrum and Physiological Origins](image1)

![Figure 2. Yield Stress vs. $T_2$ Pool size](image2)

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<th>Age</th>
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Table 1. Correlation of age, NMR, and $\mu$CT to mechanical properties.

Shading indicates Pearson’s correlation strength as follows: white for $p<0.005$, gray for $p<0.05$, and black for $p<0.05$ (not significant). Positive/negative correlations are denoted by “+”/“-” respectively. $T_2$ pool volume fractions were the strongest NMR predictors of mechanical properties, and $V_{FB}$ was a stronger predictor of each mechanical property than aBMD—a current “gold standard” for assessing bone health.