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Bone Mineral ^{31}P and Matrix-Bound Water Densities Measured by Solid-State ^1H and ^{31}P MRI

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Abstract

Bone is a composite material consisting of mineral and hydrated collagen fractions. MRI of bone is challenging due to extremely short transverse relaxation times, but solid-state imaging sequences exist that can acquire the short-lived signal from bone tissue. Previous work to quantify bone density via MRI used powerful experimental scanners. This work seeks to establish the feasibility of MRI-based measurement on clinical scanners of bone mineral and collagen-bound water densities, the latter as a surrogate of matrix density, and to examine the associations of these parameters with porosity and donors' age.

Mineral and matrix-bound water images of reference phantoms and cortical bone from 16 human donors, ages 27-97 years, were acquired by zero-echo-time ^{31}P and ^1H MRI on whole body 7T and 3T scanners, respectively. Images were corrected for relaxation and RF inhomogeneity to obtain density maps. Cortical porosity was measured by micro-CT, and apparent mineral density by pQCT. MRI-derived densities were compared to x-ray-based measurements by least-squares regression.

Mean bone mineral ^{31}P density was 6.74 ± 1.22 mol/L (corresponding to 1129 ± 204 mg/cc mineral), and mean bound water ^1H density was 31.3 ± 4.2 mol/L (corresponding to 28.3 ± 3.7 %v/v). Both ^{31}P and bound water (BW) densities were correlated negatively with porosity (^{31}P : $R^2 = 0.32$, $p < 0.005$; BW: $R^2 = 0.63$, $p < 0.0005$) and age (^{31}P : $R^2 = 0.39$, $p < 0.05$; BW: $R^2 = 0.70$, $p < 0.0001$), and positively with pQCT density (^{31}P : $R^2 = 0.46$, $p < 0.05$; BW: $R^2 = 0.50$, $p < 0.005$). In contrast, the bone mineralization ratio (expressed here as the ratio of ^{31}P density to bound water density), which is proportional to true bone mineralization, was found to be uncorrelated with porosity, age, or pQCT density.

This work establishes the feasibility of image-based quantification of bone mineral and bound water densities using clinical hardware.

Keywords

Bone; Bone mineral density; Bone matrix density; Ultra-short echo time MRI; Zero echo time MRI; Magnetic resonance imaging

1. Introduction

Bone is a composite material composed of apatite-like mineral, collagen, and water. Each constituent contributes to bone's mechanical properties; mineral provides rigidity (1), the collagen matrix contributes elasticity (2), and water stabilizes the collagen matrix, imparting viscoelasticity and increasing toughness (3,4).

The most common screening test for bone disease is dual energy x-ray absorptiometry (DXA), which provides a measurement of areal (two-dimensional) apparent bone mineral density (BMD) expressed in g/cm^2 . The term 'apparent' is used here to indicate that this density represents the mass of mineral in the volume of bone traversed by the X-ray beams, including matrix as well as pore spaces. Quantitative computed tomography (QCT) adds a third dimension, yielding volumetric apparent BMD in g/cm^3 .

However, because the mechanical properties of bone depend on both mineral and matrix, bone mineral density alone cannot fully describe bone health. For example, osteoporosis is a structural bone disorder in which deterioration of trabecular bone architecture (5) and enlargement of pores and thinning of cortical bone (6) reduce the bone's mechanical competence and increase fracture risk. Both bone mineral and matrix are lost in roughly equal proportions. In contrast, osteomalacia is a bone-demineralizing disorder in which low blood calcium or phosphorus (^{31}P) levels impair mineralization of bone matrix (7), reducing the stiffness and strength of bone (8). The differentiating factor between these disorders is bone mineralization: the mass of mineral in a volume of bone matrix only (see Figure 1). This requires knowledge of both bone mineral and matrix content. Currently, the best qualitative examination of bone mineralization is tetracycline-labeled bone biopsy (9), but this exam involves a minor surgical procedure. Synchrotron micro-computed tomography (μCT) (10) and microradiography (7) can resolve pores and may provide a quantitative measurement of bone mineralization as well, but these methods are also applicable only to biopsy cores.

Nuclear magnetic resonance (NMR) imaging of bone has the potential to detect both the mineral and matrix components of bone in vivo, but is made difficult by the extremely short effective transverse relaxation times (T_2^* s) of bone mineral ^{31}P and matrix-associated ^1H , causing their NMR signals to decay before they can be captured; bone therefore appears as a signal void in standard magnetic resonance images. Recent advances in solid-state ^{31}P and ^1H magnetic resonance imaging (MRI) have led to the possibility of quantitative measurement of bone mineral ^{31}P and collagen-bound water ^1H densities (11-17).

Wu and Ackerman first demonstrated solid-state ^{31}P imaging of bovine and ovine cortical bone mineral in 1992 (11). Since then, several groups have made strides toward quantitative bone mineral density measurement via ^{31}P NMR. Mineral density in specimens has been

quantified via ex vivo ^{31}P NMR (14) and MRI (12,13), and ^{31}P MRI of human bone mineral has been proven feasible in vivo (15-17).

Likewise, significant progress has been made in solid-state ^1H imaging and quantification of the matrix-associated component of bone water, referred to as bound water (14,18-23). Only Cao et al. (14) have quantified both ^{31}P and bound water in a single set of specimens. These two measurements, taken individually, reflect the apparent densities of bone mineral and matrix, but their ratio can provide insight into bone mineralization. In Cao's study, however, ^{31}P was quantified by non-localized NMR spectroscopy and the work was performed using animal imaging hardware with capabilities far exceeding those of clinical MRI scanners.

The objective of the present work was to quantify bone mineral ^{31}P and matrix-bound water densities in human bone on clinical scanners using solid-state MRI methods. The ratio of these densities, referred to hereafter as the bone mineralization ratio (BMR), can serve as a surrogate measure of mineralization. These measurements were compared with micro-CT (μCT)-derived porosity, pQCT-derived apparent BMD, and donor age.

2. Materials and Methods

2.1. Source of Bone Tissue

The tissue examined consisted of 16 specimens of cortical bone taken from the tibial mid-shaft of male and female human donors, aged 27-97 years (National Disease Research Interchange, NDRI). Donors with bone demineralizing disorders were excluded; only age-related structural bone loss is expected. The tibial cortex is relatively thick, while the bone lies near the surface of the lower leg, making this site uniquely suited for imaging using a small surface or volume radiofrequency (RF) coil. Whole-cross section specimens 36 mm in length were cut from thawed tibiae with a rotating blade from the region of maximum cortical bone thickness, 38% of the length of the tibia from the medial malleolus to the medial condyle. The average cortical thickness of the 16 bone specimens was 4.6 ± 1.4 mm. Thirteen specimens were placed in phosphate-buffered saline ($[^{31}\text{P}] = 12$ mM; signal contribution is negligible compared to bone $[^{31}\text{P}] \sim 7$ M) inside plastic tubes (Fig. 2), immobilized by plastic supports on each end. The tubes were centrifuged to remove air bubbles and then sealed while immersed in phosphate-buffered saline. Due to large size, three specimens were instead sealed inside rubber balloons in phosphate-buffered saline.

Each bone was scanned with solid signal intensity reference phantoms. Two cylindrical phantoms of dry, packed synthetic hydroxyapatite powder (Sigma-Aldrich, USA), with densities of 1108 and 1026 mg/cc, were used as ^{31}P reference signal intensities. These ^{31}P phantoms were encased in plastic cylinders, each 2.5 mm in diameter and 30 mm in length, and sealed on each end with epoxy. The 1108-mg/cc phantom had ^{31}P $T_1 = 93.7$ s and $T_2^* = 159.8$ μs , determined by ^{31}P saturation recovery, and the 1026-mg/cc phantom had ^{31}P $T_1 = 101.7$ s and $T_2^* = 160.0$ μs . One rectangular rubber phantom, $24 \times 15 \times 5$ mm, served as a short- T_2 ^1H reference signal sample (^1H $T_1 = 284$ ms, $T_2^* = 156$ μs). The ^{31}P and ^1H nuclear densities of these reference phantoms, in mol/L, were calibrated by scanning them in the presence of liquid calibration standards using the method described below. Pure triethyl

phosphate ($\text{C}_{24}\text{H}_{51}\text{O}_4\text{P}$, $T_1 = 709$ ms, $T_2^* = 4560$ μs , $[\text{}^{31}\text{P}] = 2.124$ mol/L) and a solution of 90% D_2O /10% H_2O doped with 28.9 mM MnCl_2 ($T_1 = 7.3$ ms, $T_2^* = 272$ μs , $[\text{}^1\text{H}] = 11.079$ mol/L) were used for ^{31}P and ^1H calibration, respectively.

2.2. Hardware

All ^{31}P scanning was performed on a 7T Magnetom whole-body MRI scanner (Siemens, Erlangen, Germany), and all ^1H scanning on a 3T Magnetom TIM Trio whole-body MRI scanner (Siemens, Erlangen, Germany). Both scanners have 40 mT/m maximum gradient strength. Because the resonance frequencies of ^{31}P at 7T (120.3 MHz) and ^1H at 3T (123.3 MHz) are similar, a single RF coil was re-tuned and used at both field strengths. This custom 3-turn transmit/receive solenoid coil, 4.5 cm in diameter and 8 cm in length, is shown in Fig. 2 with a bone specimen. In addition to the previously explained intensity reference phantoms, which are not visible in Figure 2, three samples containing trioctyl phosphate were rigidly affixed to the exterior of the coil to serve as fiducial markers in both ^{31}P and ^1H images. These landmarks were used to register each image to an RF field map.

2.3. MR Imaging

Due to its excellent SNR performance in samples with extremely short T_2^* (24), ZTE with PETRA (25), shown in Figs. 3a,b, is used in this work. This method collects k-space points on a Cartesian grid to fill the central k-space sphere that is missing from the radial k-space projection data set collected in ZTE. In the PETRA segment, the gradient magnitude and direction are adjusted in each repetition to collect a single k-space point per repetition at a time delay equal to the transmit/receive switching time. Though the addition of the PETRA segment increases the total scan time, this sequence allows collection of each k-space point at the absolute shortest time delay after excitation, limited either by maximum gradient amplitude (in the ZTE segment) or transmit/receive switching time (in the PETRA segment), without complications due to gradient waveform irregularities or slew rate limitations.

Each sample was imaged using three sequences, shown in Figure 3: ^{31}P ZTE with PETRA (25) (Figs. 3a,b), ^1H ZTE with PETRA (similar to Figs. 3a,b), and ^1H Single Adiabatic Inversion-Recovery Rapid ZTE with PETRA (SIR-rZTE, Figs. 3c,d) (22,23,26,27).

^{31}P images were acquired with the following parameters: field of view (FOV) = 128 mm isotropic, pulse duration = 12 μs , flip angle = 5.0 degrees, gradient amplitude = 36.7 mT/m, number of readout points (N) = 32, dwell time = 12 μs , TR = 20 ms, number of signal averages (NEx) = 100, 5000 projections, PETRA radius = 5 k-space points, 100 dummy scans, scan time = 3 hr 3 min. Using this gradient amplitude, the full width at half maximum (FWHM) of the point-spread function (PSF), a measurement of intrinsic image resolution, is 3.84 mm.

These images were acquired with 100 averages and a flip angle above the optimal Ernst angle condition in order to acquire sufficient signal from the three fiducial markers, which were located outside the solenoid in a region of low B_1 field strength. Only a single signal acquisition at the Ernst angle with TR = 250 ms is necessary to achieve sufficient signal-to-

noise ratio (SNR) in the interior of the coil for bone signal quantification, but the external landmarks would not be visible. The total scan time for such an image is 23 min 16 s.

^1H ZTE images were acquired with the following parameters: FOV = 80 mm isotropic, pulse duration = 32 μs , flip angle = 8.7 degrees, gradient amplitude = 18.3 mT/m, N = 40, dwell time = 16 μs , TR = 7 ms, NEx = 1, 5000 projections, PETRA radius = 4 k-space points, 100 dummy scans, scan time = 37 s. At the chosen gradient amplitude, the PSF FWHM is 1.17 mm.

^1H SIR-rZTE images were acquired with the following parameters: inversion pulse bandwidth = 5 kHz, inversion pulse duration = 5 ms, TI = 100 ms, FOV = 80 mm isotropic, 7 readouts per inversion (to lower scan time), excitation pulse duration = 32 μs , flip angle = 24-40 degrees (set to yield equal transverse magnetization in each of the seven excitations and limited by maximum RF power and pulse duration), gradient amplitude = 18.3 mT/m, N = 40, dwell time = 16 μs , TR = 300 ms, NEx = 1, 10000 projections, PETRA radius = 4 k-space points, 100 dummy scans, scan time = 26 min 45 s.

A lower than the maximally allowed gradient amplitude was used in ^1H SIR-rZTE scans to improve SNR by two routes: a lower readout bandwidth allows for longer dwell time, and also allows the use of a longer-duration (i.e. lower bandwidth) RF excitation pulse with a correspondingly higher flip angle while still avoiding signal loss toward the edge of the field of view (28). Although a lower readout gradient increases PSF broadening, the actual resolution approximately equals the reconstructed voxel resolution.

To compensate for the non-uniform sampling density inherent in radial imaging, each k-space point was multiplied by a weighting factor equal to the 'volume' of k-space occupied by that point. The k-space data were then re-mapped onto a Cartesian grid (64^3 points for ^{31}P , 80^3 points for ^1H), and Fourier transformed using the NFFT C subroutine library (29). Reconstructed isotropic voxel resolutions were 2 mm³ and 1 mm³ in ^{31}P and ^1H images, respectively.

2.4. B₁ Mapping and Registration

A map of RF amplitude and, by reciprocity, receive sensitivity was acquired by Bloch-Siegert B₁ mapping (30). This sequence was applied on the ^1H nucleus at 3T using a water-filled balloon as a sample. The balloon maximally filled the interior of the RF coil and extended out the ends of the coil's cylindrical support structure, thus maximizing the mapped volume. The spatial locations of the triethyl phosphate landmarks were obtained using a ^1H ZTE image with voxel resolution and FOV size and location identical to the bone specimen scans, and were used to register the B₁ map to each ^1H and ^{31}P bone specimen image (31).

2.5. Density Quantification

MRI signal intensity is proportional to the density of the nucleus being imaged, but also depends on the longitudinal (T_1), transverse (T_2), and effective transverse (T_2^*) relaxation times of the NMR signal and the transmit and receive radiofrequency fields (B_1). The steady-state signal acquired in the plain ZTE pulse sequence shown in Figure 3a,b is given in Eq. 1:

$$S(\vec{r}) \propto \rho(\vec{r}) \frac{1 - \exp\left(\frac{-TR}{T_1}\right)}{1 - f_z(\vec{r}) \exp\left(\frac{-TR}{T_1}\right)} f_{xy}(\vec{r}) \exp\left(\frac{-TE}{T_2^*}\right) \hat{B}_1(\vec{r}), \quad [1]$$

where $\rho(\vec{r})$ is the nuclear density, TR is the pulse repetition time, and $\hat{B}_1(\vec{r})$ is the normalized transmit RF field profile, $B_1(\vec{r})$, of the receive RF coil (representing, by reciprocity of transmit and receive B_1 fields, the reception sensitivity profile of the coil). The f_{xy} and f_z terms are mapping functions, which give the response of transverse and longitudinal magnetization, respectively, to rectangular RF pulses, which have a finite duration relative to T_2 (32):

$$f_{xy}(\vec{r}) = \exp\left(\frac{-\tau}{2T_2}\right) \gamma B_1(\vec{r}) \tau \operatorname{sinc}\left(\sqrt{(\gamma B_1(\vec{r}) \tau)^2 - \left(\frac{\tau}{2T_2}\right)^2}\right) \quad [2]$$

and

$$f_z(\vec{r}) = \exp\left(\frac{-\tau}{2T_2}\right) \left[\cos\left(\sqrt{(\gamma B_1(\vec{r}) \tau)^2 - \left(\frac{\tau}{2T_2}\right)^2}\right) + \left(\frac{\tau}{2T_2}\right) \operatorname{sinc}\left(\sqrt{(\gamma B_1(\vec{r}) \tau)^2 - \left(\frac{\tau}{2T_2}\right)^2}\right) \right], \quad [3]$$

where τ is the RF pulse duration and $B_1(\vec{r})$ is the transmit RF field amplitude.

Once T_1 , T_2 , and T_2^* of the specimen are known, and the B_1 fields of transmit and receive coils are mapped, then the image can be corrected by solving Eq. 1 for $\rho(\vec{r})$, and density can be quantified relative to a similarly corrected reference sample in the same image field of view (FOV) (12).

^{31}P T_1 of bone mineral is strongly dependent on the level of mineralization and may vary significantly among donors (24). To accurately perform this correction for ^{31}P density quantification, ^{31}P relaxation was measured in each individual bone using saturation recovery. ^1H bound water relaxation times chosen for density computation were population averages from the literature: $T_1 = 290$ ms (23) and $T_2^* = 350$ μs (26).

Unlike bone mineral ^{31}P , ^1H NMR signal in bone at 3T arises from several water compartments: long $T_2 > 1$ ms, corresponding to free water in Haversian canals and the lacuno-canalicular pore system (also denoted ‘pore water’); short $T_2 \sim 300\text{--}400$ μs , corresponding to motionally restricted water bound to bone matrix collagen (‘bound water’); and extremely short $T_2 \sim 50$ μs , corresponding to ^1H nuclei in bone matrix collagen itself (‘collagen’) (33). In practice, the collagen signal is beyond the reach of clinical hardware, even with solid-state pulse sequences. However, bound water and collagen ^1H signal both are proportional to bone matrix density (14,19-23,26,33-37), while pore water is inversely proportional to bone matrix density (21-23,36-38), and total bone water density is only weakly correlated with bone matrix density (22,36). It is therefore necessary to isolate and image only the ^1H signal components that correspond to bone matrix.

Adiabatic RF pulses can have both broad bandwidth and long duration, which enables them to saturate short- T_2 bound water ^1H signal while also being able to invert the broad band of long- T_2 pore water spins resonating over a range of frequencies (22,39,40). The response of the equilibrium longitudinal magnetization, $f_{\text{HS}} = M_z/M_0$, to a 5-kHz bandwidth, 5-ms duration hyperbolic secant adiabatic RF pulse is shown for a range of T_2 s in Fig. 4. After an appropriate inversion time delay (TI), pore water longitudinal magnetization will be nulled ($M_z \approx 0$) as a consequence of partial longitudinal (T_1) recovery of the magnetization, while bound water longitudinal magnetization will have recovered from $M_z \approx 0$ to $M_z > 0$. At this time, imaging excitation and readout can be performed, yielding an image composed only of bound water signal. Fortuitously, the same reduced molecular motion that causes bound water to have a short T_2 also results in a shorter T_1 than that of pore water, enhancing its signal recovery.

Quantification of bound water density based on an inversion-recovery image must take this adiabatic inversion into account, resulting in a more complex steady-state signal equation:

$$S(\vec{r}) \propto \rho(\vec{r}) \frac{1 - (f_{\text{HS}} - 1) \exp\left(\frac{-TI}{T_1}\right) - f_{\text{HS}} \exp\left(\frac{-TR}{T_1}\right)}{1 - f_{\text{HS}} t_z(\vec{r}) \exp\left(\frac{-TR}{T_1}\right)} f_{xy}(\vec{r}) \exp\left(\frac{-TE}{T_2^*}\right) \hat{B}_1(\vec{r}). \quad [4]$$

In Equation 4, f_z includes the cumulative effect of all seven excitation pulses, and f_{xy} is equal for each excitation.

^{31}P ZTE and ^1H SIR-rZTE images were imported into MATLAB, and volumes of interest (VOIs) were drawn to fully enclose the three landmark intensities, reference intensities, and bone, and exclude the endosteal cavity. Each image was then registered to the B_1 map and corrected for transmit and receive B_1 and for differences in relaxation times using Equations 1-4.

The reference samples and bone specimens were further masked by Otsu automatic thresholding (41) within the manually drawn VOIs. The mean corrected intensity in a central 20-mm slab of the thresholded bone was divided by the mean corrected intensity within the reference phantom, and multiplied by the calibrated ^{31}P or ^1H density of the reference. This yields a measurement of bone ^{31}P density (proportional to apparent BMD) or bound water density (proportional to apparent bone matrix density), respectively, in mol/L. The bone mineralization ratio, which is the ratio of ^{31}P density to bound water density is a surrogate of bone mineralization.

2.6. X-Ray-Based Porosity and Densitometry

For comparison to MRI-derived densities, cortical porosity was measured by micro-computed tomography (μCT). A 3D μCT image of each cortical bone specimen was acquired with 9- μm isotropic resolution using a Bruker μCT scanner (Bruker, Kontich, Belgium). The endosteal and periosteal surfaces of each bone were segmented, and the resulting image was thresholded to distinguish pore spaces from bone tissue. Porosity was quantified as the ratio of pore space volume to total bone volume, both excluding the endosteal cavity.

Apparent bone mineral density was also measured by peripheral quantitative computed tomography (pQCT). A single-slice pQCT image was acquired at the center of each cortical bone specimen with resolution $0.4 \text{ mm} \times 0.4 \text{ mm} \times 2.3 \text{ mm}$ using a Stratec XCT 2000 pQCT scanner (Orthometrix, White Plains, NY). Apparent bone mineral density was quantified in this single slice.

2.7. Data Analysis

Correlations were calculated using least squares regression. Significance of each regression was determined using one-way ANOVA, with a threshold of $p < 0.05$.

Porosity and pQCT density were regressed versus age to confirm the presence of age-related bone loss. MRI-derived densities were then each regressed versus donor age, pQCT density, and μCT porosity to determine the MRI method's ability to detect changes in bone density. pQCT density normalized by bone volume fraction (1-porosity), which provides an estimate of bone mineralization, was regressed versus age to verify that no impairment of mineralization exists in the bone specimens, and the MRI-based bone mineralization ratio was also regressed versus donor age, pQCT density, and porosity to confirm this finding. ^{31}P T_1 relaxation time was also regressed versus pQCT density and porosity to reinforce the dependence of T_1 on bone mineral density.

3. Results

A representative volume rendering of a ^{31}P ZTE image of a specimen taken from an 83-year old female donor is shown in Fig. 5, and maps of ^{31}P and bound water densities are shown in Fig. 6. These quantities are proportional to apparent bone mineral and matrix densities, respectively, and their ratio, the bone mineralization ratio, is a surrogate for bone mineralization.

Cortical porosity is known to increase with age, particularly in post-menopausal women (6). Bone porosity was correlated positively with age ($R^2 = 0.48$, $p < 0.01$) and negatively with pQCT density ($R^2 = 0.78$, $p < 0.0005$), confirming that age-related bone mineral loss is present in this set of donors. The data suggest a negative correlation between pQCT density and age, but this falls just short of statistical significance ($R^2 = 0.36$, $p = 0.053$).

Because MRI and pQCT cannot resolve individual pores (for this reason, we refer to these densities as 'apparent'), an increase in porosity manifests as a net reduction of mineral ^{31}P and bound water density, in equal proportions, within each voxel. As expected, densities are visibly lower in bone from elderly females than in bone from younger or male donors (Fig. 6).

Various measures for MRI-derived densities and x-ray-based measurements are given in Table 1. Mean bone mineral ^{31}P density was $6.7 \pm 1.2 \text{ mol/L}$ (corresponding to $1129 \pm 204 \text{ mg/cc mineral}$), and mean bound water ^1H density was $31.4 \pm 4.2 \text{ mol/L}$ (corresponding to $28.3 \pm 3.8 \text{ \% v/v}$). Coefficients of determination for correlations between measured parameters are given in Table 2. Both ^{31}P and bound water (BW) densities were correlated negatively with porosity (^{31}P : $R^2 = 0.32$, $p < 0.005$; BW: $R^2 = 0.63$, $p < 0.0005$) and age (^{31}P : $R^2 = 0.39$, $p < 0.05$; BW: $R^2 = 0.70$, $p < 0.0001$), and positively with pQCT density

(^{31}P : $R^2 = 0.46$, $p < 0.05$; BW: $R^2 = 0.50$, $p < 0.005$). These findings indicate that the MRI-based measurements are able to detect inter-subject variations in apparent mineral and osteoid density in human cortical bone.

The true density of bone mineral in the collagen matrix, variably termed ‘bone mineralization’ or ‘degree of mineralization of bone’ (42), can be inferred from apparent pQCT bone mineral density divided by the bone volume fraction (BVF), expressed as $(1 - \text{porosity})$. The tight range of volume fraction-normalized pQCT density (1213-1311 mg/cc matrix) and its lack of correlation with age ($p > 0.5$) lend support to the notion that the remaining bone tissue, even in the presence of severe bone loss, is not significantly deficient in mineral (recall that the bone studied in this work were taken from donors unaffected by bone-demineralizing disorders), as previously noted, for example, by Yeni et al. (43). As expected in fully mineralized bone, the MRI-measured ^{31}P and bound water densities (the latter scaling with matrix density) were highly correlated ($R^2 = 0.59$, $p < 0.001$, Fig. 7), and the density ratio was not correlated with age, porosity, or pQCT density.

Point-spread function (PSF) blurring is more severe in the ^{31}P maps than ^1H (Fig. 6), due to the lower gyromagnetic ratio ($\gamma = 17.24 \text{ MHz/T}$) and shorter T_2^* of bone mineral ^{31}P . Average image SNR was 12.5 in ^{31}P ZTE images, and 20.2 in ^1H SIR-ZTE images. Finally, bone mineral ^{31}P T_1 relaxation time was positively correlated with pQCT density ($R^2 = 0.62$, $p < 0.005$) and negatively with porosity ($R^2 = 0.45$, $p < 0.03$). ^{31}P T_2^* relaxation time was $130.8 \pm 1.2 \mu\text{s}$, and was not correlated with pQCT density or porosity.

4. Discussion

The adiabatic inversion recovery method takes advantage of differences in transverse relaxation between bound and pore water spins to isolate bound water signal (22). In general, the effective transverse relaxation rate, $1/T_2^*$, is composed of reversible ($1/T_2'$) and irreversible ($1/T_2$) components, such that $1/T_2^* = 1/T_2 + 1/T_2'$. Because bound water is restricted by hydrogen bonding to collagen, its transverse relaxation is chiefly due to incomplete motional averaging of dipolar interactions, such that $1/T_2^* \approx 1/T_2 \gg 1/T_2'$. In contrast, the pore water signal is the superposition of the signals from many isochromats within a single voxel, with long T_2 individually, but distributed over a broad range of resonance frequencies due to static field inhomogeneities arising from susceptibility boundaries between bone ($\chi_v = -11.3$, SI) and water ($\chi_v = -8.9$, SI) (44). This causes inhomogeneous broadening resulting in reduced pore water T_2^* , such that $1/T_2^* \approx 1/T_2' \gg 1/T_2$, with the reduction being more severe at higher field strengths. Although several other T_2^* -selective methods for distinguishing bound and pore water exist, such as bi- or multi-exponential fitting of multiple echoes or free-induction decays (FIDs) (18,20,21,35,38) or suppression of long- T_2^* signal using long, low-amplitude pulses (14,19,34,45), this work uses adiabatic inversion recovery (22,23,26,27,35), a T_2 (and partially T_1) selective method shown in Figs. 2c,d, to null pore water signal.

In the presence of various degrees of either osteoporosis or osteomalacia, negative correlations would be expected between bound water density and porosity, and between ^{31}P density and pQCT density. More importantly, the bone mineralization ratio – a measurement which, based on the present work, is anticipated to be achievable in vivo – would be

expected to correlate with bone mineralization, a parameter which is beyond the reach of standard clinical bone densitometry. Further studies in experimentally demineralized or osteomalacic bone (obtained, for example, from animal models of osteomalacia (12)) will be required to evaluate the performance of this MRI-derived bone mineralization measurement.

The ^{31}P and bound water densities given in this work were calculated using reference phantoms calibrated against standard solutions, and are therefore expressed in nuclear densities (i.e. moles of ^{31}P or ^1H per liter). To translate these measurements into bone density figures in the conventional units of mg/cc would require certain assumptions that may introduce error. For example, to convert mol $^{31}\text{P}/\text{L}$ to mg/cc mineral, one would have to assume constant chemical composition of bone mineral. Stoichiometric calcium hydroxyapatite, which has a formula mass of 502 g/mol, contains three ^{31}P atoms per molecule. This stoichiometry would yield a conversion factor of 167 g of bone mineral per mole of ^{31}P nuclei. The mean inferred mineral density of 1129 mg/cc using this conversion factor compares well to the mean bone mineral density of 1169 mg/cc by pQCT. Individual inferred values are included in Table 1.

The stoichiometry of bone mineral approaches that of calcium hydroxyapatite, but significant deviations from this composition do exist (46,47). The Ca/P ratio of bone mineral is also known to vary somewhat in bone disease (48), so the utility of this method in distinguishing between normal, osteoporotic, and osteomalacic bone in human subjects must be independently ascertained.

Similarly, conversion of bound water density to matrix density would require even more tenuous assumptions regarding the hydration of bone matrix collagen. The term ‘bound water density’ itself already implicitly assumes that collagen contributes no ^1H signal. Under this assumption, however, it is straightforward to convert the density in mol $^1\text{H}/\text{L}$ to water density in mg/cc or volume percentage using the density (1 g/mL) and molar concentration (55.3 mol/L) of pure H_2O . The average value of the inferred bound water volume fraction is $28 \pm 4\%$, somewhat greater than the total bone water volume fractions of 17% and 29% for pre- and post-menopausal women measured in vivo by Techawiboonwong, et al. (49). This discrepancy could be the result of either additional signal from protons in bone matrix collagen, or systematic errors resulting from the relaxation correction or calibration of the reference phantom.

We have previously demonstrated a reduction in T_1 after experimental removal of mineral from bone to mimic osteomalacia (24). The present work finds decreased ^{31}P T_1 with increased bone porosity as well. Bone mineral ^{31}P longitudinal relaxation is primarily due to dipolar interactions with nearby protons. As bone is mineralized, mineral crystals displace water (50), causing the ^{31}P nuclei to be surrounded by a decreased number of water protons and thus experience reduced dipolar coupling. Conversely, as bone mineral is lost from the matrix, each remaining ^{31}P nucleus interacts with a greater number of protons (51), both in its immediate vicinity and at a distance, and as a result the T_1 relaxation time is reduced.

There are two possible explanations for the observed decrease in T_1 . First, the increase in bone water content occurs chiefly in the pore spaces. Most ^{31}P nuclei in bone are distant (in

NMR terms) from these pores. The power of dipolar interaction scales with the inverse sixth power of distance, rendering the contribution of any single distant ^1H nucleus to a ^{31}P nucleus vanishingly small. However, the number of these ^1H nuclei at a given distance increases as the square of distance; therefore, the aggregate contribution of distant ^1H dipolar interactions to ^{31}P longitudinal relaxation is not negligible, and the increase in the number of distant pore water ^1H nuclei may contribute to the observed decrease in ^{31}P T_1 .

Also, age-related bone loss is most commonly due to an increase in bone turnover. Although primary mineralization occurs only days after new bone matrix is deposited in a remodeling event, it is known that enhanced bone turnover reduces the time available for bone to undergo secondary mineralization (52), in which more mineral is slowly added over many months. It is therefore possible that mineralization in more porous bone is decreased to such an extent that the slightly increased concentration of nearby ^1H nuclei manifests in increased longitudinal relaxation rate of bone mineral ^{31}P , but not so much that this decreased mineralization can be directly quantified by any of the x-ray-based methods used in this work. Nevertheless, it is interesting to note that a statistically significant correlation exists between ^{31}P T_1 and the bone mineralization ratio ($R^2 = 0.33$, $p < 0.05$).

As expected, image SNR is higher in ^1H images than in ^{31}P images, even despite an eight-fold reduction in voxel volume. The shorter T_2^* , nearly three orders of magnitude-longer T_1 , and lower gyromagnetic ratio impose serious SNR limitations on solid-state ^{31}P images of bone mineral. In general, under Ernst angle conditions, SNR efficiency is maximized at the shortest possible TR; however, in this work, TR is many orders of magnitude shorter than T_1 , and further reduction of TR and increased averaging yields diminishing returns in SNR per unit scan time. The choice of ZTE-PETRA over UTE results in higher image SNR and a narrower PSF. ZTE imaging is also not limited to gradient isocenter as is UTE with ramp-sampling.

The goal of this study was to evaluate the feasibility of MRI-based bone mineral and matrix density measurements on a clinical scanner. Gradient strength is limited on most human scanners to 40 mT/m for patient safety and comfort. Most previous work in quantifying bone mineral density via solid-state ^{31}P imaging has taken advantage of the high gradient strengths (1000 mT/m) and rapid gradient slew rates of animal and micro-imaging scanners (12). Because the PSF FWHM is inversely proportional to gradient strength, this non-clinical hardware provides an enormous advantage in intrinsic image resolution.

This work used a single small, tightly fitting solenoidal RF coil to optimize image SNR, similar to animal or micro-imaging hardware. In vivo examination would necessarily require a larger RF coil sized to enclose a human limb, thereby imposing a significant SNR penalty. A double-tuned RF coil or a combination of ^{31}P and ^1H coils, necessary to perform both density measurements in a single scanning session, would also negatively impact SNR, though SNR efficiency can be regained by reconstruction with anisotropic voxel resolution and FOV, taking advantage of the long bones' small cross section but relatively constant distribution of bone material in axial direction.

5. Conclusions

In summary, solid-state ^{31}P and bound water-selective ^1H MRI measurements reflecting apparent bone mineral ^{31}P and matrix-bound water densities correlate with porosity, age, and pQCT apparent bone mineral density in human cortical bone specimens. This work demonstrates the feasibility of image-based quantification of bone mineral and matrix densities and their ratio, the bone mineralization ratio, in whole-body scanners.

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Abbreviations

DXA	dual-energy x-ray absorptiometry
BMD	apparent bone mineral density
pQCT	peripheral quantitative computed tomography
HR-pQCT	high-resolution peripheral quantitative computed tomography
NMR	nuclear magnetic resonance
T_2^*	effective transverse relaxation time
^{31}P	31-phosphorus
^1H	1-hydrogen
MRI	magnetic resonance imaging
μCT	micro-computed tomography
BMR	bone mineralization ratio
UTE	ultra-short echo time MRI
ZTE	zero echo time MRI
SWIFT	sweep imaging with Fourier transformation
RF	radiofrequency
WASPI	water- and fat-suppressed proton projection MRI
PETRA	pointwise encoding time reduction with radial acquisition
T_1	longitudinal relaxation time
T_2	transverse relaxation time

χ_v	volume magnetic susceptibility
FID	free-induction decay
TR	pulse repetition time
τ	RF pulse duration
B₁	radiofrequency field amplitude
M_z	longitudinal magnetization
M₀	equilibrium longitudinal magnetization
TI	inversion time
t_{SR}	saturation-recovery time
SIR	single inversion recovery
FOV	field of view
N	number of readout points
FWHM	full width at half maximum
PSF	point-spread function
SNR	signal-to-noise ratio
VOI	volume of interest
ANOVA	analysis of variance
BW	bound water
γ	gyromagnetic ratio
NEx	number of signal averages

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Figure 1. Cartoon depicting apparent matrix and mineral density changes in osteoporosis and osteomalacia versus healthy bone. Apparent bone mineral density is lower in both osteoporosis and osteomalacia, but bone mineralization is reduced in osteomalacia only.

Figure 2.

Photograph of the 4.5-cm diameter solenoid coil, with three rigidly affixed landmark reference phantoms. A bone specimen, housed in its plastic tube, is also shown. One ^1H or two ^{31}P signal intensity reference phantoms are also mounted inside the solenoid, but are not visible in this photograph.

Figure 3. Imaging pulse sequences used for bone ^{31}P and bound water density quantification: ^{31}P ZTE sequence (a) with PETRA module (b), and ^1H Single Adiabatic Inversion Recovery Rapid ZTE (SIR-rZTE) sequence (c) with PETRA module (d). Relevant pulse sequence parameters are shown.

Figure 4. Simulated response, $f_{HS} = M_z/M_0$, of spins to a 5-ms, 5 kHz bandwidth adiabatic RF pulse with respect to T_2 . Ranges of bound and pore water are indicated. While this pulse largely saturates bound water ($M_z \approx 0$), it inverts pore water ($M_z < 0$).

Figure 5. Volume rendering of a ^{31}P ZTE image of a tibial cortical bone specimen from an 83 y/o female donor. Two signal intensity reference phantoms mounted inside the RF coil (right) and three landmark reference phantoms mounted outside the RF coil (top, left, bottom) are visible.

Figure 6.

Maps of bone mineral ^{31}P density (a) and bound water density (b) in central slices of 16 human tibial cortical bone specimens. Age and gender of bone specimen donors are indicated. Bone mineral ^{31}P and bound water ^1H densities are markedly lower in bones from elderly female donors than from younger females or males. ^{31}P maps also suffer from increased point spread function blurring due to the lower gyromagnetic ratio and shorter T^{2*} of ^{31}P .

Figure 7. Correlation plot of bone mineral ^{31}P density to bound water ^1H density. The two MRI-derived densities are highly correlated, as expected in a set of equally mineralized bones.

Table 1

Age (yr)	Gender	μ CT Porosity (% Pores)	pQCT Density (mg/cc)	pQCT Density/BVF (mg/cc)	^{31}P T_1 (s)	$[^{31}\text{P}]$ (mol $^{31}\text{P}/\text{L}$)	^{31}P Density (mg HA/p/cc)	Bound ^1H (mol $^1\text{H}/\text{L}$)	Bound H_2O (%v/v)	BMR ($[^{31}\text{P}]/[^1\text{H}]$)
27	F	6.9	1245	1337	93.2	6.88	1152	35.9	32.4	1.57
30	F	6.8	1236	1326	95.0	7.40	1238	38.1	34.4	1.59
37	M	8.8	1129	1238	92.2	7.98	1335	35.4	31.9	1.85
49	M	7.6	1232	1334	111.3	7.82	1308	33.9	30.6	1.89
53	M	12.5	1172	1339	101.2	8.07	1351	32.9	29.7	2.01
53	F	4.3	1242	1298	99.2	7.96	1332	34.9	31.5	1.87
65	F	10.8	1209	1356	108.9	7.88	1319	32.7	29.5	1.98
65	F	8.5	1128	1232	95.7	6.39	1069	32.7	29.5	1.61
69	M	8.8	1182	1296	102.7	7.88	1319	30.4	27.5	2.13
74	F	12.0	1084	1233	78.4	4.55	761	27.5	24.9	1.36
75	M	12.6	1165	1334	103.3	6.79	1137	32.6	29.4	1.71
82	F	12.9	1167	1339	99.1	5.28	884	24.4	22.0	1.78
83	F	17.2	1030	1245	70.7	4.81	806	23.3	21.1	1.69
83	M	14.2	1168	1361	109.8	6.47	1084	30.1	27.2	1.76
93	M	16.0	1159	1380	95.9	6.56	1099	27.7	25.0	1.94
97	F	11.2	1149	1294	90.3	5.20	870	28.8	26.0	1.48
64.7 ± 21.5		10.7 ± 3.6	1169 ± 59	1309 ± 49	96.7 ± 10.8	6.74 ± 1.22	1129 ± 204	31.3 ± 4.2	28.3 ± 3.7	1.76 ± 0.21

Table 2

	Age	Porosity	pQCT	pQCT/BVF	³¹ P T ₁	³¹ PD	³¹ PD/BVF	BWD	BWD/BVF
BMR	2.5x10 ⁻³	3.4x10 ⁻⁹	0.09	0.18	0.33*	0.50**	0.62***	8.8x10 ⁻³	0.02
BWD/BVF	0.59***	0.40*	0.41*	0.04	0.21	0.59**	0.51**	0.94***	
BWD	0.70***	0.63***	0.50**	0.01	0.19	0.59**	0.45**		
³¹PD/BVF	0.26*	0.15	0.36*	0.17	0.47**	0.96***			
³¹PD	0.39*	0.32*	0.46**	0.11	0.45**				
³¹P T₁	0.02	0.09	0.51**	0.45**					
pQCT/BVF	4.2x10 ⁻³	0.02	0.39*						
pQCT	0.29*	0.48**							
Porosity	0.54**								