Non-invasive Predictors of Human Cortical Bone Mechanical Properties: T2-Discriminated 1H NMR Compared with High Resolution X-ray

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Abstract

Recent advancements in magnetic resonance imaging (MRI) have enabled clinical imaging of human cortical bone, providing potentially powerful new means for assessing bone health with molecular-scale sensitivities unavailable to conventional X-ray-based diagnostics. To this end, 1H nuclear magnetic resonance (NMR) and high-resolution X-ray signals from human cortical bone samples were correlated with mechanical properties of bone. Results showed that 1H NMR signals were better predictors of yield stress, peak stress, and pre-yield toughness than were the X-ray derived signals. These 1H NMR signals can, in principle, be extracted from clinical MRI, thus offering the potential for improved clinical assessment of fracture risk.

Introduction

Current bone diagnostics are incomplete. The estimate of areal bone mineral density (BMD) by dual energy x-ray absorptiometry (DXA) does not fully predict fracture risk: for a given DXA score, there is an unexplained increase in fracture risk with age [1,2], as well as with progression of various disease states, such as diabetes [3]. The limitations of DXA related to BMD depending on bone size [4] may be somewhat overcome by quantitative computed tomography imaging, but, ultimately, any X-ray based diagnostic is only sensitive to the mineral portion of the bone, which accounts for only \( \approx 43\% \) of bone by volume. The remaining soft-tissue components of bone, including collagen and collagen-bound water, are essentially invisible to DXA and quantitative computed tomography. In contrast, clinical magnetic resonance imaging (MRI), which is based on the 1H NMR signal, cannot directly detect bone mineral but is sensitive to the soft tissue of bone. Further, a recent study has demonstrated that 1H NMR transverse relaxation time constants (T2) distinguishes proton signals from collagen, collagen-bound water, and pore water [5]. With this technology and the idea that the presence and hydration-state of collagen play a critical role in dissipating energy in bone [6], we hypothesized that 1H NMR can report on the material strength of bone, and we present here compelling experimental observations of 1H NMR, X-ray CT and mechanical tests of cadaveric bone samples which indicate that MRI has the potential to better diagnose fracture risk than DXA.

Results

Figure 1 shows the mean (and standard deviation and range) spectrum of 1H NMR transverse relaxation time constants (T2) spectrum from 40 cadaveric bone samples. In this mean spectrum and in each individual sample spectrum, signals from three distinct domains of T2 were readily identified, as previously found [5]: 50 \( \mu \text{s} < T2 < 150 \mu \text{s} \), defined as pool A, due primarily to collagen methylene protons; 150 \( \mu \text{s} < T2 < 1 \text{ ms} \), pool B, due primarily to collagen-bound water protons; and 1 ms \( < T2 < 1 \text{ s} \), pool C, due to water protons in pores in lipid protons. From these three signals, six parameters were extracted: 3 signal amplitudes \( (S_A, S_B, S_C) \), in absolute units of mole \( \text{1H} \) per liter bone) and 3 corresponding mean relaxation rate constants \( (R2,A, R2,B, R2,C \text{ in } \text{s}^{-1}) \). Note that while the signal amplitudes are computed in absolute units of concentration, the correspondence between signal amplitudes, \( S_A \), \( S_B \), and \( S_C \), and actual concentrations of collagen methylene protons, bound water protons, and pore-water or lipid protons, respectively, is potentially affected by a number of factors, including the line shape of the methylene protons, the magnetization exchange rate between bound and methylene protons, and overlap of T2 components from different sources.

Each of the three NMR signal amplitudes \( (S_A, S_B, S_C) \) was found to linearly correlate \( (r^2 = 0.34, 0.68, 0.61, p<0.05) \) with peak stress (Fig. 2), but note that the sum of all three signals did not \( (r^2 = 0.06, p>0.05) \). Similar pair-wise linear correlations (and lack thereof) also existed between NMR signal amplitudes and the other three
measured mechanical properties. These findings indicate that peak cortical bone stress, and the other measured mechanical properties, are directly related to the amount of collagen and collagen-bound water in bone, and inversely related to the bone pore volume. Micro-computed tomography ($\mu$CT)-derived measures of bone porosity and the apparent volumetric bone mineral density (avBMD, akin to DXA) were also found to linearly correlate with mechanical properties, but $S_A$ and $S_B$ were better predictors (i.e., higher $r^2$ values) than $\mu$CT-porosity for three of four mechanical properties (flexural modulus being the exception), and better predictors than avBMD (i.e., DXA) for all four mechanical properties. Table 1 summarizes the pairwise linear correlations between imaging measure ($^1$H NMR and X-ray) and the four mechanical properties.

Note that without the two apparent outlier data (peak stress $<100$ MPa), the predictive power of $S_B$ and $S_C$ decreased to $r^2$ values of 0.52 and 0.49, respectively, but the $r^2$ of avBMD with peak stress decreased to a greater extent (to 0.16). That is, the relative predictive power of $S_B$ and $S_C$ compared with avBMD increased without these two data points. Also note that multiple linear regression analysis told a similar story: combination of NMR signal parameters ($R_B$ and $S_C$) best predicted of three of four mechanical properties (adjusted $R^2$: 0.56-0.70, again, flexural modulus was the exception), and better predicted all four mechanical properties than did avBMD.

Discussion

As a surrogate to radiation-based CT, MRI has been developed to characterize trabecular volume and architecture as a means to assess fracture risk [7,8]. For example, such MRI-derived measurements of bone volume fraction and trabecular thickness
correlated with the compressive strength of human trabecular bone, although the correlations were not as strong as that between CT-derived BMD and strength [9]. These MRI techniques do not assess the inherent quality of the bone tissue, and this is a significant shortcoming given the importance of ultrastructural characteristics of the extracellular matrix (e.g., collagen integrity) to the fracture resistance of bone [10]. From ex vivo studies of bone, various quantifications of water by NMR have been correlated with the mechanical properties. In a rabbit model of diet-induced hypomineralization, a $^1$H NMR-derived measurement of water content was directly related to the bending strength of cortical bone [11]; however, in a study of ovariectomized and treated mice, only those correlations were found across pooled data from 60 bones, which may be explained by the findings of total $^1$H signal shown here (Fig. 2). Also, an NMR technique known as “decay from an internal field” (DDIF) found an inverse correlation between this NMR-derived pore water parameter and the yield stress of bovine trabecular bone in compression [12], in rough agreement with the present observations of pore-water. Prior to the present study though, only one study attempted to correlate NMR measurements of both pore water and water bound to the extracellular matrix to the mechanical properties of human bone [13]. That study used $T_2$-discriminated rather than $T_2$-discriminated (used herein) $^1$H NMR signals at low static magnetic field, and while a direct relationship existed between the so-called $T_2^*$-defined bound water and peak stress, it described a much lower fraction of the peak stress variance ($r^2 = 0.36$, compared to 0.68, above). Also, the translation of $T_2^*$ based discrimination to clinical imaging may be problematic due to the presence of lipid in bone [5,11], and the inability of $T_2^*$ to discriminate bone $^1$H pools at clinical field strengths (no discrimination was found at 4.7T [5] and no discrimination has been reported at clinical field strengths (≥1.5 T)).

Current uTE protocols on human MRI systems use echo times <100 μs [14] (and references therein), more than short enough to capture the majority of the bound water signal and some of the collagen proton signal, but the translation of the present findings to clinical MRI will require practical imaging methods of distinguishing these short-$T_2$ signals from the longer-$T_2$ pore water and lipid signals. There are numerous strategies for integrating $T_2$-selective magnetization preparation into a clinically practical uTE-type sequence [15,16,17], and the optimal approach for bone imaging has not yet been determined. However, Fig. 3 shows two $T_2$ spectra from one bone specimen. The solid line shows the normal $T_2$ spectrum, as used in the above analysis, while the dotted line shows the spectrum that results following the complex average of two CPMG signals, with and without the preceding hyperbolic secant radiofrequency (RF) pulse. This RF pulse effectively inverts only the long $T_2$ signals while largely saturating the collagen proton and bound-water signal, so the complex average cancels only the long $T_2$ signals and results in a net NMR signal that is ≈95% derived from protons with $T_2$<1 ms. This result demonstrates in principle that a simple RF pre-pulse, which can be readily integrated into a standard uTE pulse sequence, can distinguish pore water from collagen protons and collagen bound water protons in bone. Once implemented on clinical scanners, such an MRI method can then assess both the contribution of structure to whole bone strength as well as the contributions of collagen integrity and porosity, thus proving a more complete assessment of fracture risk than current X-ray based methods.

### Materials and Methods

**Human cortical bone processing**

The Musculoskeletal Tissue Foundation (Edison, NJ), a non-profit tissue allograft bank, and the Vanderbilt Donor Program...
transverse relaxation (T$_2$) characteristics were measured and which provided 90° structural support was used (similar to the coil described in [18]), Drive Receiver. An in-house loop-gap style RF coil with Teflon Varian/Magnex 4.7 T horizontal bore magnet with a Direct protons in pores [5]. All NMR measurements were performed in a methylene protons, collagen-bound water protons, and water recently identified as being primarily derived from collagen of 10000 echoes were collected at 100° signal).

between 20° decaying exponential functions (with time constants log-spaced empirically determined to be a suitable minimum threshold for both maximizing the range of T$_2$ detection while minimizing spin-locking effects. Echo magnitudes were fitted to a sum of 128 decaying exponential functions (with time constants log-spaced between 20 μs and 10 sec) in a non-negative least-squares sense, subject to a minimum curvature constraint, which produced a so-called T$_2$ spectrum [19]. In order to quantitatively compare the absolute signal amplitudes of T$_2$ components across days, a reference sample with long T$_2$ (≈2 s) and known proton content was included in each CPMG measurement. The presence of the reference sample, together with the known specimen volumes, enabled the calculation of proton concentrations in the bulk bone specimens for each CPMG relaxation component by comparing integrated areas of each T$_2$ spectral component to the area of the marker. As a simple demonstration of the potential for acquiring signal from a specific T$_2$ domain without the full CPMG acquisition, from one bone specimen, an additional CPMG measurement was acquired with a preceding a 10-ns duration, 3500 Hz bandwidth hyperbolic secant inversion pulse [20], so chosen to selectively invert the long-T$_2$ $^1$H signal.

μCT

The second specimen from each donor sample (~ volume of 40 mm$^3$) was studied at high resolution (6 μm), with low noise micro-CT (μCT) to quantify apparent volumetric bone mineral density (avBMD) and intracortical porosity (for pores ≥6 μm in diameter). Note that for a given specimen size avBMD is a volumetric analog to areal BMD as measured by DXA, and intracortical porosity at this resolution is not readily determined from clinical radiographs or QCT including high-resolution peripheral QCT scanners (which obtain resolutions of 80–100 μm) [21]. The specimen was scanned by acquiring 1000 projections per 180° at 70 keV using a Scanco, model μCT-40. From an hydroxyapatite (HA) phantom image (acquired weekly), linear attenuation coefficients derived from the μCT images were equated to volumetric bone mineral density (vBMD) in units of mg-HA/cm$^3$. Using the Scanco software, the outer perimeter of the sample was defined to determine the total bone volume. The avBMD was defined as the mean of vBMD for all voxels within the total bone volume. The bone tissue volume was segmented from air or soft tissue at a threshold of 800 mg-HA/cm$^3$ to determine the porosity (= 1 minus bone tissue volume per total bone volume) (Fig. 5).

Figure 4. From each cadaveric bone studied, one strip of cortical bone was extracted, three separate pieces of which were used for NMR, μCT, and mechanical testing. doi:10.1371/journal.pone.0016359.g004

Figure 5. Axial μCT images are shown for cortical bone specimens from a 48 y.o. male donor (left) and an 82 y.o. male donor (right). For the 48 and 82 y.o. donors, respectively, avBMD was 1222 and 1135 mg-HA/cm$^3$, and porosity was 4% and 11.3%. doi:10.1371/journal.pone.0016359.g005
determined by the 0.2% offset, or to the peak force endured by the temperature. Applying the flexure formula to the yield force, as The span was 35 mm, and all tests were performed at room 50 Hz, as the hydrated bone was loaded to failure at 5 mm/min. cell and the linear variable differential transformer, respectively, at recorded the force-displacement data (Fig. 6) from a 100 N load material testing system (Dynamight 8841, Instron, Canton, OH) properties relevant to fracture risk in bone: yield stress, peak stress, flexural modulus, and pre-yield or elastic toughness. A material testing system (Dynamight 8841, Instron, Canton, OH) recorded the force-displacement data (Fig. 6) from a 100 N load cell and the linear variable differential transformer, respectively, at 50 Hz, as the hydrated bone was loaded to failure at 5 mm/min. The span was 35 mm, and all tests were performed at room temperature. Applying the flexure formula to the yield force, as determined by the 0.2% offset, or to the peak force endured by the bone specimen, and applying the deflection equation to the slope of the linear section of the force-displacement curve provided the material properties, yield stress, peak stress, and flexural modulus, respectively [6]. Pre-yield or elastic toughness was the area under the force-displacement curve from zero displacement to the yield displacement divided by the cross-sectional area of the bone sample to account for slight differences in specimen dimensions.

**Mechanical**

Finally, we subjected the third, parallelpiped specimen (nominal dimensions of 2 mm × 3 mm × 40 mm) from each donor sample to a three point bending test, and measured four mechanical properties relevant to fracture risk in bone: yield stress, peak stress, flexural modulus, and pre-yield or elastic toughness. A material testing system (Dynamight 8841, Instron, Canton, OH) recorded the force-displacement data (Fig. 6) from a 100 N load cell and the linear variable differential transformer, respectively, at 50 Hz, as the hydrated bone was loaded to failure at 5 mm/min. The span was 35 mm, and all tests were performed at room temperature. Applying the flexure formula to the yield force, as determined by the 0.2% offset, or to the peak force endured by the bone specimen, and applying the deflection equation to the slope of the linear section of the force-displacement curve provided the material properties, yield stress, peak stress, and flexural modulus, respectively [6]. Pre-yield or elastic toughness was the area under the force-displacement curve from zero displacement to the yield displacement divided by the cross-sectional area of the bone sample to account for slight differences in specimen dimensions.

**Author Contributions**

Conceived and designed the experiments: RAH DFG JSN MDD. Performed the experiments: RAH. Analyzed the data: RAH. Contributed reagents/materials/analysis tools: RAH DFG JSN MDD. Wrote the paper: JSN MDD. Supervised the project: JSN MDD.

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