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***In Vivo* Bone ^{31}P Relaxation Times and Their Implications on Mineral Quantification**

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Abstract

Purpose—The inter-subject variations in bone ^{31}P T_1 and T_2^* , as well as their implications on *in vivo* ^{31}P MRI-based bone mineral quantification were investigated at 3 T field strength.

Methods—A technique that isolates the bone signal from the composite *in vivo* ^{31}P spectrum was first evaluated by simulation and experiments *ex vivo*, and subsequently applied to measure the T_1 of bone ^{31}P collectively with a spectroscopic saturation-recovery (SR) sequence in a group of healthy subjects aged 26 to 76 years. T_2^* was derived from the bone signal line width. The density of bone ^{31}P was derived for all subjects from ^{31}P ZTE images acquired in the same scan session using the measured relaxation times. Test-retest experiments were also performed to evaluate repeatability of this *in vivo* MRI-based bone mineral quantification protocol.

Results—The T_1 obtained *in vivo* using the proposed spectral separation method combined with SR sequence is 38.4 ± 1.5 s for the subjects studied. Average ^{31}P density found was 6.40 ± 0.58 mol/L (corresponding to 1072 ± 98 mg/cm³ mineral density), in good agreement with an earlier study in specimens from donors of similar age range. Neither the relaxation times ($p=0.18$ for T_1 , $p=0.99$ for T_2^*) nor ^{31}P density ($p=0.55$) was found to correlate with subject age. Average coefficients of variation for the repeat study were 1.5%, 2.6%, 4.4% for bone ^{31}P T_1 , T_2^* and density, respectively.

Conclusion—Neither ^{31}P T_1 nor T_2^* varies significantly in healthy adults across a 50-year age range, therefore obviating the need for subject-specific measurements.

Keywords

bone mineral; phosphorus; solid state; short echo time; relaxation time

Introduction

Recent advances in solid-state MRI have made possible imaging of the mineral portion of bone by means of phosphorus-31 (^{31}P) ultra-short echo-time (UTE) and zero echo-time (ZTE) pulse sequences (1, 2) including methods for quantification of ^{31}P density (3, 4). The resulting measurements typically yield apparent density only, similar to 3-D CT. However, MRI has the potential to quantify true mineralization density, often referred to as degree of mineralization of bone (DMB) (5), which so far could be obtained only on specimens by means of autoradiography (6) or synchrotron radiation microtomography (7). An assessment of DMB requires knowledge of the unmineralized fraction of the bone, essentially the density of the collagen matrix (8). The protons of collagen have transverse relaxation times on the order of 50 μs , and are therefore not amenable to detection by *in vivo* MRI methods. However, as pointed out in a recent paper by Seifert et al, the collagen-associated water can be detected and quantified, thereby possibly serving as a surrogate for collagen itself (3).

Knowledge of DMB is of interest as this quantity would allow differentiation of osteoporosis from osteomalacia. The former is characterized by loss of bone volume with the remaining bone tissue properly mineralized, while in the latter case the osteoid is poorly mineralized even though the total volume may be normal. Of note is that current x-ray based densitometric techniques are unable to make this clinically important distinction. Both conditions are common in older people but the treatment of the two disorders is very different.

The practicality of accurate quantification of collagen-bound water (usually referred to as 'bound water') has been demonstrated by several laboratories (4, 9, 10). Quantification of mineral phosphorus by MRI in live human subjects, however, is far more difficult, even though its feasibility has been reported recently (4). Detection sensitivity scales as the ratio of T_2/T_1 ($\sim 10^{-5}$ for ^{31}P in apatite-like solids such as bone mineral), which is compounded by the ^{31}P nucleus' smaller gyromagnetic ratio ($\gamma_{^{31}\text{P}}/\gamma_{^1\text{H}} = 0.401$), lowering achievable SNR by another order of magnitude (11). Further, quantification requires co-imaging a density calibration sample, for instance containing synthetic calcium hydroxyl apatite. Since the T_1 of the reference sample differs from that of bone, a signal intensity correction is needed to account for their differences. Thus, accurate knowledge of the ^{31}P relaxation properties of the bone is required to derive its density.

^{31}P T_1 relaxation in bone mineral is dominated by dipole-dipole relaxation with nearby protons, most likely, crystal water or the hydroxyl ion. The relaxation rate ($1/T_1$) is proportional to the product of the squares of the gyromagnetic ratios of the interacting spins (^1H and ^{31}P) and inversely proportional to the sixth power of the inter-nuclear distance. It is the relative remoteness of nearby protons and their scarcity that renders this mechanism relatively ineffective. Nevertheless, as shown previously at 3 T 80% of the relaxation rate is governed by ^1H - ^{31}P dipole-dipole interaction, as determined by deuterium exchange of the labile protons (12). On the other hand, the transverse relaxation rate ($1/T_2$) responsible for the very large line width of mineral ^{31}P has been attributed to homonuclear ^{31}P - ^{31}P dipole-dipole coupling (13)

A number of previous studies have reported bone ^{31}P T_1 relaxation times (3, 12, 14, 15). Seifert et al measured bulk ^{31}P T_1 in lamb cortical bone (12) and subsequently in human bone specimens (3) using a saturation-recovery method. Earlier, Robson et al had reported image-based ^{31}P T_1 measurements in the human tibia *in vivo* with both saturation-recovery and variable flip-angle UTE (14) at 1.5 T finding average values of 8.6 seconds with the latter method in seven subjects. More recently, Wu et al measured ^{31}P T_1 at 3 T in two subjects by means of a progressive saturation method, one spectroscopically (i.e. non spatially resolved) and the other using a low-resolution image based method, reporting values of 17 and 19 seconds, respectively (15). The much longer values found at 3 T suggest a strong field dependence, which was studied systematically more recently by Seifert et al (12) who found T_1 to increase monotonically with field strength in lamb cortical bone from 12.8 ± 0.5 s at 1.5 T to 97.3 ± 6.4 s at 11.7 T.

However, most prior measurements were either conducted *ex vivo* in various species or *in vivo* resulting in very long scan times. In addition, measurements *in vivo* may be affected by contributions from soft tissue ^{31}P signals arising from phosphorus metabolites. Robson et al (14) discounted such effects as significant given that bone ^{31}P concentrations are larger by about three orders of magnitude than those of muscle metabolites. While in the work by Wu et al the experiments were conducted in the wrist (15), which is covered by a thin layer of soft tissue only, in the tibia the volume fraction of muscle adjacent to bone is much larger and contribution of ^{31}P signal from metabolites could become more significant.

The primary objectives of the present work was to investigate means to isolate and measure bone ^{31}P T_1 values and to determine the implications of inter-subject variations in T_1 on quantification of bone ^{31}P content. To this end, a technique to separate bone ^{31}P signal from those of the metabolites was first evaluated via simulation and *ex vivo* experiments, and subsequently applied *in vivo* to measure bone ^{31}P T_1 relaxation times in a group of healthy subjects of wide age range. Subsequently, the measured relaxation properties were used to estimate bone ^{31}P density from ZTE images. Finally, repeatability of the MRI-based bone mineral quantification protocol incorporating relaxation measurements was evaluated *in vivo*.

Methods

T_1 Measurement Sequence

T_1 of most tissues can be measured accurately by means of the inversion recovery strategy. However, due to its extremely short T_2 , it is impossible to adequately invert the magnetization of bone ^{31}P . Therefore, a saturation recovery (SR) technique was used to measure ^{31}P T_1 (12). Furthermore, the inherently low SNR of bone ^{31}P would make reliable image-based T_1 measurements prohibitively long. For this reason, a spatially non-selective spectroscopy-based method was implemented.

The SR spectroscopic sequence used starts off with a saturation (SAT) block to saturate the ^{31}P spins (Figure 1). Each of the twelve SAT pulses is followed by a spoiler gradient to enhance saturation and reduce echo formation using multiple gradient orientations (3). After a saturation recovery delay (TSR), an FID is collected. TSR is stepped from one acquisition

to the next to sample the T_1 recovery process. The set of TSRs chosen were: 2, 4, 8, 16, 32, 64, 128 and 256 s, resulting in a total scan time of 8.5 minutes. A $^1\text{H}/^{31}\text{P}$ dual-frequency birdcage coil (Rapid Biomedical, Rimpf, Germany) was used. At maximum allowable peak power, a 60° nominal flip angle could be achieved at 120 μs pulse duration, which is less than ^{31}P T_2 in bone mineral. This flip angle was used for both saturation and excitation.

Bloch equation simulations show that twelve 60° constant-phase RF pulses are able to achieve $> 99.5\%$ saturation for ^{31}P from both bone and metabolites. Signal was acquired 20 μs after excitation with a dwell time of 4 μs , and a total of 4096 data points were collected, but the first 8 points affected by coil ringing during T/R switching were removed before reconstruction.

Whole-volume spectroscopy with short nonselective RF pulses was indicated to capture the short, low-SNR ^{31}P signal. However, this approach entails two potential problems: First, because the signal is the integral over the entire sensitivity region of the transmit/receive coil, ^{31}P signal from all tissues are detected, not only from bone. As demonstrated in Figure 2, ^{31}P peaks from muscle metabolites are superimposed on the broad bone spectrum. Accurate measurement of bone phosphorus requires proper isolation of the bone peak from all other phosphorus signals. Second, whole-volume measurement is sensitive to B_1 inhomogeneity, which could adversely affect saturation. Both issues were investigated via simulation as well as experimentally.

Isolation of the Bone ^{31}P Spectrum

In order to separate the bone spectrum from the composite signal shown in Figure 2, a spectral subtraction method was used yielding two spectra at each TSR during post-processing. Removal of the first 150 data points (corresponding to fast-decaying bone signal) results in a spectrum dominated by the much narrower metabolite signal components (Spec_Metabolite). The other spectrum was generated after removing 150 points from the end of the original FID, resulting in a composite spectrum consisting of both bone and metabolite peaks (Spec_Total). In this manner both FIDs have the same number of data points. After appropriate phase correction (both zero and first order), subtraction between the real parts of the two spectra yielded the bone ^{31}P spectrum (Spec_Subtract). Subsequently, the bone ^{31}P spectrum from each TSR was fitted to a Lorentzian. The processing steps are schematically illustrated in Figure 3. The total peak area is then fitted to the three-parameter model to estimate T_1 (Eqn. 1):

$$S = S_0 \cdot (1 - (1 - Q) \cdot e^{\frac{-\text{TSR}}{T_1}}) + N \quad [1]$$

where S_0 is the equilibrium signal of bone ^{31}P , Q represents the proportion of unsaturated ^{31}P spins in bone following the SAT pulses, which should ideally be zero for a perfect saturation, and N is noise. T_2^* of bone ^{31}P was estimated as $1/(\pi \times \text{FWHM})$, where FWHM is the full width at half maximum of the fitted Lorentzian. Although chemical shift anisotropy, homonuclear and heteronuclear scalar coupling are expected to cause deviations

from Lorentzian line shape (16), we found the latter to be a reasonable approximation. Lastly, the Lorentzian conforms to the concept of T_2^* as the time constant for monoexponential decay.

Simulation

Simulation experiments were carried out to investigate the accuracy of the proposed T_1 measurement method in the presence of ^{31}P -containing metabolites. Based on the *in vivo* ^{31}P spectrum observed both in this study as well as those reported in the literature (17), five ^{31}P sources from metabolites— α -ATP, β -ATP, γ -ATP, phosphocreatine (PCr) and inorganic phosphate (Pi) were considered. Chemical shifts of the metabolites were measured *in vivo* while relaxation properties are based on values reported previously by Meyerspeer et al (18). T_1 and T_2^* of bone ^{31}P were assumed to be 35 s and 200 μs based on an initial *in vivo* test scan. Bone ^{31}P concentration measured *ex vivo* by Seifert et al (3), calf muscle metabolites concentrations measured *in vivo* by Kemp et al (17), and volume of cortical bone and muscle in the lower leg measured from conventional 3D gradient echo (GRE) images of four healthy subjects were used to estimate the proportions of bone and metabolites in the total signal. The simulation also incorporated B_1 field inhomogeneity along the z-axis (where most variation is expected for non-selective whole-volume measurements). The volume coil used is of a fixed birdcage design with an inner diameter of 17.6 cm, which is large enough to pass the foot through. It consists of two concentric-element coils, tuned to ^1H and ^{31}P , respectively. The two coils have the same physical size and number of rungs (eight), with the two sets rotated relative to each other by 22.5° . Therefore, the B_1 fields of the two coils are expected to have similar characteristics. Since it is difficult to map the B_1 of the ^{31}P channel due to the inherently low SNR, the proton channel of the dual-frequency coil was used for B_1 mapping, which was accomplished with the Bloch-Siegert technique (19) by means of a homogeneous doped water phantom. Because the phantom was not sufficiently long to cover the entire sensitivity region of the coil, the measured B_1 profile was fitted to a Gaussian function to estimate B_1 in the missing outer-most regions. Receive sensitivity (B_1^-) was assumed to be the same as transmit B_1 (B_1^+) from the principle of reciprocity as the same birdcage structure was used for both transmission and reception. Parameters of the simulated SR sequence were the same as described above.

Ex Vivo Validation

For further validation, two sets of *ex vivo* experiments were performed on a 3 T (49.9 MHz) TIM Trio system (Siemens Medical Solutions, Erlangen, Germany) with the $^1\text{H}/^{31}\text{P}$ dual-frequency coil. The first experiment was aimed at investigating the effect of inhomogeneous B_1 on the accuracy of T_1 measurements. Four lamb tibiae (with soft tissue and articular cartilage removed) were cut into small sections of approximately 5 cm length and T_1 and T_2^* were measured with two different specimen placements: 1) all bone fragments were positioned within about 5 cm from the coil center, at which location the flip angle should be close to the nominal value; 2) the fragments were evenly distributed ± 20 cm along the coil axis, which represents the entire sensitive region of the coil (Figure 4a), so as to cover the range of flip angles from the nominal value to near zero. In the second experiment, the performance of the proposed method for bone signal isolation was evaluated. To this end, a lamb hind leg, including femur, tibia and surrounding muscles, was scanned in two different

ways. T_1 and T_2^* measurements were first done with soft tissue intact, and the scan was centered at the tibial mid-shaft. This measurement was repeated for four times with repositioning each time to test repeatability. Subsequently, the surrounding soft tissues (mostly muscle) were removed and the femur and tibia were harvested. The relaxation times of the two specimens were measured separately, with the specimen aligned along the long axis of the coil. Each bone was scanned three times with repositioning. These measurements were carried out within 24 hours of soft tissue removal, and caution was taken to maintain the hydration level of the bone during storage as well as the experiment. All specimens were collected from freshly slaughtered animals from the local market.

In Vivo ^{31}P Imaging and Quantification

Ten healthy subjects of both genders (2 males, 8 females, age range: 26 to 76 years, mean (SD)=50.4 (16.6) y/o, BMI<30) were recruited under an Institutional Review Board approved protocol. The left mid-tibia (centered at 38% of tibia length from medial malleolus), where the cortical bone is the thickest (3), of each subject was imaged with the same MRI system and coil used for the *ex vivo* experiment. After localization, ^{31}P transmit power was calibrated manually by incrementing the flip angle and maximizing the equilibrium calf muscle phosphocreatine signal (4). Next, the SR-prepared spectroscopic sequence described above was applied to measure T_1 of bone ^{31}P . 3D ^{31}P images were subsequently acquired with a custom-designed ZTE-PETRA pulse sequence (4). The ^{31}P ZTE-PETRA sequence was optimized for the experimentally observed relaxation parameters. Initial test scans in the tibia indicated an approximate bone ^{31}P T_1 of around 35 s, therefore, FA was set to the Ernst angle of 5.3° (16- μs hard pulse) for a 150 ms TR. Sampling frequency bandwidth was 125 kHz filling 3D spherical k-space with 8000 spokes and 895 SPI points (PETRA radius=6, hence the effective TE was dwell time $\times$ PETRA radius=48 μs), resulting in a total scan time of 22.5 min. An FOV of (250 mm) 3 was prescribed, and 50 points along each spoke were acquired for reconstruction, leading to a nominal isotropic spatial resolution of 2.5 mm. Additionally, a ^1H image at the same location was also acquired with the manufacturer's 3D GRE sequence, from which the tibial cortex was segmented using ITK-SNAP (20) to derive the bone volume, which was used for ^{31}P density calculation. Aside from the localizer and the 3D GRE sequence, all custom sequences were implemented in SequenceTree (21).

^{31}P ZTE-PETRA image data were acquired in the presence of a synthetic hydroxyapatite reference sample (Sigma-Aldrich, St. Louis, United States) with a known ^{31}P density of 9.5 mol/L (T_1 =42.2 s, T_2^* =145 μs) placed adjacent to the tibia. Following image reconstruction, signal intensity of cortical bone was compared to that of that of the reference sample to determine the amount of ^{31}P in bone (Eqn. 2):

$$\rho_{\text{bone}} = \rho_{\text{ref}} \cdot \frac{I_{\text{bone}} F_{\text{ref}}}{I_{\text{ref}} F_{\text{bone}}} \cdot e^{-TE \cdot \left(\frac{1}{T_{2,\text{ref}}^*} - \frac{1}{T_{2,\text{bone}}^*} \right)} \quad [2]$$

where I_{ref} and I_{bone} are image intensities measured within the reference sample and cortical bone, while F_{ref} and F_{bone} are calculated steady-state signals using their respective relaxation properties, given by Eqn. 3:

$$F = f_{xy} \cdot \frac{1 - e^{-\frac{TR}{T_1}}}{1 - f_z \cdot e^{-\frac{TR}{T_1}}} \quad [3]$$

f_{xy} , f_z are the mapping functions that describe the response of the magnetization to a constant-amplitude RF pulse, applicable when the pulse width τ is comparable to or less than T_2^* (22):

$$f_{xy} = \gamma B_1 \tau e^{-\frac{\tau}{2T_2}} \text{sinc}\left(\sqrt{(\gamma B_1 \tau)^2 - \left(\frac{\tau}{2T_2}\right)^2}\right) \quad [4]$$

$$f_z = e^{-\frac{\tau}{2T_2}} \left[\cos\left(\sqrt{(\gamma B_1 \tau)^2 - \left(\frac{\tau}{2T_2}\right)^2}\right) + \frac{\tau}{2T_2} \text{sinc}\left(\sqrt{(\gamma B_1 \tau)^2 - \left(\frac{\tau}{2T_2}\right)^2}\right) \right] \quad [5]$$

Bone ^{31}P meets these requirements (3, 4) and under some conditions also bone water constituents (23).

In order to investigate repeatability, the above protocol was performed three times in three of the ten subjects with repositioning between scans. Coefficients of variation (CV) were calculated for cortical bone volume estimated from ITK-SNAP segmentation, relaxation times, and density of bone ^{31}P for each of the three test-retest subjects.

Results

Simulation

The B_1^+ field of the coil along its axis measured by the Bloch-Siegert phase shift method (19) and the fitted Gaussian function are shown in Figure 4a. Figure 4b depicts the simulated saturation profile for various ^{31}P -containing species in the presence of such B_1 inhomogeneity following the 12-pulse SAT module of the SR sequence. Due to the gradual decrease in the strength of the transmit field toward the edges of the coil, the saturation profile is characterized by a relatively large transition band (shaded area). As a result of its much shorter T_2 , which causes the flip angle to deviate from the nominal value (24), the bone has a narrower full saturation region than that of the metabolites. Figure 4c shows the simulated profile of the received bone ^{31}P signal strength as a function of spatial location for different TSRs. It is worth noting that, although saturation is incomplete toward the edges of

the coil as a result of rapid B_1 field drop-off, the contribution from the unsaturated spins is small because of the low coil sensitivity in those regions (Figure 4c).

Figure 5a shows the simulation results of the effects of metabolite signal fraction on the bone phosphorus relaxation times computed using the proposed spectral separation method, taking into account the B_1 inhomogeneity along the coil axis. T_1 is increasingly underestimated with increasing fraction of metabolite ^{31}P in the total signal, while T_2^* is overestimated. If 15% of the total signal were to arise from metabolites, the error in T_1 and T_2^* would be approximately -10% and $+24\%$ respectively. According to Figure 5b, which plots the error in quantified $[^{31}\text{P}]$ as a function of T_1 and T_2^* error, the above relaxation time errors result in $[^{31}\text{P}]$ being underestimated by approximately 9.7% . Note that in the absence of metabolites, T_1 and T_2^* errors are less than 1% and 3% respectively even in the presence of large B_1 field variations along the length of the coil.

Ex Vivo Validation

In the *ex vivo* experiment that investigated the effect of B_1 inhomogeneity on relaxation measurement, the T_1 and T_2^* were 26.7 s and $167.7\text{ }\mu\text{s}$ when all the lamb tibiae fragments were placed near the coil center. When the fragments evenly spanned the entire coil sensitivity region along its long axis, T_1 and T_2^* were found to be 25.1 s and $173.6\text{ }\mu\text{s}$ respectively. In the second *ex vivo* experiment that evaluated the impact of presence of soft tissue, the T_1 and T_2^* of bone were $27.3\pm 0.6\text{ s}$ and $229.6\pm 1.5\text{ }\mu\text{s}$ in the presence of surrounding soft tissue. After soft tissue removal, T_1 and T_2^* of tibia were measured to be $28.2\pm 0.8\text{ s}$ and $170.9\pm 0.8\text{ }\mu\text{s}$, while those of femur were $26.7\pm 0.3\text{ s}$ and $168.7\pm 1.0\text{ }\mu\text{s}$.

In Vivo ^{31}P Measurement and ^{31}P Quantification

Figure 6 illustrates the T_1 estimation using the proposed spectral separation method from an SR spectroscopic data set of one subject. Figure 7 displays structural GRE images acquired from three subjects as well as the segmented tibia using ITK-SNAP for computing cortical bone volumes. Phosphorus images used for $[^{31}\text{P}]$ quantification are also shown in the figure. Bone ^{31}P relaxation times and $[^{31}\text{P}]$ measured for all ten subjects are given in Table 1. R^2 for Lorentzian fitting of the isolated bone spectrum (following subtraction of the metabolite spectrum) and subsequent monoexponential T_1 fitting was greater than 0.97 and 0.99 , respectively. To facilitate comparisons with x-ray based imaging modalities, the table also includes bone mineral density (BMD) computed from our MR-measured $[^{31}\text{P}]$ assuming $\text{Ca}_5(\text{PO}_4)_3(\text{OH})$ as the mineral stoichiometry. While BMD was not measured independently by quantitative computed tomography, the ^{31}P density derived values are within the expected range of densitometric measurements.

Given the similarity in relaxation times across the ten subjects, ^{31}P concentrations were also computed using population-averaged T_1 and T_2^* values, resulting in an average difference of about 2.4% compared to values computed using individually measured relaxation times. Since the muscle-to-bone ratio is expected to be largely invariant (see, for instance (25)), a retrospective correction of the derived ^{31}P concentration which accounts for the metabolite fraction can also be applied. It is preferable, however, to apply the correction to the relaxation constants since the apparent ^{31}P concentrations are pulse sequence dependent

(Eqs. 2 and 3). Doing so on the basis of 92.5% average bone ^{31}P fraction for the study group yielded T_1 and T_2^* errors of about -4.3% and $+10.5\%$, respectively, resulting in a ^{31}P density error of approximately 4.6% . Relaxation times corrected for this error as well as ^{31}P concentrations calculated using an average of the corrected T_1 and T_2^* values are given in Table 1 as well. None of the parameters, $[^{31}\text{P}]$ ($p=0.55$), T_1 ($p=0.18$) or T_2^* ($p=0.99$), were correlated with subject age. CVs for cortical bone volume, T_1 , T_2^* and $[^{31}\text{P}]$ were 2.2% , 1.5% , 2.6% and 4.4% , respectively.

Discussion

The present work sheds some light on the question of whether or not bone mineral ^{31}P T_1 and T_2^* need to be measured individually to ensure quantitative accuracy in the measurement of phosphorus mineral density by solid-state MRI techniques or whether the use of global averages is adequate for quantification. The data obtained in the present work in the tibial cortex of ten healthy test subjects ranging in age from 26 to 76 years suggest only minimal inter-subject variations without a dependence on subjects' age or gender (Table 1).

The measurement of the relaxation times, in particular of T_1 was found not to be entirely straightforward. An image-based measurement, consisting of an appropriate saturation-recovery preparation module preceding the ZTE excitation and readout would be desirable as it would provide the spatial dependence of the relaxation times. However, such an approach is entirely impractical due to excessive scan times. We therefore elected a spectroscopic approach forgoing spatial selectivity, an approach previously chosen by Seifert et al for T_1 quantification in human specimens (3). The loss of spatial selectivity, one could argue, is acceptable, given the systemic nature of most degenerative bone disorders. However, the presence of major muscle groups in the tibia poses unique challenges to the measurement of bone ^{31}P T_1 *in vivo*. The spectrum is a superposition of the spectra from bone and muscle phosphorus metabolites and even though the latter are far lower in concentration than mineral phosphate, this disparity is partially offset by the muscles' much larger volume. *In vivo* concentrations of three main ^{31}P -containing metabolites, PCr, Pi and ATP, are approximately 33, 4.5, 8.2 mM, respectively, in human calf muscle (17). Average cortical bone ^{31}P concentration reported by Seifert et al (3) was $6.74 \pm 1.22 \text{ mol/L}$ in the tibial cortex of 16 donors covering the entire adult age range. These data, along with muscle and bone volumes estimated by standard gradient-echo imaging in all ten subjects suggest tibial bone phosphorus to account for $>90\%$ of total amount of ^{31}P present *in vivo* at the tibial location. However, the much greater visibility of the metabolite signals given their ^{31}P signal line widths being at least two orders of magnitude lower than those of the phosphate resonances from bone (18, 26), means that their contributions to the total signal cannot be ignored. Here, the authors made use of the large differential in T_2^* between the two ^{31}P species and thus the lifetime of their FIDs for their separation. Another issue examined is the effect of RF inhomogeneity in the non-spatially selective measurement as the B_1 field falls off in axial direction from the center toward the coil boundaries, which raises the question as to the effectiveness of saturation of spins in more distant locations. The data, obtained by both simulation and experimentally, show, however, that the 12-pulse saturation train is

effective across a large range of excitation flip angles as they occur along the coil axis yielding accurate T_1 and T_2^* values in the absence of metabolites.

With bone comprising >90% of the total signal, T_1 and T_2^* errors are limited to about 6% and 15%, respectively (Figure 5a). The underestimation of T_1 and overestimation of T_2^* both lead to lower computed phosphorus density. Since ZTE-PETRA is essentially a steady-state gradient-echo sequence (Eqn. 3), and therefore inherently T_1 -weighted, lower computed T_1 translates into a higher calculated steady-state signal level (F_{bone} in Eqn. 2), and therefore lower estimated ^{31}P density, which scales as the ratio of observed image intensity to its predicted steady-state value. Overestimated T_2^* , according to Eqn. 2 (due to the exponential term), will also translate into lower computed ^{31}P density.

Several observations are worth noting from the specimen experiments. First, the very similar values of T_1 and T_2^* obtained from the two arrangements of tibia specimens confirm the simulation results indicating that relaxation measurements are relatively insensitive to B_1 inhomogeneity. The likely reason for this finding is that, although inhomogeneous transmit fields cause imperfect saturation, the unsaturated spins have relatively small contribution to the total detected signal due to lower coil sensitivity in those regions. This holds true as long as the same coil is used for both transmission and reception, due to the principle of reciprocity, as it applies to the birdcage coil used. The close agreement between the bone T_1 values measured in the presence of soft tissue (27.3 ± 0.6 s) and those measured with soft tissue removed (tibia $T_1 = 28.2 \pm 0.8$ s, femur $T_1 = 26.7 \pm 0.3$ s) confirms the effectiveness of the proposed method at isolating bone spectrum from the composite signal. Furthermore, the T_1 values of lamb cortical bone measured *ex vivo* in the current study are consistent with those reported previously by Seifert et al ($T_1 = 26.0 \pm 1.4$ s, $T_2^* = 189 \pm 2.2$ μs), which were also obtained from the same animal species, anatomic location and field strength (12). On the other hand, the obviously elevated T_2^* of bone in the presence of soft tissue is consistent with the result of the simulation experiment, and may lead to underestimation in ^{31}P .

As noted above, the variation of T_1 across subjects (age range = 26–76 years) observed in this study is unexpectedly small (38.4 ± 1.5 s), a result suggestive of little variation in chemical make-up of the mineral over adult life. In fact, as noted above, there was no T_1 versus age correlation. This finding is consistent with the relatively small variation in T_1 for a group of 16 tibia specimens from donors aged 27 to 97 years (96.7 ± 10.8 s measured at 7 T), where no T_1 -age correlation was found either (3). These observations indicate that relaxation measurements for bone ^{31}P quantification may be unnecessary. In addition, group average ^{31}P quantified in the present study (6.40 ± 0.58 mol/L) is close to measurements *ex vivo* for a similar age range (6.74 ± 1.22 mol/L) (3), where the experiment was free from interference of metabolites signal. For comparison, bone ^{31}P was also quantified using relaxation times reported by Wu et al ($T_1 \sim 18$ s, $T_2^* \sim 160$ μs) (15) yielding an average ^{31}P of 4.9 mol/L for the ten subjects, which is around 20% lower as predicted (Figure 5b). Bone ^{31}P relaxation times published by Wu et al were also used in an earlier work that investigated the feasibility of MRI-based bone matrix and mineral evaluation (4), and the average ^{31}P of 5.29 mol/L found in the group of subjects for that study was likely an underestimation based on the relaxation data of the current study.

The absence of an association with age of the ^{31}P mineral density is unexpected but is likely due to limited power with $N = 10$ subjects (in spite of the intentionally chosen relatively large age range). Interestingly, an earlier *ex vivo* study of mid-tibia specimens ($N = 16$) from donors aged 27 – 97 years showed the expected negative correlation of MR-measured bone phosphorus density with age ($R^2 = 0.39$, $p < 0.05$) (3). However, when specimens of donors aged > 76 years are removed from their analysis, significance disappears ($N = 11$, $R^2 = 0.14$, $p = 0.26$). Thus, it is likely that an age dependence of phosphorus density would be observed for a greater number of study subjects or by expanding the age range.

Previously, Wu et al performed both spectroscopic and image-based ^{31}P T_1 measurement of the bones in the wrist *in vivo* at 3 T. In their experiment, the steady-state ^{31}P signal was acquired by progressively increasing TR (0.5, 1, 2, 3 and 5 s). A T_1 range of 17 s to 19 s was reported in two healthy subjects, which is about half the value found in the current work (average of 38.4 s in ten subjects). In contrast, *ex vivo* experiments by Seifert et al, T_1 of ^{31}P in lamb tibia yielded 26 s and 66 s at 3 T and 7 T respectively, while T_1 of ^{31}P in the human tibia was found to be 96.7 s at 7 T (3, 12). Since the T_1 of human bone is longer than that of lamb bone at 7 T, it is reasonable to expect a qualitatively similar relation at 3 T. Therefore, our value of 38.4 s is likely to be more accurate (> 26 s). There are several possible reasons for the disparity with our data: First, their spectroscopic measurement might have been affected by metabolite signal (Figure 5a) although the fraction of soft tissue is much less in the wrist than in the calf. Second, the relatively narrow range of TRs used, in particular the lack of a value close to equilibrium (2–3 times T_1), is well known to underestimate T_1 with the progressive saturation technique (even though an image-based measurement should be relatively immune to metabolite interference).

Conclusions

Quantification of bone ^{31}P concentration is critically dependent on T_1 and T_2^* , which was measured *in vivo* in the tibia at 3 T field strength. The results show the two parameters to vary insignificantly across the adult age range, therefore obviating the need for subject-specific measurements.

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Figure 1.

Diagram of the SR-prepared spectroscopy sequence, consisting of a saturation block of twelve 120- μ s 60° hard pulses, 2 ms apart, and each followed by a spoiler. Spoilers are applied along different orientations for optimal de-phasing (3). After a delay of TSR, signal is excited with another 120- μ s 60° hard pulse and an FID is acquired at 250 kHz bandwidth. For further details, see text.

Figure 2.
In vivo ^{31}P spectrum obtained from the calf of a healthy subject at 3 T. The broad peak corresponds to the ^{31}P in bone mineral, while the superimposed narrow peaks are from muscle metabolites.

Figure 3.

Workflow for isolating the bone ^{31}P spectrum from the composite signal containing both bone ^{31}P and ^{31}P metabolites. Spec_Total (overall signal) and Spec_Metabolite (predominantly metabolite signal) are generated from different portions of the FID. After phase correction, bone spectrum is obtained via subtraction of the two which is subsequently fitted to a Lorentzian.

Figure 4.

(a) Measured B_1^+ profile along z-axis of the coil and the fitted Gaussian. (b) Simulated saturation profile following the SAT module of the SR sequence in the presence of B_1^+ inhomogeneity depicted in (a) for different ^{31}P signal sources. (c) Relative amplitude of the signal as a function of spatial location plotted versus TSR. The pair of small peaks appearing at the shorter TSRs is due to incomplete saturation at the most distal coil locations.

Figure 5.

(a) Error in bone phosphorus relaxation times measured with the proposed method as a function of the fraction of metabolites in the total signal. (b) Error in quantified $[^{31}\text{P}]$ as a function of error in T_1 and T_2^* .

Figure 6.

(a) Total and (b) metabolite spectra generated from an SR dataset of one of the subjects; (c) isolated bone spectra and corresponding Lorentzian fits; (d) T_1 estimation via mono-exponential fitting of the integral of Lorentzian lines in (c).

Figure 7.

Axial 3D GRE images of the calf from (a) 30 y/o female, (b) 44 y/o male and (c) 51 y/o female; (d–f) corresponding segmented cortical bone volumes; (g–i) ^{31}P ZTE image resulting from averaging of 18 slices.

Cortical bone ^{31}P relaxation times and concentrations: MR = measured; CR = systematic error corrected using population-averaged relaxation times (for detail see text); $[\text{}^{31}\text{P}]$ (%) = estimated systematic error; BMD = bone mineral density computed from ^{31}P densities using stoichiometric calcium hydroxyapatite (for detail see text).

Table 1

		MR		CR		$[^3\text{P}]$ (mol/L)		Error	BMD (mg/cc)	
Age/Gender	T ₁ (s)	T ₂ * (μs)	T ₁ (s)	T ₂ * (μs)	MR	CR	$[^3\text{P}]$ (%)		MR	CR
26 M	36.1	185.7	37.7	168.1	5.56	5.81	4.50		931	973
30 F	35.7	202.7	37.3	183.4	6.45	6.94	7.60		1080	1162
33 M	34.7	213.9	36.3	193.6	5.97	6.61	10.72		1000	1107
44 M	38.7	190.1	40.4	172.0	7.57	7.68	1.45		1267	1286
51 F	37.8	191.8	39.5	173.5	6.50	6.69	2.98		1088	1120
58 F	37.6	188.6	39.3	170.7	5.92	6.08	2.70		991	1018
60 F	36.7	185.7	38.3	168.1	5.70	5.91	3.68		954	990
61 F	35.0	206.4	36.6	186.8	5.62	6.13	9.07		941	1026
65 F	36.9	206.5	38.6	186.9	5.61	5.96	6.24		939	998
76 F	38.5	198.4	40.2	179.5	6.02	6.19	2.82		1008	1036
Mean	36.8	197.0	38.4	178.3	6.09	6.40	5.18		1020	1072
SD	1.4	10.0	1.5	9.0	0.62	0.58	3.09		103	98