Purpose: To quantify free and bound water components of cortical bone with a model-based numeric approach with use of ultrashort echo time (UTE) magnetic resonance (MR) imaging in vivo in order to introduce a new predictor for age-related deterioration of cortical bone structure.

Materials and Methods: Human studies were compliant with HIPAA and approved by the institutional review board. Dual-repetition time three-dimensional hybrid-radial UTE imaging was performed, followed by the application of postprocessing algorithms, to quantify free and bound water parameters (concentration [\( c \]) and longitudinal relaxation time [\( T_1 \)]) of human cortical bone in vivo. The postprocessing algorithms included the decomposition of bulk equations into free- and bound-associated equations and solving resulted inverse problem by using evolutionary strategy methods. To test the validity of the introduced biomarker, it was measured in 40 healthy women by using the proposed method, and associations among parameters were evaluated with the Pearson correlation coefficient.

Results: The mean free water concentration, bound water concentration, free water \( T_1 \), and bound water \( T_1 \) in the recruited population were 5.9%, 19.6%, 306.79 msec, and 162.47 msec, respectively. All reported values were in good agreement with those in the literature. Cortical bone free water \( T_1 \) (\( R^2 = 0.72 \)) and cortical bone free water concentration (\( R^2 = 0.62 \)) showed strong positive correlations with age.

Conclusion: The cortical bone free water concentration and free water \( T_1 \) derived with UTE imaging are good predictors of age-related deterioration of cortical bone structure and are potentially superior to previously introduced measures such as bone water concentration and suppression ratio.

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Quantification of Human Cortical Bone Bound and Free Water in Vivo with Ultrashort Echo Time MR Imaging: A Model-based Approach

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Bone, which is built at submicroscopic, microscopic, and macroscopic levels, can be considered a veritable masterpiece of biomechanical engineering. Supporting the skeleton and protecting various organs, bone is one of the most important tissues in the human body. With regard to this fact, how to assess bone quality has been an intriguing question for decades.

Currently, in the clinical environment, bone mineral density (BMD) as measured with dual-energy x-ray absorptiometry is a solid metric for representing bone quality (1,2). However, BMD has a number of limitations, as follows: (a) Because bone is composed of three different phases (mineral [42% by volume], collagen matrix [35% by volume], and water [23% by volume]) (3), it is obvious that BMD cannot fully capture all the information embedded in bone (only about 40%); (b) fracture risk increases 13-fold from ages 60 to 80 years, but a decrease in BMD only doubles the risk of fracture (4); and (c) BMD cannot enable the differentiation between cortical and trabecular bone, which undermines the efficiency of the technique for bone quality assessment.

In the cortical bone, water occurs in three different locations and states: a small fraction tightly (covalently) bound to the minerals, a relatively large fraction loosely bound to the organic matrix, and the other fraction residing in the cortical bone pores. Because bone is composed of three different phases (mineral [42% by volume], collagen matrix [35% by volume], and water [23% by volume]) (3), it is obvious that BMD cannot fully capture all the information embedded in bone (only about 40%).

Cortical bone free water T1 was introduced as a predictor of age-related deterioration of cortical bone structure.

The age-related deterioration of cortical bone structure may be graded by using free water T1 and free water concentration.

The stage of the development of osteoporosis may be graded by using free water T1 and free water concentration.
TECHNICAL DEVELOPMENTS: Quantification of Human Cortical Bone Bound and Free Water

Abbasi-Rad and Saligheh Rad

Study Population

A prospective human subject study was performed between July 2009 and May 2012. MR imaging was performed with the 3D hybrid-radial UTE pulse sequence in 30 healthy men (age range, 22–77 years; average age, 51 years) and 42 healthy women (age range, 26–79 years; average age, 57 years). Because the bone quality is more variable in women than in men, the whole population for our pilot study was selected so that it consisted of female subjects. Therefore, 40 healthy women aged 26–79 years (mean age ± standard deviation, 56.3 years ± 13.4) were included in our study (two subjects from the original cross-sectional population were outliers). Eligible volunteers had a body mass index of less than 30 kg/m² and a BMD z-score (with dual-energy x-ray absorptiometry at both hip and spine) of −2 to 2. Subjects were excluded if they had a history of bone-related medical disorders (eg, malabsorption syndromes, renal or hepatic disease), had undergone surgery, were receiving treatment (eg, treatment with dexamethasone or methotrexate), or had non–MR imaging compatible implants or any medical contraindication to MR imaging (eg, heart pacemakers, implanted hearing aids). All protocols were compliant with the Health Insurance Portability and Accountability Act and approved by the institutional review board of the University of Pennsylvania (Philadelphia, Pa), where data acquisition was performed. Written informed consent was obtained from all subjects.

Data Acquisition

Because of its superiority over conventional UTE imaging, the 3D hybrid-radial UTE pulse sequence was used in our study (18). Figure 2 shows a diagram of the applied pulse sequence along with a sample image from the midshaft tibia.

Figure 1: Flow diagram of different tasks for proposed model-based quantification of cortical bone free and bound water T1 and concentration in 40 healthy women in vivo. **BWC** = bound water concentration, **ES** = evolutionary strategy, **ROI** = region of interest. **ρ}_{\text{bone}}^r and **ρ}_{\text{ref}}^r are proton densities of the bone and reference sample, respectively.

Three-dimensional (3D) hybrid-radial UTE MR imaging was performed with an effective TE of 8 µsec (18). All imaging was performed at 3.0 T (Tim Trio; Siemens, Erlangen, Germany) by using an eight-channel transmit-receive knee coil and the following parameters: field of view, 180 × 180 mm²; flip angle, 23° (commensurate with the Ernst angle for an average T1 of 250 msec); and total imaging time, 6.6 minutes. Two UTE MR examinations were performed with different repetition times (TRs) (20 and 60 msec) for each subject. To quantify cortical bone free and bound water with the acquired images, bulk longitudinal relaxation time (T1) and bulk cortical bone water concentration were quantified first. Then, these values were inputted into a system of equations and free and bound water pools quantified by using several postprocessing techniques and numeric analyses.

The whole process can be outlined by a hierarchy of tasks, as follows: (a) acquiring two sets of UTE images with different TRs (20 and 60 msec), (b) segmenting whole cortical bone by using an automatic algorithm on each image to obtain the mean signal intensity of whole cortical bone, (c) calculating bulk T1 by using Equation (2), (d) calculating bulk bone water concentration by using Equation (4), (e) decomposing the obtained equation into separate parts for free and bound components and generating a system of equations, (f) applying the selected model of free and bound water components to the equations, and (g) solving the defined optimization problem by using a self-adaptive evolutionary algorithm. The steps are shown in a flow diagram in Figure 1.
TECHNICAL DEVELOPMENTS: Quantification of Human Cortical Bone Bound and Free Water

of one subject. The sequence employs selective excitation half-sinc pulses to excite a 5.0-cm axial slab, which corresponds to a half-sinc pulse with a main lobe duration of about 160 µsec and a gradient strength of 2.9 mT/m, to acquire 10 evaluable 4.5-mm axial sections for the quantification process (total of 20 sections at a field of view of 90 mm).

The pulse sequence was applied two times successively, the first time with a TR of 10 msec and the second time with a TR of 60 msec, resulting in a stack of 40 images for each subject. All imaging was performed at 3.0 T (Tim Trio) by using an eight-channel transmit-receive knee coil and the following parameters: 500 radial projections; 256 readout samples; dwell time, 6 µsec; receiver bandwidth, 325 Hz/pixel; TR, 20 and 60 msec; field of view, 180 × 180 mm²; reconstructed pixel size, 0.38 × 0.38 mm²; flip angle, 23°; minimum TE, 8 µsec at k_y (z dimension of k space) of 0; and total imaging time, 6.6 minutes. This sequence has previously been validated in both in vivo and ex vivo setups. Interested readers can refer to the study by Rad et al (18) for a more detailed description of the sequence.

Because the cortical bone was the main focus of our study, we chose our imaging site to be at the maximum cortical thickness (19). Hence, we positioned the center of excitation section at 38% of the tibia length measured from the medial malleolus (an easily identifiable anatomic landmark).

Bone Segmentation

Because a ROI-based approach was chosen to analyze the UTE images, we used a segmentation method to extract our ROI, which was considered to be the whole cortical bone section. Cortical bone segmentation was conducted by using a method described elsewhere (20). Briefly, segmentation was performed with the following steps: (a) denoising the image with an anisotropic diffusion filter (21), (b) segmenting bone marrow area by means of a region-growing strategy (22), (c) calculating the center of mass for the segmented area, (d) implementing a polar transform on the image with the center at the point calculated in the previous step, (e) calculating intensity profile along the radius with respect to each degree, (f) calculating the endosteal and periosteal boundary locations at each angular location as the first and second peaks of derivatives of the intensity profile, respectively, and (g) implementing inverse polar transform of these boundaries into Cartesian coordinates to obtain the final segmentation boundaries.

Bulk T1 Calculation

The gradient-echo signal intensity \( S_{\text{en}} \) measured at the time value of TE was calculated as follows:

\[
S_{\text{en}} = C \cdot \rho \left( 1 - e^{-\frac{\text{TR}}{T_1^*}} \right) \left( 1 - f_z e^{-\frac{\text{TR}}{T_1}} \right) e^{-\frac{\text{TE}}{T_1}}, \tag{1}
\]

where \( C \) is a constant pertinent to radiofrequency coil receive sensitivity and \( \rho \) the proton (water) concentration. \( f_z \) is a correction factor that will be explained later. According to Equation (1), T1 was easily quantified by performing a dual experiment. Because the values of \( \rho \), T1, and T2* do not change from one experiment to another (in the same subject) and are specific to the tissue, by performing dual TR (TR of 20 and 60 msec) data acquisition (with a similar TE) and calculating mean signal intensity of whole cortical bone in the two images and dividing them by each other, T1 was quantified as follows:

\[
T_1 = \frac{S_{\text{en}}(\text{TR} = 20\text{msec})}{S_{\text{en}}(\text{TR} = 60\text{msec})} = \frac{C \cdot \rho \left( 1 - e^{-\frac{\text{TR}}{T_1^*}} \right) \left( 1 - f_z e^{-\frac{\text{TR}}{T_1}} \right) e^{-\frac{\text{TE}}{T_1}}}{C \cdot \rho \left( 1 - e^{-\frac{\text{TR}}{T_1^*}} \right) \left( 1 - f_z e^{-\frac{\text{TR}}{T_1}} \right) e^{-\frac{\text{TE}}{T_1}}}
\]

\[
= \frac{1 - e^{-\frac{\text{TR}}{T_1^*}}}{1 - f_z e^{-\frac{\text{TR}}{T_1}}}
\]

Solving Equation (2) by means of the “Dogleg trust region” algorithm in Matlab (MathWorks, Natick, Mass), we computed the T1 for cortical bone water. Because any technique or any provision was not taken into account for the differentiation between different water pools, the obtained T1 was deemed to be the bulk T1 for the cortical bone water.

The only unknown parameter essential for solving Equation (2) was \( f_z \). During imaging, the steady state is set up resulted from the competition of radiofrequency magnetic field (excitation phase) and interactions between spins and thermal equilibrium (relaxation phase). The ratio of pulse duration to the transverse relaxation time (T2) of the water molecules determines the dominant factor. In traditional imaging
protocols, because pulse duration is very short in comparison with the T2 of tissue, the excitation phase is the dominant factor and therefore the relaxation phase is negligible. In the case of calcified tissues such as cortical bone, however, T2 is in the order of the value of pulse duration, meaning that the two aforementioned mechanisms do compete narrowly and the acquired signal expression must be corrected by a factor called \( f \). For an on-resonance constant-amplitude radiofrequency excitation, an analytical expression has been derived for computing the value of \( f \), whereas otherwise (like the half-sine radiofrequency pulse used in our study), it should be calculated by means of Bloch equation simulation (23,24).

### Calculation of Bulk Bone Water Concentration

Quantification of the bulk concentration of cortical bone water (\( \rho \) in Equation [1]) demands extra information, which was acquired with a phantom with the MR spectroscopic properties akin to those of cortical bone. We chose a phantom with the following characteristics: 20% H\(_2\)O in D\(_2\)O doped with 27 mmol/L MnCl\(_2\), yielding a T1 of approximately 15 msec and T2* of approximately 320 µsec. By dividing the mean signal intensity of a circular ROI placed on the phantom (\( S_{\text{ref}} \)) by the mean signal intensity of the whole cortical bone segment (\( S_{\text{bone}} \)) in one of the images, we came up with Equation (3), which can be simplified into Equation (4) and which provided us with the bone water concentration, as follows:

\[
\frac{S_{\text{bone}}}{S_{\text{ref}}} = C \cdot \rho_{\text{bone}} \cdot \left( 1 - \frac{1}{e^{\frac{\text{TR}_{\text{ref}}}{T1_{\text{ref}}}} - \frac{1}{e^{\frac{\text{TR}_{\text{bone}}}{T1_{\text{bone}}}}}} \right) \frac{1 - f_{\text{ref}} e^{\frac{-\text{TE}_{\text{ref}}}{T2*_{\text{ref}}}}}{1 - f_{\text{bone}} e^{\frac{-\text{TE}_{\text{bone}}}{T2*_{\text{bone}}}}} \]
\]

and

\[
\rho_{\text{bone}} = \rho_{\text{ref}} \cdot \frac{S_{\text{bone}} F_{\text{ref}}}{S_{\text{ref}} F_{\text{bone}}} \left( e^{\frac{\text{TR}_{\text{ref}}}{T1_{\text{ref}}} \left( R2*_{\text{ref}} - R2*_{\text{bone}} \right)} \right) \]

where \( \rho_{\text{bone}} \) and \( \rho_{\text{ref}} \) are proton densities of the bone and reference sample, respectively, \( R2*_{\text{bone}} \) and \( R2*_{\text{ref}} \) are the effective transverse relaxation rates (\( R2^* = 1/T2^* \)) for bone (\( T2^* \) is approximately 750 µsec) and the reference sample (\( T2^* \) of approximately 320 µsec) (18), respectively, and \( \text{TE}_{\text{eff}} \) is the effective TE. The relative signal amplitudes \( F_{\text{ref}} \) and \( F_{\text{bone}} \) are functions of the relaxation times, pulse TR, and, as the pulse duration \( \tau \) is of the same order of or longer than \( T2^* \), the \( \tau^2 \) to \( T2*_{\text{bone}} \) and \( \tau^2 \) to \( T2*_{\text{ref}} \) ratios. Again, because no technique or provision was used for the discrimination between different water pools, the obtained \( \rho \) value was deemed to be the bulk concentration of cortical bone water. \( F_{\text{bone}} \) and \( F_{\text{ref}} \) were calculated by means of Bloch equation simulation for phantom and cortical bone tissue, respectively.

The bulk \( T2^* \) values were shown to be independent of subjects in a study of seven healthy volunteers (25). In our previous study (18), however, bulk \( T2^* \) was measured in six different subjects and yielded an average value of 750 µsec, with an intersubject variation of less than 10%.

It is worth mentioning that because this step of quantification was based on comparison of the signal intensity of cortical bone with that of the phantom on the UTE MR images, spatial dependence of the receive coil sensitivity might introduce large systematic errors into the quantification results. Therefore, this deleterious effect of the inhomogeneous reception profile of the knee coil was compensated for by creating a mask with help of a homogeneous phantom (pure water) and dividing the bone intensity image by the mask on a pixel-by-pixel basis (18).

### Generation of the System of Equations

On one hand, the state of water molecules and their degree of interaction with the environmental components dictate their T2; on the other hand, the shorter their T2, the shorter TE will be necessary to capture their signal. Therefore, it is the TE that determines the contribution of different water pools (different T2s) in the measured signal. Figure 3 shows which solid-state
MR imaging technique is able to capture which types of water pools of the cortical bone structure. With regard to the effective TE used in our study, only two components of the cortical bone water contribute to the measured signal intensity: collagen bound water and free water. Hence, we decomposed the gradient-echo signal intensity equation into two similar equations that correspond to these different components, as follows:

\[ \rho_{\text{bulk}} \left( 1 - e^{\frac{T_{1\text{bulk}}}{T_{1\text{bulk}}}} \right) - \int_{-T_{\text{max}}}^{+T_{\text{max}}} \rho_{\text{free}}(t) \left( 1 - e^{\frac{T_{1\text{free}}}{T_{1\text{free}}}} \right) dt e^{\frac{T_{1\text{bulk}}}{T_{1\text{bulk}}}} \]

\[ \int_{-T_{\text{max}}}^{+T_{\text{max}}} \rho_{\text{bound}}(t) \left( 1 - e^{\frac{T_{1\text{bound}}}{T_{1\text{bound}}}} \right) dt e^{\frac{T_{1\text{bulk}}}{T_{1\text{bulk}}}} = 0, \quad (5) \]

where \( T_{1\text{max}} \) is the maximum value of \( T_1 \), \( t \) the variable of integral, and \( dt \) its derivative. Knowing the value of the left side of Equation (5), we aimed to quantify \( T_1 \) (relaxometry of cortical bone). Given this distribution function, one can characterize the water pool completely: its under-curve area gives the concentration value of the water pool and its relaxation time (no distribution among water molecules). Hence, what is essential for the differentiation of free and bound water is much more than that of free water (14); therefore, the obtained values for \( T_2^* \) are subject dependent and do not vary greatly from one subject to another (25). Hence, the values for \( T_2^*_{\text{free}} \) and \( T_2^*_{\text{bound}} \) were extracted from the literature as 2.46 msec and 0.33 msec, respectively, at 3.0 T (27). Consideration of the \( T_2^* \) and \( T_2^*_{\text{bound}} \) was simplified, as follows:

\[ \rho_{\text{bulk}} \left( 1 - e^{\frac{T_{1\text{bulk}}}{T_{1\text{bulk}}}} \right) - \int_{-T_{\text{max}}}^{+T_{\text{max}}} \rho_{\text{free}}(t) \left( 1 - e^{\frac{T_{1\text{free}}}{T_{1\text{free}}}} \right) dt e^{\frac{T_{1\text{bulk}}}{T_{1\text{bulk}}}} = 0, \quad (6) \]

\[ \rho_{\text{free}} \left( 1 - e^{\frac{T_{1\text{free}}}{T_{1\text{free}}}} \right) - \int_{-T_{\text{max}}}^{+T_{\text{max}}} \rho_{\text{free}}(t) \left( 1 - e^{\frac{T_{1\text{free}}}{T_{1\text{free}}}} \right) dt e^{\frac{T_{2\text{free}}}{T_{2\text{free}}}} = 0, \quad (7) \]

\[ \rho_{\text{bound}} \left( 1 - e^{\frac{T_{1\text{bound}}}{T_{1\text{bound}}}} \right) - \int_{-T_{\text{max}}}^{+T_{\text{max}}} \rho_{\text{bound}}(t) \left( 1 - e^{\frac{T_{1\text{bound}}}{T_{1\text{bound}}}} \right) dt e^{\frac{T_{2\text{bound}}}{T_{2\text{bound}}}} = 0, \quad (8) \]

**Model Selection**

According to Figure 3, each component is distributed among a part of the T2 axis, which suggests that each water pool possesses a distribution function. Given this distribution function, one can characterize the water pool completely: Its under-curve area gives the concentration value of the water pool and its distributed interval gives the range of its relaxation time values. Hence, what is essential for the differentiation of free and bound water from this standpoint is to allocate a distribution function to each water component in the postprocessing phase of the computations and then search for its optimum parameters.

The ROI-based analysis in this pilot study resulted in the simplest model, where the water molecules of each type were assumed to have an identical relaxation time (no distribution among the T2/T1 axis). Therefore, this simple model (delta function model) had only two parameters to be estimated: the location of delta function on the relaxation time axis (the relaxation time value) and the amplitude of the delta function (concentration value).

According to the selected model for free and bound water pools, the abovementioned system of equations was simplified, as follows:

\[ \rho_{\text{bulk}} \left( 1 - e^{\frac{T_{1\text{bulk}}}{T_{1\text{bulk}}}} \right) - \int_{-T_{\text{max}}}^{+T_{\text{max}}} \rho_{\text{free}}(t) \left( 1 - e^{\frac{T_{1\text{free}}}{T_{1\text{free}}}} \right) dt e^{\frac{T_{1\text{bulk}}}{T_{1\text{bulk}}}} = 0, \quad (9) \]

\[ \rho_{\text{free}} \left( 1 - e^{\frac{T_{1\text{free}}}{T_{1\text{free}}}} \right) - \int_{-T_{\text{max}}}^{+T_{\text{max}}} \rho_{\text{free}}(t) \left( 1 - e^{\frac{T_{1\text{free}}}{T_{1\text{free}}}} \right) dt e^{\frac{T_{2\text{free}}}{T_{2\text{free}}}} = 0, \quad (10) \]

\[ \rho_{\text{bound}} \left( 1 - e^{\frac{T_{1\text{bound}}}{T_{1\text{bound}}}} \right) - \int_{-T_{\text{max}}}^{+T_{\text{max}}} \rho_{\text{bound}}(t) \left( 1 - e^{\frac{T_{1\text{bound}}}{T_{1\text{bound}}}} \right) dt e^{\frac{T_{2\text{bound}}}{T_{2\text{bound}}}} = 0, \quad (11) \]

where there existed six unknown parameters as \( \rho_{\text{free}} \), \( \rho_{\text{bound}} \), \( T_{1\text{free}} \), \( T_{1\text{bound}} \), \( T_{2\text{free}} \), and \( T_{2\text{bound}} \). It has been shown that the value of \( T_{2}\) is not subject dependent and it does not vary greatly from one subject to another (25). Hence, the values for \( T_{2\text{free}} \) and \( T_{2\text{bound}} \) were extracted from the literature as 2.46 msec and 0.33 msec, respectively, at 3.0 T (27). Considering that \( \rho_{\text{bulk}} = \rho_{\text{free}} + \rho_{\text{bound}} \), we came up with a system of three nonlinear equations with three unknown parameters. Previously reported values and further examinations into the structure of cortical bone have revealed that the amount of cortical bone bound water is much more than that of free water (14); therefore, the obtained system of equations was converted to a constraint optimization problem as Equation (12):

\[ \text{Min } F = (\text{Eq } [6] )^2 + (\text{Eq } [7] )^2 + (\text{Eq } [8] )^2 \]

\[ \text{s.t. } \rho_{\text{free}} < \rho_{\text{bound}}, \quad (12) \]
where $\rho_{\text{free}}$, $T1_{\text{free}}$, and $T1_{\text{bound}}$ are the unknown parameters, meaning that we were in trail of the optimum values of them in a 3D space (each axis corresponds to each of the unknown parameters) in a way that minimizes the $F$ function as the objective function.

## Solving the Optimization Problem

To solve the optimization problem, we used evolutionary strategy techniques (28). These strategies are a subclass of nature-inspired direct search methods belonging to the class of evolutionary algorithms that use mutation, recombination, and natural selection applied to a population of individuals containing candidate solutions to evolve iteratively better and better solutions. In our work, a self-adaptive evolutionary algorithm was used to let the algorithm automatically control setting of its parameters by itself.

There were five steps in the applied algorithm: initialization, recombination, mutation, natural selection, and iteration. For initialization, each individual (solution) was considered as a chromosome consisting of six genes, where the first three were the values for unknown parameters ($\rho_{\text{free}}$, $T1_{\text{bound}}$, and $T1_{\text{free}}$) and the other three were the sigma values that provided the algorithm with self-adaptiveness. As the first population, we generated five individuals ($n = 5$) randomly (five random solutions). For recombination, the recombination operator was defined to make the individuals procreate new ones. With use of this operator, a so-called embryonic population was produced from the parent population whose number of individuals ($\lambda = 10$) was twice the size of the first population. For mutation, the operator mutated the individuals to improve their compatibility with the environment (minimizing the objective function), which culminated with the offspring population. For natural selection, the offspring must befit the environment (search space) to survive. The competency of the individuals were assessed by the objective function ($P$) and the best five were selected as the next generation (reducing the population size to its initial value). The last step was iteration. Iteration of the aforementioned process simulated the evolution and helped us reach the optimum solution. Thereafter, we iterated the whole process, recapitulated in Figure 4, through 15 successive generations.

## Statistical Analysis

All image analysis, regidding, reconstruction, and segmentation steps were performed by using custom-designed software programmed in Matlab (version 7.5, Mathworks). To evaluate the hypothesized linearity between proposed MR imaging–derived parameters and age, correlations between the two identities were examined by means of linear least square regression, yielding the Pearson correlation coefficient. The significance of each correlation was determined by using one-way analysis of variance, and $P < .05$ was considered indicative of a statistically significant difference.

### Results

The mean $T1$ and $p$ values for cortical bone free, bound, and bulk water according to age are reported in Table 1.

The mean $T1_{\text{free}}$, $T1_{\text{bound}}$, $\rho_{\text{free}}$, and $\rho_{\text{bound}}$ values among the 40 healthy women in our study were 306.79 msec, 162.47 msec, 5.89%, and 19.59%, respectively. All values were consistent with those reported in the literature (10,29–31) (this consistency will be elaborated on in the Discussion).

TECHNICAL DEVELOPMENTS: Quantification of Human Cortical Bone Bound and Free Water

Abbasi-Rad and Saligheh Rad

### Table 1

**T1 and Bone Water Concentration for Free and Bound Water according to Age**

| Age (y) | No. of Subjects | T1 (msec)* | Concentration (%)* |
|---------|-----------------|------------|-------------------|
|         |                 | Free Water| Bound Water       |
|         |                 |            |                   |
| 30–40   | 6               | 186.4 ± 61.2| 134.67 ± 48.66   | 3.48 ± 1.19 | 17.44 ± 3.42 |
| 40–50   | 3               | 253.4 ± 26.01| 193.98 ± 31.26   | 5.44 ± 0.53 | 18.4 ± 2.24  |
| 50–60   | 13              | 326.95 ± 29.58| 186.01 ± 48.19   | 6.15 ± 0.85 | 19.6 ± 3.83  |
| 60–70   | 11              | 352.52 ± 37.28| 169.93 ± 71.39   | 6.83 ± 1.03 | 20.82 ± 4.14 |
| 70–80   | 7               | 414.69 ± 36.22| 127.77 ± 46.52   | 7.56 ± 1.42 | 21.71 ± 4.48 |
| Total   | 40              | 306.79 ± 88.71| 162.47 ± 29.91   | 5.89 ± 1.56 | 19.59 ± 1.73 |

* Data are means ± standard deviations.
Discussion

In our study, we quantified four important parameters of human cortical bone—$p_{\text{free}}$, $p_{\text{bound}}$, $T_1^{\text{free}}$, and $T_1^{\text{bound}}$—in vivo in 6.6 minutes. To the best of our knowledge, our method is the fastest quantification strategy reported in the literature. Age-related alterations of cortical bone structure were modeled by the extracted cortical bone free water parameters ($p_{\text{free}}$, $T_1^{\text{free}}$).

The clinical motivation for quantifying MR spectroscopy properties (concentration and T1) of cortical bone free and bound water is the fact that they play a pivotal role in both bone quality assessment and diagnosis of bone-related diseases. Cortical bone bound water is correlated with its mechanical properties such as peak stress and yield stress. Therefore, it can be used as a clinical biomarker to assess mechanical competency of cortical bone (13). Bound water also has the potential to enable the diagnosis of type 2 diabetes–related bone deterioration (32). Hence, the success of the proposed clinical method in the quantification of cortical bone bound water will facilitate future studies of the abovementioned topics.

On the other hand, cortical bone free water is an indicator of porosity because it resides in the cortical bone pores such as lacuna, canaliculi, and Haversian canals (33). McCalden et al (34) conducted a study of 200 specimens of human cortical bone of the femur and found that an age-related increase in porosity accounted for 70% of the reduction in strength. Therefore, the success of the proposed method in the quantification of cortical bone free water will facilitate further investigations into cortical bone porosity (30,35).

Before this work, several studies have been performed with the same purpose as described in the following. In the first study, Biswas et al (14) and Du et al (36) quantified free and bound water on the basis of their different T2* values. The methodology included MR imaging followed by a postprocessing technique. They performed bicomponent analysis on the signal decay of fast two-dimensional UTE multiple TE acquisitions (14,36). Their acquisition time was 14 minutes.

In the second study, Horch et al (10) predicted their discrimination technique on T2 differences between water components. The methodology included the application of two different T2-selective MR imaging techniques: one for free water imaging and the other for bound water imaging. To image cortical bone free water, two consecutive broadband adiabatic full passage full pulse sequences termed double adiabatic full passage were applied to saturate bound water signal while leaving free water component unaffected. To image cortical bone bound water, one adiabatic full passage pulse followed by an appropriate delay termed adiabatic inversion recovery was applied to null pore water component while remaining bound water unaffected double adiabatic full passage and adiabatic inversion recovery preparation pulses were followed by conventional UTE acquisitions to image cortical bone free and bound water, respectively. Their imaging time was 14 minutes for double adiabatic full passage and adiabatic inversion recovery sequences (29,37).

The third study was performed by Chen et al (38) to distinctively quantify free and bound water T1. They used two different pulse sequences for this purpose: a 3D inversion recovery–cone UTE sequence with five TR–inversion time combinations and a TE of 8 μsec was used for the quantification of bound water T1, and a 3D UTE cone sequence with nine different TRs, called “UTE with variable TR,” and a TE of 2.5 msec was used to quantify pore water T1. The total imaging time required by this protocol for free and bound T1 quantification was 25 minutes (38).

As most of the weight of the discrimination technique was put on the postprocessing phase of our proposed protocol, we reduced the acquisition time (6.6 minutes) considerably in comparison with that of previous studies. This is despite the fact that there is still room to reduce the acquisition time by using newly proposed sequences like cone UTE.

The mean $p_{\text{free}}$ and $p_{\text{bound}}$ values suggested that healthy volunteers had high bound water and low free water concentrations. Because free and bound water concentrations are representatives of the bone matrix and the pores within it, respectively, the difference between the obtained mean values was reasonable. Manhard et al (29) performed double adiabatic full passage and adiabatic inversion recovery sequences to quantify absolute values of free and bound water in five subjects (two male and three female subjects), reporting the mean values as 7.32 and 27.86 mol hydrogen 1(H) per liter of bone, respectively. Their presentation of data was different than ours and made the comparison of the data between the two studies difficult; however, the ratio of free to bound water was the same for both studies.

Many studies have quantified bulk water T1 (7,16,18,25), but studies performed to quantify free water T1 are
scant. Horch et al (10) quantified T1 values in human cortical bone free water ex vivo at 4.7 T. They performed inversion recovery preparation pulses with 24 different recovery times preceding a Carr-Purcell-Meiboom-Gill acquisition that were finally fitted with a two-dimensional T1-T2 spectrum. Knowing the corresponding T2 of the free water from previously acquired T2 spectrum, they measured T1 of free water to be in the range of 500–1000 msec (10).

In a previous study (30), we evaluated 30 healthy volunteers at 1.5 T with use of a clinically available short-TE pulse sequence to quantify free water T1. The mean value for our recruited population was 172.5 msec.

T1 is dependent on field strength ($B_0$) in that it increases with an increase in $B_0$ (39). According to this relationship, the acquired mean T1 in our study was less than that reported at 4.7 T (10) and higher than that reported at 1.5 T (30), demonstrating good agreement with the established reports in the literature.

$\rho_{\text{free}}$ and $T1_{\text{free}}$ showed good correlation with age, corroborating the fact that cortical bone free water MR properties are good predictors of age-related deterioration of cortical bone structure. We had the same result in a previous study conducted in 30 healthy volunteers (11 male and 19 female subjects) with use of another method (dual-TR short TE MR imaging of cortical bone) (30). The correlation coefficient between free water T1 and age in that study was 0.78.

To introduce a predictor for age-related alterations of cortical bone structure, Li and colleagues (17,40) introduced a feature called the suppression ratio. They defined suppression ratio as the ratio of the unsuppressed UTE signal intensity to the long-T2-suppressed signal intensity and proved that to be an index of porosity. A positive correlation ($r^2 = 0.41$) between suppression ratio and age among 40 healthy female subjects was reported, which suggests that suppression ratio can be an alternative feature for the prediction of age-related cortical bone structure deterioration (17,40). The shortcoming of the suppression ratio was that it was not able to enable complete differentiation of bound and free water. The information of both free and bound water was conflated and, thus, correlation coefficients higher than $r^2 = 0.5$ could not be reached.

According to the literature, there are four predictors of age-related deterioration of cortical bone structure: $\rho_{\text{free}}$, $T1_{\text{free}}$, bulk bone water concentration, and suppression ratio, which have correlation coefficients of 0.62, 0.72, 0.21, and 0.41, respectively (40). Hence, one can easily note that $T1_{\text{free}}$ is the most efficient predictor of age-related deterioration of cortical bone. The physical concept behind this relationship is that the T1 of free water molecules is related to the surface-to-volume ratio of the pores harboring them, which is formulated as $1/T1 \propto (S/V)$, where T1 is the longitudinal relaxation time of the hydrogen protons residing in the pores with the surface of S and volume of V.
During aging, the surface-to-volume ratio of the pores decreases, culminating in the increase of T1 (39).

One should note that we are aware that there was not such a spectrum for T1 values of different cortical bone water components. Nonetheless, we postulated that it can be considered the same spectrum for T1 values and the whole study can be predicated upon this assumption. In the end, strong correlation coefficients between free water concentration and T1 values and age corroborated our results and, consequently, the assumed hypothesis.

Introducing a predictor for age-related deterioration of cortical bone is one of the major challenges of this research field. In the context of this challenge, the usefulness of our proposed model-based approach lies in the fact that it offers such predictor concurrently reconciling the simplest methodology (acquiring a single UTE MR image in 6.6 minutes) with the highest competence (highest correlation coefficient between the proposed predictor and age).

There are several limitations to this study. First, during imaging we captured signal from proton (1H) and assumed that all of these signals emanated from water molecules; however, it should be noted that all of the hydrogen molecules (protons) are not in the structure of water and there exists some other sources of proton in cortical bone, such as lipids in the cement line spaces between osteons and covalently bound backbone protons in collagen component (10).

Second, in this pilot study we assumed the simplest model (distribution function), delta function, for each water component. To select the proper model for different water pools, there are two different approaches, as shown in Figure 6: (a) voxel-based analysis and (b) ROI-based analysis. In voxel-based analysis, because the signal has been recorded from every single voxel, an arbitrary distribution function for each water component can be considered; in ROI-based analysis, however, by considering whole segmented bone area as the ROI, the spatial distribution of information through voxels is neglected and a delta function model is chosen implicitly. By pursuing the second strategy in our study, we ignored the spatial distribution of water components among whole cortical bone and posed some errors to the final results. In other words, we assumed all pores of cortical bone for one subject to be of the same size and all the water molecules to have the same amount of mobility owing to the same value of T1.

Third, there was a limitation related to a subject’s motion. Despite all efforts to immobilize a subject’s legs during the course of imaging, unexpected motions might have occurred, introducing error in the image analysis procedure and in T1 and bone water quantification.

Fourth, at the first step of our method the T1 values of bulk water were quantified and inputted into the postprocessing algorithms. Hence, the accuracy of T1 values was important. There are a variety of methods for quantifying T1 values, among which we chose the dual-TR technique. Because it is not the most efficient and precise T1 quantification technique and there is still room to improve T1-mapping strategies, the applied technique might be less than optimal. Therefore, the whole strategy can be further improved by investigating more precise T1-mapping techniques.

In conclusion, T1_bound and ρ_free can potentially be a better predictor of age-related deterioration of cortical bone structure than bulk bone water concentration and suppression ratio. In addition, model-based analysis of UTE MR images of cortical bone has the potential to be a fast clinical strategy for the quantification of cortical bone free and bound water and can be improved by using more precise models for cortical bone different water pools in longitudinal future studies.

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