A Quantitative MRI Protocol for Assessing Matrix and Mineral Densities and Degree of Mineralization of Human Cortical Bone

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A Quantitative MRI Protocol for Assessing Matrix and Mineral Densities and Degree of Mineralization of Human Cortical Bone

Abstract
Two categories of bone disease, osteoporosis and osteomalacia, affect bone in different ways: bone mineral and matrix are lost in roughly equal proportions in osteoporosis, while only mineral is depleted in osteomalacia. The difference between these disorders is in bone mineralization: the mass of mineral per volume of bone matrix, excluding pore spaces.

Standard clinical examinations measure x-ray attenuation to infer mineral density. However, bone mineral density alone cannot fully describe bone health. Advances in solid-state 31P and 1H magnetic resonance imaging (MRI) have enabled quantification of the densities of extremely short-lived bone mineral 31P and matrix-bound water 1H signals as surrogates for bone mineral and matrix densities. The ratio of these two measurements provides the degree of mineralization of bone (DMB).

In this dissertation, the relaxation properties of bone mineral 31P and water 1H were analyzed, the surrogacy of bound water concentration for bone matrix density was established, and measurements of bone mineral 31P and matrix-associated water 1H densities in human bone specimens were designed and implemented on clinical scanners.

Although bone mineral 31P longitudinal relaxation time (T1) increased and effective transverse relaxation time (T2*) decreased with increasing field strength, the predicted signal-to-noise ratio (SNR) increased slightly. Also, the short-T2* fraction of bone water calculated by 1H bi-component fitting was correlated with porosity and matrix density at 1.5 T, but these associations weakened as field strength increased. In contrast, short-transverse relaxation time (T2) fraction was highly correlated with gold-standard measurements, suggesting the superiority of T2-based methods for separation of bound and pore water fractions. Additionally, single adiabatic inversion-recovery zero echo time (SIR-ZTE) 1H density was correlated negatively with porosity and positively with matrix and mineral densities, suggesting that this MRI method provides a surrogate measure of bone matrix density. Finally, both bone mineral 31P and matrix-associated 1H densities in human cortical bone specimens were correlated negatively with porosity and age, and positively with peripheral quantitative computed tomography (pQCT) density. As expected, DMB was uncorrelated with porosity, age, or pQCT density.

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

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A QUANTITATIVE MRI PROTOCOL FOR ASSESSING MATRIX AND MINERAL DENSITIES
AND DEGREE OF MINERALIZATION OF HUMAN CORTICAL BONE

Alan C. Seifert

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2015

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A QUANTITATIVE MRI PROTOCOL FOR ASSESSING MATRIX AND MINERAL DENSITIES
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Alan Charles Seifert
“To my family, especially Dad, for teaching me how to build an RF circuit when I was still in elementary school, and to Steph, for not laughing too hard when I try to do statistics.”
I would like to thank everyone who helped me to reach this point. First and foremost, I thank my advisor, Prof. Felix Wehrli, for his mentorship. He sets a very high bar for each of his students, and we benefit from his patience and dedication in helping us to clear it. I also thank Prof. Alex Wright for bringing me into this lab on a summer rotation and getting me started on this project; Dr. Suzanne Wehrli for her advice and hands-on help in planning and carrying out so many experiments; Dr. Tom Connick for his substantial help with RF hardware; Dr. Henry Ong, Dr. Yusuf Bhagat, Prof. Michael Langham, and Prof. Chamith Rajapakse for their guidance and advice in various parts of this project; Holly Flachs for all the effort she puts into making our lab run smoothly; and my lab mates, Dr. Alvin Tsai, Dr. Varsha Jain, Dr. Cheng Li, Zach Rodgers, Erin Englund, Francisco Contijoch, and Xia Zhao. I especially thank Cheng Li: this project would not have been possible without your patience, dedication, and enormous knowledge.

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ABSTRACT

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Alan C. Seifert
Felix W. Wehrli, Ph.D.

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# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acknowledgements</td>
<td>iv</td>
</tr>
<tr>
<td>Abstract</td>
<td>v</td>
</tr>
<tr>
<td>Table of Contents</td>
<td>vii</td>
</tr>
<tr>
<td>List of Tables</td>
<td>xi</td>
</tr>
<tr>
<td>List of Figures</td>
<td>xiii</td>
</tr>
<tr>
<td>Preface</td>
<td>xx</td>
</tr>
<tr>
<td><strong>Chapter 1: Introduction</strong></td>
<td>1</td>
</tr>
<tr>
<td>1.1. Bone Anatomy</td>
<td>1</td>
</tr>
<tr>
<td>1.2. Bone Disease: Osteoporosis and Osteomalacia</td>
<td>5</td>
</tr>
<tr>
<td>1.3. Measurement of Bone Health</td>
<td>7</td>
</tr>
<tr>
<td>1.4. MRI of Bone</td>
<td>12</td>
</tr>
<tr>
<td>1.5. Outline of Thesis Chapters</td>
<td>18</td>
</tr>
<tr>
<td><strong>Chapter 2: $^{31}$P NMR Relaxation of Cortical Bone Mineral at Multiple Magnetic Field Strengths and Levels of Demineralization</strong></td>
<td>20</td>
</tr>
<tr>
<td>2.1. Abstract</td>
<td>20</td>
</tr>
<tr>
<td>2.2. Introduction</td>
<td>21</td>
</tr>
<tr>
<td>2.3. Methods</td>
<td>23</td>
</tr>
<tr>
<td>2.3.1. Specimens</td>
<td>23</td>
</tr>
<tr>
<td>2.3.2. MRI Scanners and RF Coils</td>
<td>24</td>
</tr>
<tr>
<td>2.3.3. $T_1$ Measurements</td>
<td>25</td>
</tr>
<tr>
<td>2.3.4. $T_2^*$ Measurements</td>
<td>27</td>
</tr>
<tr>
<td>2.3.5. Multiple Fields and SNR Estimation</td>
<td>28</td>
</tr>
<tr>
<td>2.3.6. Deuterium Exchange</td>
<td>30</td>
</tr>
<tr>
<td>2.3.7. Partial Demineralization</td>
<td>30</td>
</tr>
<tr>
<td>2.3.8. Spectroscopy Processing Details</td>
<td>32</td>
</tr>
<tr>
<td>2.3.9. Imaging</td>
<td>32</td>
</tr>
</tbody>
</table>
CHAPTER 3: BI-COMPONENT $T_2^*$ ANALYSIS OF BOUND AND PORE BONE WATER FRACTIONS FAILS AT HIGH FIELD STRENGTHS .......... 45

3.1. Abstract ......................................................................................... 45

3.2. Introduction .................................................................................. 46

3.3. Materials and Methods .................................................................. 48
  3.3.1. Specimen Source and Preparation ........................................... 48
  3.3.2. $^1$H NMR Spectroscopy .......................................................... 49
  3.3.3. $^2$H NMR Spectroscopy ............................................................ 52
  3.3.4. $\mu$CT Imaging ........................................................................ 53
  3.3.5. Gravimetry ................................................................................ 54

3.4. Results .......................................................................................... 54
  3.4.1. $^1$H NMR Spectroscopy ............................................................ 54
  3.4.2. $^2$H NMR Spectroscopy ............................................................ 59
  3.4.3. $\mu$CT and Gravimetry ............................................................... 60

3.5. Discussion ..................................................................................... 63

3.6. Conclusion ..................................................................................... 70

CHAPTER 4: SINGLE ADIABATIC INVERSION RECOVERY ZERO ECHO TIME MRI IS A SURROGATE MEASURE OF BONE MATRIX DENSITY ...... 71

4.1. Abstract ......................................................................................... 71

4.2. Introduction .................................................................................. 72

4.3. Materials and Methods .................................................................. 74
  4.3.1. Specimens and Scanners .......................................................... 74
  4.3.2. NMR Spectroscopy ................................................................. 75
CHAPTER 5: BONE MINERAL $^{31}$P AND MATRIX-BOUND WATER DENSITIES MEASURED BY SOLID-STATE $^1$H AND $^{31}$P MRI .................................................. 102

5.1. Abstract.................................................................................................................. 102

5.2. Introduction ............................................................................................................. 103

5.3. Materials and Methods ......................................................................................... 105
  5.3.1. Source of Bone Tissue ...................................................................................... 105
  5.3.2. Hardware .......................................................................................................... 107
  5.3.3. MR Imaging ....................................................................................................... 107
  5.3.4. $B_1$ Mapping and Registration .................................................................... 111
  5.3.5. Density Quantification ...................................................................................... 112
  5.3.6. X-Ray-Based Porosity and Densitometry ..................................................... 116
  5.3.7. Data Analysis ..................................................................................................... 117

5.4. Results .................................................................................................................... 117

5.5. Discussion .............................................................................................................. 122

5.6. Conclusions ............................................................................................................ 127
# CHAPTER 6: CONCLUSIONS AND FUTURE WORK

6.1. Conclusions ........................................................................................................................................... 128

6.2. Future Work ........................................................................................................................................ 130
   6.2.1. Technical Development .................................................................................................................. 130
   6.2.2. Translation to the Clinic .................................................................................................................. 131

# BIBLIOGRAPHY ...................................................................................................................................... 133
LIST OF TABLES

Table 1.1: Changes in mineral density, matrix density, and mineralization in osteoporosis and osteomalacia. .......................................................................................................................... 7

Table 2.1: Bone mineral phosphorus relaxation times in solid bone samples before and after D₂O exchange. .................................................................................................................. 37

Table 2.2: Bone mineral phosphorus relaxation times at 9.4 T in bone powder samples before and after three stages of demineralization. .................................................. 37

Table 3.1: Bone properties measured by µCT, ³¹H IR, gravimetry, SR-CPMG NMR at 9.4 T, SR-FID NMR at four field strengths, and SIR-FID NMR at three field strengths. Bone labels are composed of the two-digit age and one-letter gender of the donor. .................................................................................................. 55

Table 3.2: Inter-parameter correlations (R²) of measured bone properties. All correlations are statistically significant (p < 0.05) unless italicized.............. 61

Table 4.1: ZTE, SIR-ZTE, and non-exchangeable (i.e., not removed by ²H₂O exchange) 
³¹H concentration measurements by MRI, and reference measurements by µCT, gravimetry, and ³¹H and ²H NMR. Sample labels indicate age and gender. ............. 85

Table 4.2: Inter-parameter correlations (R²) of measured bone properties. All correlations are statistically significant unless italicized (*p<0.05, †p<0.005, ‡p<0.0005). .................................................................................................................. 91

Table 5.1: Measured bone parameters with means and standard deviations. ³¹P density in mg of hydroxyapatite per cc and bound water volume fraction are inferred from MRI-derived densities based on certain assumptions described in the discussion. Abbreviations: BVF, bone volume fraction; HAp, hydroxyapatite; BMR, bone mineral ratio. .............................................................................................................. 120

Table 5.2: Correlation matrix of R² values. All correlations are statistically significant unless italicized (*p<0.05, †p<0.005, ‡p<0.0005). Abbreviations: BMR, bone
mineralization ratio; BWD, bound water density; \(^{31}\)PD, \(^{31}\)P density; BVF, bone volume fraction = 1 - porosity.
LIST OF FIGURES

Figure 1.1: A diagram depicting the osteonal organization and pore structure of cortical bone. Figure adapted from (6). ................................................................. 2

Figure 1.2: Structural organization of bone over multiple size scales. Figure from (7).... 3

Figure 1.3: Collagen triple helix (a, in yellow, orange, and red) with multiple hydration layers of adsorbed water molecules (b, c, d, in blue). Internal single- and double-water bridges between collagen molecules are not visible. Figure is taken from (8). ........................................................................................................................................ 4

Figure 1.4: DXA images for measurement of bone mineral density at the proximal femur (a) and lumbar spine (b) in a 53-year-old male (39). .......................................................... 8

Figure 1.5: Quantitative computed tomography image of a lumbar vertebra (40). Calibration standards are visible in the table pad below the patient. ......................... 9

Figure 1.6: HR-pQCT image of the wrist at 82µm in-plane resolution, showing the distal radius and ulna (40). ........................................................................................................ 10

Figure 1.7: Comparison of a synchrotron-radiation µCT image with 7.5-µm isotropic resolution (top) to an HR-pQCT image of the same site with 82-µm isotropic resolution (43,44). ........................................................................................................ 11

Figure 1.8: UTE (a) and ZTE (b) imaging pulse sequences and corresponding k-space acquisition schemes. In ZTE, central k-space points are lost during transmit/receive dead time, and this region must be re-filled. The pointwise encoding time reduction with radial acquisition (PETRA) method is shown in panel (b) using dotted lines in G and ADC to indicate single-point acquisitions at lower gradient strengths (57). ..... 14

Figure 1.9: Cartoon $T_2$ relaxation spectrum diagramming the three major $^1$H NMR signal pools in bone. Pore water has $T_2 > 1$ ms, while bound water has $T_2 \sim 300$-500 µs. Collagen signal, at $T_2 \sim 40$-60 µs, is below the detection limit at clinical field strengths, but becomes visible using micro-imaging and spectroscopic hardware. As porosity increases, collagen and bound water decrease, while pore water
increases and shifts to longer $T_2$ values due the smaller surface-to-volume ratio of enlarged pores. This figure is adapted from Li et al. (74).

Figure 2.1: Example standardized RF coil used at field strengths from 1.5 T to 7 T. Coils are transmit/receive parallel dual-conductor solenoids.

Figure 2.2: Saturation-recovery pulse sequence used for bulk $T_1$ relaxation time measurements.

Figure 2.3: Sample $^{31}$P relaxation analysis data in specimen of lamb cortical bone at 7 T: a) lineshape (solid line) with Lorentzian fit (dashed line); b) saturation recovery data (points) with exponential fit (dashed line).

Figure 2.4: Imaging pulse sequences used for SNR comparisons to predicted trends: a) ramp-sampled ultra-short echo time (UTE), and b) zero echo time (ZTE) PETRA, with single-point sampling of k-space center.

Figure 2.5: $^{31}$P (a) $T_1$ (squares) and $T_2^*$ (circles) relaxation times and (b) $R_1$ (squares) and $R_2^*$ (circles) relaxation rates of bone mineral phosphorus in fully hydrated solid bone samples at six field strengths. Lines connecting points are a visual guide only, and do not represent data or predictions. Error bars are included, but do not extend beyond markers.

Figure 2.6: Predicted (solid markers) relative bone mineral $^{31}$P SNR based on measured relaxation times at multiple field strengths at $t_{dead} = 20$ and 40 µs, normalized to the value at 1.5 T for each condition, and power fits (solid lines) at $t_{dead} = 40$ µs. Relative SNR trends calculated from actual UTE and ZTE imaging acquisitions (open markers), also normalized to the value at 1.5 T for each condition.

Figure 2.7: UTE and ZTE images (1.21 mm isotropic voxel resolution) of a longitudinal section through the center of a cylindrical solid cortical bone specimen (7 mm diameter, 30 mm length) at 1.5 T, 3 T, and 7 T, with SNR values. Specimen dimensions are indicated with a dashed line.
Figure 3.1: Cartoon $^1$H NMR $T_2^*$ relaxation spectrum of bone at multiple field strengths. Note that as field strength increases, the $T_2^*$ of pore water becomes shorter and merges with the short-$T_2^*$ bound water pool. ................................................................. 48

Figure 3.2: $^1$H SR-FID (a), SR-CPMG (b), SIR-FID (c), and $^2$H IR-FID (d) NMR pulse sequences. In (a) and (b), the saturation-recovery time, $T_{SR}$, is arrayed logarithmically from 3 ms to 6 s in 12 steps. In (b), the number of refocusing pulses, $N$, is arrayed logarithmically from 0 to 5000 in 20 steps............................................. 50

Figure 3.3: Bar graphs showing trends in average (a) short-$T_2^*$ relaxation times and (b) long-$T_2^*$ relaxation times by 1D $T_2^*$ bi-component fitting of FIDs, and (c) short-$T_2^*$ fractions by 1D $T_2^*$ and 2D $T_1-T_2^*$ bi-component fitting at four field strengths. Error bars indicate standard deviation......................................................... 56

Figure 3.4: 2D $T_2^*$-$T_2$ and $T_1-T_2$ $^1$H relaxation spectra at 9.4 T, and 2D $T_1-T_2^*$ relaxation spectrum at 3 T, generated using the MERA software package (124). Spectra are from a bone specimen taken from a 37 year old male donor. The $T_2^*$-$T_2$ spectrum is generated from CPMG data, the $T_1-T_2$ spectrum from SR-CPMG data, and the $T_1-T_2^*$ spectrum from SR-FID data................................................................. 58

Figure 3.5: $^2$H spectra showing the bound and pore D$_2$O components (inset is magnified vertically and truncated). Pore water (narrow central peak in green) is calculated by subtracting the bound water spectrum obtained by inversion-recovery nulling of pore water (the quadrupolar coupled split peaks shown in red) from the fully relaxed spectrum (shown in blue). This spectrum is taken from a specimen from a 27 year old female donor with the osteonal axis orthogonal to $B_0$. A splitting of 4.8 kHz is observed, consistent with the orientation-dependent quadrupolar splitting observed by Ong et al. (71)....................................................................................... 59

Figure 3.6: Scatter plots displaying the correlations of the 1D bi-component short-$T_2^*$ $^1$H signal fraction to organic matrix density measured by gravimetry. Correlations become significantly worse as field strength increases................................................. 62
Figure 3.7: Scatter plots displaying the correlations of 1D bi-component (a) short-$T_2^*$ $^1$H signal fraction by fitting of FID data and (b) short-$T_2$ $^1$H signal fraction by fitting of CPMG data at 9.4 T. Short-$T_2$ fraction is very strongly associated with organic matrix density, while short-$T_2^*$ has no association with matrix density................. 63

Figure 3.8: Log-magnitude FID at 7 T of a cortical bone specimen from a 53 y/o female donor (a). Note the irregular oscillation of the signal, which causes failure of bi-exponential fitting (red line, $R^2 = 0.999592$): 97.4% short-$T_2^*$ signal fraction, versus 86.6% by 2D $T_1$-$T_2$ bi-exponential fitting at 9.4 T. Fat at 7 T is 1040 Hz off-resonance. A FID from a 53 y/o male donor (b) not exhibiting these oscillations is also shown for comparison ($R^2 = 0.999901$). Similar plots of fitted CPMG echo amplitudes for the same 53 y/o female (c) and male (d) donors are also shown.... 65

Figure 4.1: Schematic $T_2$ relaxation spectrum diagramming the three major $^1$H NMR signal pools in bone. Pore water has $T_2 > 1$ ms and is broadly distributed, while bound water has $T_2 \sim 300-500 \mu$s. Collagen signal, at $T_2 \sim 40-60 \mu$s, is below the detection limit at clinical field strengths, but becomes visible using micro-imaging and spectroscopic hardware. As porosity increases, as shown in the inset µCT images of bone specimens from 27 y/o and 83 y/o female donors (dense and porous bone, respectively) collagen and bound water content decrease while pore water content increases and shifts to longer $T_2$ values due the smaller surface-to-volume ratio of enlarged pores................................................................. 73

Figure 4.2: SR-CPMG pulse sequence. Saturation-recovery times ($T_{SR}$) were arrayed logarithmically in 12 steps from 3 ms to 6 s, the number of refocusing pulses, $N$, was arrayed logarithmically from 0 to 5000 in 20 steps, and one signal acquisition was performed................................................................. 76

Figure 4.3: NMR data (points) from a bone specimen from a 53-year-old male with bi-component fits (curves). Panel (a) shows a $T_1$ fit of saturation-recovery data, (b) shows a $T_2$ fit of CPMG echo amplitudes, and (c) shows a $T_2^*$ fit of a FID. Although only one-dimensional data are shown, fits were performed using the two-dimensional methods given in the methods section (a,b: $T_1$-$T_2$; c: $T_2$-$T_2^*$)................ 78
Figure 4.4: ZTE (a) and SIR-ZTE (b) imaging pulse sequences. ZTE parameters: 51896 projections, TR = 2 ms, 1 min 43 sec scan time. SIR-ZTE parameters: 6588 projections, TR = 200 ms, 21 min 58 sec scan time. FOV = 64 mm isotropic, resolution = 500 µm isotropic, and 1 signal acquisition for both................................. 79

Figure 4.5: Volume rendering of the pore spaces (in white) within four representative bone specimens. Note the increased number and size of pores in bone specimens from elderly female donors................................................................. 86

Figure 4.6: Maps of ZTE, SIR-ZTE, and non-exchangeable ZTE $^1$H concentrations, in mol/L, in bone specimens from four representative donors. Age and gender of the donors are given within each quadrant, and the endosteal surface of each specimen faces left. Arrows indicate a region of high porosity, which has elevated total water and reduced matrix densities, and correspondingly increased ZTE and decreased SIR-ZTE $^1$H concentrations. .................................................................................................. 88

Figure 4.7: Scatter plots displaying the correlations of MRI-derived ZTE (a,c,e) and SIR-ZTE (b,d,f) $^1$H concentrations versus µCT porosity (a,b), gravimetric water density (c,d), and gravimetric organic matrix density (e,f). ZTE $^1$H concentration is positively correlated with porosity and gravimetric water density and negatively with matrix density, while SIR-ZTE correlations show the opposite behavior. Clustering of data is due to severe bone loss being present in a small subset of bones from post-menopausal female donors)............................................................................................................ 90

Figure 4.8: a) The modulation transfer function (MTF) describes the $T_2^*$ decay of the MRI signal over the course of signal acquisition. b) The Fourier transform of the MTF is the point spread function (PSF), which describes how a single infinitesimally small point source of signal is blurred due to attenuation of higher spatial frequencies. Shorter $T_2^*$ results in a more rapid decay of the MTF and a broader PSF. c) A rectangular profile with several gaps, representing a 1D cross-section through a porous bone, is convolved with the PSFs of ZTE, SIR-ZTE, and non-exchangeable ZTE. These gaps are more severely blurred in cases of shorter $T_2$. ....................... 95
Figure 5.1: 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. .............................. 103

Figure 5.2: 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 $^1$H or two $^{31}$P signal intensity reference phantoms are also mounted inside the solenoid, but are not visible in this photograph. ......................... 106

Figure 5.3: Imaging pulse sequences used for bone $^{31}$P and bound water density quantification: $^{31}$P ZTE sequence (a) with PETRA module (b), and $^1$H Single Adiabatic Inversion Recovery Rapid ZTE (SIR-rZTE) sequence (c) with PETRA module (d). Relevant pulse sequence parameters are shown. .......................... 109

Figure 5.4: 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 ($M_z < 0$). ................................................... 115

Figure 5.5: 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. .................................................. 118

Figure 5.6: 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 $^1$H 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. ............................................. 118
Figure 5.7: Correlation plot of bone mineral $^{31}$P density to bound water $^1$H density. The two MRI-derived densities are highly correlated, as expected in a set of equally mineralized bones.
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CHAPTER 1: INTRODUCTION

1.1. Bone Anatomy

Bone is a composite material composed of apatite-like mineral, collagen, water, and several types of cells arranged over multiple size scales. Each constituent contributes uniquely to bone’s mechanical properties; mineral provides rigidity (1), collagen matrix contributes elasticity (2), water stabilizes the collagen matrix, imparting viscoelasticity and increasing toughness (3,4), and cells maintain bone health.

The two main structural types of bone are trabecular and cortical. Trabecular bone is a mesh-like network of interconnected rods and plates, while cortical bone is more compact. On the largest scale, long bones in the appendicular skeleton consist of a shaft, the diaphysis, which widens into the metaphysis, containing the growth plate, and epiphysis at each end. The diaphysis consists of thick cortical bone surrounding the marrow cavity. The epiphysis contains predominantly trabecular bone interspersed with marrow, covered by a thinner shell of cortical bone.

The structure of bone is directly related to its mechanical function. Wolff’s law, a theory developed by the 19th century anatomist Julius Wolff, states that bone adapts its structure to the mechanical demands placed on it. If the stress placed on a certain site is increased, the trabecular bone’s anisotropic structure will re-orient to better bear the load placed on it, the cortical bone at that site will thicken, and overall bone density will increase. The inverse is also true: if less stress is placed on a bone, that bone will lose density to reduce its metabolic cost (for example, during extended immobilization due to injury or exposure to microgravity).
Though cortical bone changes more slowly in response to loading, its importance must not be underestimated. Even at sites of predominantly trabecular bone, such as the femoral neck, approximately half of the bone’s failure load is explained by the cross-sectional area and mineral content of the thin cortical bone layer (5).

Cortical bone tissue is further organized into cylindrical osteons surrounding a central Haversian canal containing blood vessels. Along with the longitudinal Haversian canals and transverse Volkmann’s canals, cortical bone’s pore system also consists of canaliculi, which allow diffusion of nutrients to and waste away from cells, and lacunae, which contain osteocytes, bone cells which reside within the bone tissue. A diagram of the pore system in cortical bone is shown in Figure 1.1.

![Diagram of the pore system in cortical bone](image)

**Figure 1.1:** A diagram depicting the osteonal organization and pore structure of cortical bone. Figure adapted from (6).

Osteons are in turn composed of wrapped sheet-like layers of bone, or lamellae, 3-7 µm thick. Type-1 collagen fibers within these lamellae are parallel within a layer, but are oriented obliquely to adjacent layers. Each collagen fiber contains many collagen fibrils,
each of which is composed of many individual collagen molecules periodically interrupted by gaps containing mineral crystals. This complex organization is diagrammed in Figure 1.2.

![Diagram of bone structure](image)

Figure 1.2: Structural organization of bone over multiple size scales. Figure from (7).

Collagen molecules are composed of a cross-linked triple-helix structure, which is energetically stabilized by hydrogen-bonded water molecules. Some water molecules reside in the interior of the triple helix, where they form bridges between electrostatic charges on adjacent collagen molecules, while a larger proportion envelops the exterior of the collagen molecule in several hydration layers, shown in Figure 1.3.
Three main types of cells are present in bone: osteocytes, osteoclasts, and osteoblasts. These cells, collectively called the basic multicellular unit (BMU), contribute to the continuous process of bone maintenance and remodeling in complementary ways. Osteocytes, which are embedded throughout bone tissue, monitor the health and structural integrity of their surrounding bone, and signal to initiate a bone remodeling event to replace old, brittle bone with new tissue (9). Osteoclasts, large multi-nucleated cells, resorb this old bone, either stripping it away from the surface of trabeculae or boring outward from the interior of a Haversian canal (10). Osteoblasts follow, laying down new un-mineralized bone matrix, called osteoid. This osteoid is then mineralized in two stages, neither of which require the direct involvement of bone cells (11). First,
primary mineralization occurs over the course of a few days, with 50-70% of bone mineralization being completed (12). Next, secondary mineralization occurs over several weeks to months, in which mineral crystals increase further in number and size until full mineralization is achieved (13). This process occurs continuously to maintain bone homeostasis, with about 10% of the skeleton being replaced each year.

Bone mineral itself is a calcium apatite, most closely resembling calcium hydroxyapatite \((\text{Ca}_{10}(\text{OH})_2(\text{PO}_4)_6)\) (14,15) and carbonatoapatite type B, but bone mineral does not conform to a fixed stoichiometry (16). Several prior studies (17-21) have detected the presence of a protonated phosphate group \((\text{HPO}_4^{2-})\), and identified a deficiency in hydroxyl \((\text{OH}^-)\) groups compared to hydroxyapatite (22). Though the ratio of calcium to phosphorus varies somewhat even within humans (23), the composition of bone mineral is relatively consistent among different mammalian species.

1.2. Bone Disease: Osteoporosis and Osteomalacia

Osteoporosis is a common bone disorder, affecting over 10 million adults in the United States (24). In this disease, bone remodeling increases in frequency, and bone resorption outpaces bone deposition (25,26). Osteoporosis (from Greek, ‘oste’, which means bone, and ‘poros’, which means pore) is particularly prevalent in post-menopausal females, in whom the decrease in estrogen causes more frequent initiation of bone remodeling events (27,28). The result of this dysregulation is thinning of the cortex and enlargement of cortical pores (29), degradation and disconnection of trabecular rods and plates (30), and an overall decrease in the structural and mechanical competence of bone. The increase in bone remodeling frequency also decreases the time available for secondary mineralization to occur, leading to a decrease in the degree
of mineralization of bone (DMB), or mass of bone mineral per unit volume of bone matrix (12,31). Many drug treatments for osteoporosis exist (30), and combinations of drugs can effectively treat this disease in many patients. Bisphosphonates reduce the frequency of initiation of bone remodeling events, and intermittent administration of exogenous parathyroid hormone (PTH) stimulates bone formation by osteoblasts.

Osteomalacia (‘malacia’ being Greek for softness) is a bone-demineralizing disorder in which decreased levels of blood calcium or phosphorus impair the proper mineralization of bone matrix (26). This reduces the stiffness and static strength of bone, making it less mechanically competent (32-34). Vitamin D deficiency, the most common cause of osteomalacia (35), can cause poor intestinal uptake of calcium, leading to low levels in the blood. Vitamin D deficiency may occur in as many as 25% of elderly patients (36). Logically, the main treatment for this type of osteomalacia is vitamin D supplementation. Diseases of the kidneys may also cause osteomalacia due to wasting of phosphorus. In these cases, treatment involves dietary supplementation of phosphate, and may include supplementation of 1,25-dihydroxyvitamin D.

The essential difference between these two disorders is in bone mineralization. As shown in Table 1.1, mineral density is decreased in both osteoporosis and osteomalacia, while matrix is lost only in osteoporosis. This means that in osteomalacia, bone mineralization is severely decreased, while in osteoporosis, mineralization is only slightly decreased due to increased bone turnover and the consequently attenuated secondary mineralization (37).
7

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Table 1.1: Changes in mineral density, matrix density, and mineralization in osteoporosis and osteomalacia.

1.3. Measurement of Bone Health

Standard clinical measurement of bone density is performed using dual-energy x-ray absorptiometry (DXA). In this method, projection x-ray images, most commonly at the proximal femur and lumbar spine, are acquired at two different photon energies. This allows the contribution of soft tissue to total x-ray attenuation to be removed, leaving only the attenuation of bone mineral. Example DXA images are shown in Figure 1.4. This method, therefore, measures apparent areal (two-dimensional) bone mineral density (BMD), expressed in grams per cm². DXA BMD measurements are typically expressed as T-scores, the multiple of standard deviations above or below the average BMD of a thirty-year-old female (942 ± 122 mg/cm²) (38). A T-score > -1.0 is considered normal, while a T-score less than -2.5 is defined by the World Health Organization as the diagnostic criterion for osteoporosis.
Figure 1.4: DXA images for measurement of bone mineral density at the proximal femur (a) and lumbar spine (b) in a 53-year-old male (39).

BMD obtained in this manner is ‘apparent’ in that it lacks sufficient spatial resolution to image individual pore spaces, and so the measured density represents mass of bone mineral per unit total bone area (including pore spaces) rather than per unit of matrix. DXA density is therefore affected both by changes in porosity (mesoscopic scale) and tissue mineralization (microscopic scale); the loss of total bone tissue in osteoporosis and demineralization in osteomalacia appear identical on DXA.

Quantitative computed tomography (QCT) is less commonly used as a screening tool, but has one important advantage over DXA: resolution in three dimensions. This method uses a standard x-ray computed tomography (CT) scanner, which is calibrated to allow conversion of x-ray attenuation in Hounsfield Units (HU) to bone mineral density
values in mg/cc. An example of a QCT image, with density calibration standards in the pad below the patient, is shown in Figure 1.5. Resolution in QCT is on the order of several hundred µm.

QCT can also be performed using a scanner designed specifically to measure bone mineral density at a peripheral skeletal site, such as the radius or tibia; the examination is then termed peripheral QCT (pQCT). The effective x-ray dose is a tissue- and organ-weighted measurement of the health risk of exposure to ionizing radiation. By applying QCT to the limbs, where the effective dose for a given absorbed dose of ionizing radiation is lower, resolution can be improved through use of greater x-ray exposure and a specialized high-resolution scanner. In-plane resolution in this high-resolution peripheral QCT (HR-pQCT) method can be improved to better than 100 µm, sufficient for
visualization of individual trabeculae and the largest cortical pores (see Figure 1.6), but still insufficient to quantify DMB. Specialized HR-pQCT scanners are also not in widespread use, and are used mainly for research.

Figure 1.6: HR-pQCT image of the wrist at 82µm in-plane resolution, showing the distal radius and ulna (40).

Full resolution of even the smallest pores in bone is possible only with micro-computed tomography (µCT, Figure 1.7). This ex vivo method uses the same imaging principles as CT, but with much higher exposures necessary to achieve isotropic resolution finer than 10 µm. This method, therefore, can image the pore structure in cortical bone, including individual osteocyte lacunae. Micro-CT is the gold-standard method for analysis of bone structure in specimens and small animals (41,42), but is not applicable in vivo in humans. An analogous two-dimensional method, microradiography, can visualize individual pores in histologically-prepared samples (12), but has fallen out of routine use due to the proliferation of µCT. Accurate quantification of DMB also requires
a monochromatic x-ray source to avoid beam-hardening artifacts; this can be performed using a synchrotron x-ray source, but such facilities are very rare. Synchrotron μCT is therefore not commonly performed, even for research studies.

![Figure 1.7](image)

Figure 1.7: Comparison of a synchrotron-radiation μCT image with 7.5-μm isotropic resolution (top) to an HR-pQCT image of the same site with 82-μm isotropic resolution (43,44).

Double tetracycline-labeled bone biopsy is currently the best qualitative clinical assessment of bone mineralization (45). The antibiotic tetracycline binds reversibly to newly mineralized bone at the bone/osteoid interface, and is permanently incorporated as mineralization progresses. In this examination, the patient receives two doses of tetracycline spaced approximately two weeks apart. Several days after the second dose,
a bone biopsy is taken. Under fluorescent microscopy, areas of active mineralization show two bands of fluorescence, with the distance between them giving the rate of bone mineralization. However, the link between labeling and active bone formation is less reliable in osteomalacic bone, complicating the interpretation of this test (46). For example, the rate of bone mineralization may be so slow that only a single band of tetracycline appears, or tetracycline may bind to areas where no actual mineralization is occurring. Bone biopsy is also an invasive procedure, making this test unsuitable for repeated measurements, and does not provide a quantitative measurement of DMB.

A method for in vivo measurement of DMB could be designed in two ways: a single high-resolution measurement to spatially differentiate bone matrix from pore spaces, or as the ratio of paired measurements of apparent bone mineral and matrix densities. X-ray-based measurements with high resolution rely on high radiation dose, and are not applicable to human subjects. A paired-measurement method may therefore hold more promise for in vivo use.

1.4. MRI of Bone

In magnetic resonance imaging, a strong magnetic field first polarizes the nuclear spins, which are then manipulated in a spatially dependent manner by applied radiofrequency (RF) and magnetic gradient fields (47). The weak RF electromagnetic field produced by the resulting magnetization as it rotates in a plane transverse to the main magnetic field is then recorded and reconstructed into an image. The three basic steps of an MRI imaging experiment, known as a pulse sequence, are to excite, encode, and acquire the nuclear magnetic resonance (NMR) signal.
After signal excitation, however, the NMR signal decays throughout the experiment with a time constant called the effective transverse relaxation time ($T_2^*$). $T_2^*$ depends on, among other effects, the homogeneity of the internal magnetic field within the sample and the degree of molecular motion, which allows for averaging of the local magnetic field experienced by the spins over the course of the NMR experiment. The portion of this decay due to static field inhomogeneity can be removed using RF pulses to refocus spins in the transverse plane, thus isolating the transverse relaxation time ($T_2$). MRI of bone is made difficult by the extremely short $T_2^*$ of highly inhomogeneous, motionally restricted bone tissue. The excited NMR signal, therefore, decays to below the noise level before it can be sufficiently encoded and acquired by standard MRI pulse sequences. Bone, therefore, appears dark in standard magnetic resonance images.

Three solid-state pulse sequences have recently been developed for imaging short-$T_2^*$ tissues: ultrashort echo-time (UTE) (48), zero echo-time (ZTE) (49-55), and sweep imaging with Fourier transformation (SWIFT) (56).
Figure 1.8: UTE (a) and ZTE (b) imaging pulse sequences and corresponding k-space acquisition schemes. In ZTE, central k-space points are lost during transmit/receive dead time, and this region must be re-filled. The pointwise encoding time reduction with radial acquisition (PETRA) method is shown in panel (b) using dotted lines in G and ADC to indicate single-point acquisitions at lower gradient strengths (57).

In 3D UTE (Figure 1.8a), the time delay between the end of signal excitation and the beginning of encoding and acquisition is reduced by simultaneously beginning signal acquisition and ramping the gradient to full strength immediately after the MRI system's transmit/receive (T/R) switching dead time (a hardware dependent parameter) has elapsed. K-space is sampled along radial center-out half-projections in all three dimensions. This method is simple to implement, but may be complicated by inevitable deviations from a perfectly trapezoidal gradient profile due to eddy currents. This may
be dealt with by mapping the gradient waveform and relocating the acquired k-space points to their true, rather than assumed, locations (58).

The ZTE pulse sequence (Figure 1.8b) further reduces the delay between signal excitation and acquisition for each k-space point. In this method, the gradient is first ramped up to full strength, and then a short (less than two dwell times) RF excitation pulse is applied. Signal encoding begins simultaneously with excitation, but the time delay to signal acquisition is still dictated by the T/R dead time. This delay results in the loss of one or more data points near the center of k-space. These points can either be inferred by k-space oversampling and extrapolation (59), or recaptured by performing a second set of acquisitions with lower gradient amplitudes either in a radial (60) or single-point Cartesian (57) scheme. The pointwise encoding time reduction with radial acquisition (PETRA, Figure 1.8b) method acquires each and every k-space point, in both the radially sampled outer region and the Cartesian-sampled inner sphere, at the shortest possible delay time after signal excitation. Though ramping the gradient to full strength and allowing it to stabilize before excitation avoids the complications related to eddy currents, it renders any RF pulse slab-selective. The pulse must either be short enough to reasonably assume non-selectivity (limiting this method to small flip angles), or the non-uniform excitation across the imaging field of view (FOV) must be corrected (61,62). Due to the changing slab orientation, depending on the direction of the readout gradient, such correction is not trivial.

While UTE and ZTE can be performed on standard clinical hardware with minor hardware modifications, SWIFT requires more extensive modification to perform signal
excitation, encoding, and acquisition in a finely interleaved or simultaneous manner, and will therefore not be discussed further.

Both $^1$H, present in bone water, and $^{31}$P, a major component of bone mineral, have spin $= \frac{1}{2}$ and are NMR-active. Both components of a paired measurement of apparent bone mineral and matrix densities are therefore possible using solid-state MRI, despite their extremely short $T_2^*$ and, in the case of bone mineral $^{31}$P, very long longitudinal relaxation time ($T_1$).

Wu and Ackerman first performed $^{31}$P imaging of bone mineral in animal specimens in 1992 (55). Cao et al. later quantified mineral density in specimens by $^{31}$P NMR (63) with separate determination of bone volume. Anumula et al. reported imaging-based quantification of $^{31}$P in animal specimens using experimental hardware (64,65). While the feasibility of in vivo $^{31}$P MR imaging of human bone mineral has been proven (66-68), quantification using clinical hardware had not been performed prior to the work in Chapter 5 of this thesis.

Bone $^1$H signal arises from three major pools (69), illustrated in Figure 1.9. Free water within pores has the longest $T_2$ relaxation times, ranging from 1 ms to 1 s. Within this pool, water in small pores, which have greater surface to volume ratios, experiences greater surface relaxation and thus has shorter $T_2$ than water in larger pores (70).

Water hydrogen-bonded to bone matrix is more tightly restricted in its movement, and its protons experience less motional averaging of their local magnetic environments. The $^1$H signal from bound water has a shorter $T_2$ of approximately 300-400 µs (69,71,72).
A third pool of $^1$H signal with extremely short $T_2 < 100 \, \mu s$ also exists (73). This pool encompasses protons in matrix collagen molecules, water within bone mineral crystals, and possibly other macromolecules. Clinical MRI equipment is unable to capture signal from this extremely short-$T_2$ pool, even using specialized solid-state pulse sequences.

![Diagram](image.png)

Figure 1.9: Cartoon $T_2$ relaxation spectrum diagramming the three major $^1$H NMR signal pools in bone. Pore water has $T_2 > 1 \, ms$, while bound water has $T_2 \sim 300$-500 $\mu s$. Collagen signal, at $T_2 \sim 40$-60 $\mu s$, is below the detection limit at clinical field strengths, but becomes visible using micro-imaging and spectroscopic hardware. As porosity increases, collagen and bound water decrease, while pore water increases and shifts to longer $T_2$ values due to the smaller surface-to-volume ratio of enlarged pores. This figure is adapted from Li et al. (74).

Several groups have also made progress toward imaging and quantification of the fraction of total bone water that is hydrogen-bonded, and therefore is assumed to exist in proportion to bone matrix. There are two classes of methods to isolate this matrix-associated bone water pool. In bi-component fitting methods, signal can be acquired at several $TE$s and fitted to a sum of two weighted exponential functions. The weights of
these exponential decays have been hypothesized to represent bound and pore water signal amplitudes (75-78).

Alternatively, specifically designed RF pulses can be employed to differentially affect the magnetization of the two pools. A long, low-amplitude $T_2^*$-selective RF pulse can selectively saturate the long-$T_2^*$ magnetization of pore water while only slightly affecting the short-$T_2^*$ magnetization of bound water (60,63,79). In a similar method, a $T_2$-selective adiabatic pulse can invert the long-$T_2$ magnetization of pore water, while largely saturating the short-$T_2$ magnetization of bound water. After a delay time, the magnetization of pore water will pass through zero, while the magnetization of bound water will have recovered to a positive and measurable value (80-84).

1.5. Outline of Thesis Chapters

In this dissertation, a combined MRI method for quantification of bone mineral and matrix densities, and their ratio, the degree of mineralization of bone, is introduced. In Chapter 2, the uniquely unfavorable relaxation properties of bone mineral $^{31}$P are systematically examined at multiple magnetic field strengths and levels of demineralization, and the results are used to predict the magnetic field at which SNR will be optimized. Chapter 3 evaluates the performance of bi-component $T_2^*$ fitting for quantification of bound and pore bone water fractions in comparison to other experimental NMR-based techniques and two gold-standard validation methods. Chapter 4 validates $^1$H signal density in single adiabatic inversion-recovery-prepared ZTE (SIR-ZTE) MRI as a surrogate measure of bone matrix density in human bone specimens. Finally, in Chapter 5, a combined examination of bone mineral and matrix densities using $^{31}$P ZTE and $^1$H single adiabatic inversion-recovery-prepared rapid ZTE (SIR-rZTE) is introduced, and the
degree of mineralization of bone is quantified in sixteen human tibial cortical bone specimens using a clinical scanner.
CHAPTER 2: $^{31}$P NMR RELAXATION OF CORTICAL BONE MINERAL AT MULTIPLE MAGNETIC FIELD STRENGTHS AND LEVELS OF DEMINERALIZATION

2.1. Abstract

Recent work has shown that solid-state $^1$H and $^{31}$P MRI can provide detailed insight into bone matrix and mineral properties, thereby potentially enabling differentiation of osteoporosis from osteomalacia. However, $^{31}$P MRI of bone mineral is hampered by unfavorable relaxation properties. Accurate knowledge of these properties is critical to optimizing MRI of bone phosphorus.

In this work, $^{31}$P MRI signal-to-noise ratio (SNR) was predicted on the basis of $T_1$ and $T_2^*$ (effective transverse relaxation time) measured in lamb bone at six field strengths (1.5 – 11.7 T) and subsequently verified by 3D ultra-short echo-time and zero echo-time imaging. Further, $T_1$ was measured in deuterium-exchanged bone and partially demineralized bone.

$^{31}$P $T_2^*$ was found to decrease from 220.3 ± 4.3 µs to 98.0 ± 1.4 µs from 1.5 to 11.7 T, and $T_1$ to increase from 12.8 ± 0.5 s to 97.3 ± 6.4 s with increasing field strength. Deuteron substitution of exchangeable water showed that 76% of the $^{31}$P longitudinal relaxation rate is due to $^1$H-$^{31}$P dipolar interactions. Lastly, hypomineralization was found to decrease $T_1$, which may have implications for $^{31}$P MRI based mineralization density quantification.
Despite the steep decrease in the $T_2^*/T_1$ ratio, SNR should increase with field strength as $B_0^{0.4}$ for sample-dominated noise and as $B_0^{1.1}$ for coil-dominated noise. This was confirmed by imaging experiments.

2.2. Introduction

Bone mineral is a nanocrystalline, non-stoichiometric, highly substituted calcium apatite (16), most closely resembling calcium hydroxyapatite ($\text{Ca}_{10}(\text{OH})_2(\text{PO}_4)_6$) (14,15) and carbonatoapatite type B (85-89), in which some phosphate ions are substituted by carbonate. Phosphorus-31 ($^{31}\text{P}$) has spin $I=\frac{1}{2}$ and 100% natural abundance, and as a major component of bone mineral, it therefore should be ideally suited for quantitative evaluation of bone mineral.

Prior $^{31}\text{P}$ NMR studies of bone mineral by magic-angle spinning (MAS) and cross-polarization (CP) from $^1\text{H}$ (17-21) have detected the presence of a protonated phosphate group ($\text{HPO}_4^{2-}$) having an isotropic chemical shift similar to that in octacalcium phosphate ($\text{Ca}_8(\text{HPO}_4)_2(\text{PO}_4)_4\cdot5\text{H}_2\text{O}$), and chemical shift anisotropy similar to brushite ($\text{CaHPO}_4\cdot2\text{H}_2\text{O}$). Wu, et al. argued that this makes $\text{HPO}_4^{2-}$ in bone mineral uniquely different from any such groups in synthetic models of bone mineral (21). Using 2D $^1\text{H}$-$^{31}\text{P}$ heteronuclear correlation spectroscopy, Cho, et al. determined that bone mineral is also severely deficient in hydroxyl ($\text{OH}^-$) groups, having approximately 21% of the $\text{OH}^-$ content of stoichiometric hydroxyapatite (22).

More recent work has shown that solid-state $^1\text{H}$ and $^{31}\text{P}$ MRI have the potential to quantify bone matrix and mineral densities, thereby providing information that is not available using current x-ray based clinical methods, but may enable differentiation of
osteoporosis from osteomalacia. \(^{31}\)P NMR (63) and MRI (64,65,90) have shown substantial potential for quantification of bone mineral density, and \(^{31}\)P MRI of bone mineral has been proven feasible in vivo (66,68).

However, magnetic resonance imaging of solid-state phosphorus in bone is greatly complicated by unfavorable relaxation parameters. Greater net magnetization and higher Larmor frequency at higher magnetic fields generally increase signal strength, but prolonged \(T_1\) (longitudinal relaxation time) and reduced \(T_2^*\) (effective transverse relaxation time) as field strength increases may outweigh possible gains. Several groups have measured \(T_1\) and \(T_2^*\) of bone mineral \(^{31}\)P (63,64,66,91) under various conditions and selected field strengths, but no systematic study across field strengths has so far been performed.

The purpose of this work was to predict the dependence of bone mineral phosphorus signal-to-noise ratio (SNR) on static magnetic field strength and level of bone mineralization to aid in the design of an in vivo MRI protocol for measurement of bone mineral density. To accomplish this goal, the \(^{31}\)P \(T_1\) and \(T_2^*\) relaxation times were measured using NMR in unmodified, deuterium-exchanged, and partially demineralized lamb tibial cortical bone samples at six magnetic field strengths ranging from 1.5 T to 11.7 T using comparable transmit/receive radiofrequency (RF) coils. Relaxation times of unmodified bones were then used to predict the theoretically achievable image SNR at each field strength, and these predictions were compared with SNR measured in ultra-short echo-time (UTE) and zero echo-time (ZTE) images. Finally, the mechanisms responsible for longitudinal relaxation and the effect of demineralization on bone mineral
\(^{31}\)P \(T_1\) were evaluated using measurements in deuterium-exchanged and partially demineralized bone, respectively.

### 2.3. Methods

#### 2.3.1. Specimens

Lamb tibiae were obtained fresh from a local butcher, cleaned of soft tissue, marrow, and periosteum, wrapped in saline-soaked gauze and aluminum foil, and kept frozen at -20 °C until specimens were cut. Five cylindrical specimens of the tibial cortex (4 mm diameter, 10 mm length) were sectioned such that the specimen axis was parallel to the osteonal axis, and then scanned to determine the dependence of \(T_1\) and \(T_2^*\) relaxation times of bone mineral phosphorus on static magnetic field strengths. These specimens were later subjected to deuterium exchange to investigate the mechanisms responsible for longitudinal relaxation. A sixth, larger cylindrical cortical specimen (7 mm diameter, 30 mm length) was sectioned from a lamb tibia and imaged for comparison with predicted SNR trends. An additional five samples of cortical bone, weighing 300 mg each, were ground from the lamb tibiae in a mortar and pestle under liquid nitrogen. The resulting powders were used to measure the effect of partial demineralization on the relaxation times of bone mineral phosphorus. The initial \(T_1\)s of bone powders (84.97 ± 1.57 \(\mu\)s) were similar to those of intact bone specimens (82.97 ± 2.18 \(\mu\)s), though a slight but statistically significant decrease in \(T_1\) was observed upon addition of saline (79.91 ± 2.44 \(\mu\)s). All specimens were stored in saline until the time of scanning. Intact specimens were gently blotted dry before scanning in a sealed vessel to prevent dehydration. Powder specimens were mixed with saline to facilitate transfer into 5 mm
NMR tubes, centrifuged, the supernatant removed, and the precipitate scanned. All data were acquired at room temperature.

2.3.2. MRI Scanners and RF Coils

Experiments were performed on the following scanners: 1.5 T, 3 T, and 7 T whole-body MRI scanners (Siemens, Erlangen, Germany); a 4.7 T horizontal-bore animal MRI scanner (Varian, Palo Alto, CA); a 9.4 T vertical-bore NMR spectrometer and micro-imaging system (Bruker, Billerica, MA); and a 11.7 T vertical-bore NMR spectrometer (Varian, Palo Alto, CA).

For all except the 9.4 T and 11.7 T systems, a standardized set of transmit/receive (T/R) RF coils were constructed and used (1 cm diameter, 3 cm length, 10 turns per cm, shown in Figure 2.1). Each coil incorporated two interleaved solenoids connected in parallel, each with 5 turns per cm, to reduce the accumulation of phase along the electrical length of the coil. These coils were designed to have sensitivity and homogeneity properties similar to high-resolution NMR probes used in spectrometers. At 9.4 T and 11.7 T, vendor-supplied RF/gradient probes with one gradient axis (for spoiling) were used.
2.3.3. $T_1$ Measurements

As $T_2^*$ approaches the duration of an RF pulse, spins experience relaxation during the pulse and are rotated by less than the nominal flip angle, $\alpha = \omega_1 \tau_{RF}$. To minimize the duration of RF excitation pulses relative to $T_2^*$, saturation-recovery rather than inversion-recovery was used for $T_1$ measurements. A series of six 90-degree rectangular RF saturation pulses were applied, each followed by a spoiler gradient (duration = 2 ms, amplitude = 20 mT/m). Bloch equation simulations show that six repetitions of the saturation pulse-spoiler series saturates longitudinal magnetization to 0.1% of initial magnetization in the presence of a flip angle error of ±20%, and 0.0015% in the
presence of a flip angle error of ±10%. After a saturation-recovery time ($t_{SR}$) following the final saturation pulse, a 90-degree rectangular RF excitation pulse was applied, and free-induction decay (FID) acquisition was begun 20 µs after the end of this pulse. Transmit/receive dead time was taken into account during reconstruction by dropping points acquired before T/R switching was completed (a typical dead time is 40 µs). All 90-degree pulses were identical within each sequence, and RF pulse durations at 1.5, 3, 4.7, 7, 9.4, and 11.7 T were 20, 10, 12, 10, 7, and 12 µs, respectively. 2048 complex points were read out at a dwell time of 5 µs. After acquisition, a final spoiler gradient (duration = 5.2 ms, amplitude = 20 mT/m) was applied. TR was minimized for each repetition, leaving only 1 ms before the first saturation pulse and 1 ms after the final spoiler gradient. This sequence is diagrammed in Figure 2.2.

Figure 2.2: Saturation-recovery pulse sequence used for bulk $T_1$ relaxation time measurements.
$T_1$s were calculated by non-linear least squares fitting of peak amplitudes (processing details are given below) to Equation 2.1, where $a$, $b$, and $T_1$ are fitted parameters:

$$S(t_{SR}) = a - b \exp \left( \frac{-t_{SR}}{T_1} \right)$$

[2.1]

2.3.4. $T_2^*$ Measurements

$T_2^*$ was measured using a pulse-acquire FID acquisition module identical to that of the saturation-recovery sequence used for $T_1$ measurements. For fully mineralized bone experiments, this sequence was implemented separately from the saturation-recovery sequence. For deuterium exchange and partial demineralization measurements, the FID acquisition after the longest $t_{SR}$ was taken from the saturation-recovery data.

The resulting spectra (processing details are given below) fit a Lorentzian better than a Gaussian function; the quality of fit is shown in Figure 2.3. $T_2^*$ was calculated from the full width at half maximum (FWHM) of the fitted Lorentzian:

$$T_2^* = (\pi \text{FWHM})^{-1}$$

[2.2]
2.3.5. Multiple Fields and SNR Estimation

Each fully hydrated solid bone specimen was gently blotted dry and placed into a 5 mm sealed plastic tube (1.5 T - 7 T) or glass NMR tube (9.4 T, 11.7 T), and $T_1$ and $T_2^*$ measurements were performed. To estimate achievable spectral SNR, measured relaxation times were incorporated into a signal equation (92), which was modified to account for loss of phase coherence during the RF pulse and to include frequency-dependent sample-dominated and coil-dominated noise terms:

$$SNR_{coil\_dom} = \frac{1 - \exp\left(\frac{-1}{T_1}\right)}{1 - f_x(\alpha, \tau_{RF}) \exp\left(\frac{-1}{T_1}\right)} \exp\left(\frac{-t_{dom}}{T_2^*}\right)$$

[2.3]
and

\[ SNR_{\text{sample-dom}} = \omega f_{xy}(\alpha, \tau_{RF}) \frac{1 - \exp\left(-\frac{\tau_{RF}}{T_1}\right)}{1 - f_z(\alpha, \tau_{RF}) \exp\left(-\frac{\tau_{RF}}{T_2}\right)} \exp\left(-\frac{t_{\text{dead}}}{T_2}\right) \]  \hspace{1cm} [2.4]

where

\[ f_{xy}(\alpha, \tau_{RF}) = \exp\left(-\frac{-\tau_{RF}}{2T_2}\right) \cos \left(\sqrt{\alpha^2 - \left(\frac{\tau_{RF}}{2T_2}\right)^2}\right) \]  \hspace{1cm} [2.5]

and

\[ f_z(\alpha, \tau_{RF}) = \exp\left(-\frac{-\tau_{RF}}{2T_2}\right) \left(\cos \left(\sqrt{\alpha^2 - \left(\frac{\tau_{RF}}{2T_2}\right)^2}\right) + \frac{\tau_{RF}}{2T_2} \sin \left(\sqrt{\alpha^2 - \left(\frac{\tau_{RF}}{2T_2}\right)^2}\right)\right) \]  \hspace{1cm} [2.6]

describe the response of transverse and longitudinal magnetization, respectively, to a square, on-resonance RF pulse of nominal flip angle \(\alpha\) and duration \(\tau_{RF}\) (93). These equations represent purely coil- or sample-dominated noise cases; in practice, actual noise dominance falls between these two extremes, and so these equations represent the upper and lower bounds of expected experimental conditions.

The duration of the RF pulse is much less than \(T_2^*\), so \(f_{xy} \approx \sin(\alpha)\) and \(f_z \approx \cos(\alpha)\) and the Ernst angle equation, \(\cos(\alpha) = \exp(-TR/T_1)\), is therefore valid. The optimal flip angle, calculated using \(TR = 250\) ms and the measured \(T_1\), was used as the nominal flip angle at each field strength, and a 10 \(\mu\)s RF pulse duration was assumed. SNR was estimated using dead times \((t_{\text{dead}})\) ranging from 0 to 400 \(\mu\)s. SNR at each dead time was normalized to the value at 1.5 T to allow for comparison across field strengths and fitted to an empirically chosen power law, \(y = aB_0^b\).
2.3.6. Deuterium Exchange

To determine the contribution of $^{1}\text{H}-^{31}\text{P}$ dipolar interaction to the longitudinal relaxation rate ($R_T = T_1^{-1}$) of bone mineral phosphorus, relaxation measurements were repeated on the intact bone specimens at 3 T and 7 T after deuterium oxide ($\text{D}_2\text{O}$) exchange. The solid bone specimens were thoroughly blotted dry, immersed in 3 mL of $\text{D}_2\text{O}$ saline (a 25-fold volume excess) at 4 °C for 72 h, and scanned upon removal from the solution. The fraction of longitudinal relaxation rate attributed to $^{1}\text{H}-^{31}\text{P}$ heteronuclear dipolar interaction was quantified as

$$R_{1;\text{H}^{+31}\text{P}} = \frac{R_{1;\text{H},\text{O}} - R_{1;\text{D},\text{O}}}{(1 - 0.0629) R_{1;\text{H},\text{O}}}$$  [2.7]$$

which follows from the ratio of the strengths of $^{1}\text{H}$ and $^{2}\text{H}$ dipolar coupling (given by the direct dipole-dipole spin Hamiltonian) (94):

$$R_{1;\text{H}^{+31}\text{P}} = \frac{\hbar}{\hbar} \left( \frac{I_{1\text{H}}(I_{1\text{H}} + 1)}{I_{2\text{H}}(I_{2\text{H}} + 1)} \right) \gamma_2^{2\text{H}} = 0.0629 R_{1;\text{H}^{+31}\text{F}}$$  [2.8]$$

where $I_{1\text{H}} = \frac{1}{2}$, $I_{2\text{H}} = 1$, $\gamma_{1\text{H}} = 42.58$ MHz/T, and $\gamma_{2\text{H}} = 6.54$ MHz/T are the spin quantum numbers and gyromagnetic ratios of the two nuclei (47).

2.3.7. Partial Demineralization

A solid piece of lamb tibial cortical bone was immersed in liquid nitrogen and ground to powder with a pestle in a mortar. Five 300 mg portions of the powdered samples were measured and combined with 1.2 mL of saline, transferred to 5 mm NMR tubes, centrifuged to settle the powder, and the supernatant removed. Relaxation
measurements of the precipitate were performed at 9.4 T, and the powder slurries were then transferred back to storage vials.

The slurries were again centrifuged and the saline supernatant was drawn off and saved. 1.2 mL of 1% ethylenediaminetetraacetic acid (EDTA) solution was added to each sample, and the samples were agitated and left at room temperature for three days to allow partial demineralization to occur. After three days, the samples were centrifuged and the EDTA solution was drawn off, saved, and replaced with 1.2 mL of fresh 1% EDTA solution. The samples were again agitated and left for another three days at room temperature, centrifuged, and the EDTA solution again drawn off and saved. The powders were rinsed with three 1.2 mL changes of water to remove any remaining EDTA and liberated phosphates. In each rinse cycle, water was added, the samples were thoroughly agitated, centrifuged, and the rinse water drawn off and saved. Thereafter, the precipitates were combined with 1.2 mL saline, transferred to 5 mm NMR tubes, and centrifuged. Relaxation measurements were performed on the precipitates at 9.4 T, and the saline and powder slurries transferred back to storage vials. All saline, phosphorus-containing EDTA solution, and rinse water which had been in contact with the bone powders was combined, lyophilized, and redissolved in 0.5 mL of water. The resulting solutions were scanned with a calibrated methylene diphosphonate (MDP) capillary using high-resolution $^{31}$P NMR to quantify the amount of phosphorus removed from each sample (number of dummy scans = 4, number of scans = 50, flip angle = 90 degrees, number of points = 65536, bandwidth = 13021 Hz, $TR = 47.5$ s).

The cycle comprising removal of saline to re-addition of saline, relaxation measurement, and high-resolution $^{31}$P NMR spectroscopy, was repeated two more times for a total of
three levels of demineralization. Subsequently, 7 mL of 1.23M HCl was added to each powder specimen to dissolve all remaining bone mineral. After two days at room temperature, 10 mL of water and 1 mL of D_2O was added to each sample, and high-resolution ^31P NMR spectra were run to quantify the amount of phosphorus remaining in each sample of bone powder after the final round of EDTA demineralization. The sum of this amount and the amount of phosphorus removed during all rounds of partial demineralization is the total amount of bone mineral phosphorus initially present in the bone. The degree of demineralization after each round was quantified as the concentration of phosphorus in the saline, EDTA, and rinse liquids divided by this sum.

2.3.8. Spectroscopy Processing Details

Two signal averages were used for deuterium exchange measurements at 7 T, and four for all other measurements. Data were Fourier transformed and automatically phase-corrected based on peak symmetry. All reconstruction and processing were performed using custom scripts programmed in MATLAB (MathWorks, Natick, MA).

2.3.9. Imaging

To test predicted SNR behavior, a sixth, larger bone sample was imaged using two forms of radial pulse sequences: 3D ramp-sampled UTE (48) and 3D ZTE (49-53), so called in order to specify whether gradients are ramped up before or after RF excitation, respectively (54). These imaging sequences, shown in Figure 2.4, were implemented in SequenceTree (95) at 1.5 T, 3 T, and 7 T using the custom-built solenoidal RF coils described earlier. These clinical scanners all have maximum gradient amplitude of 40 mT/m and slew rate of 180 T/m/s.
Figure 2.4: Imaging pulse sequences used for SNR comparisons to predicted trends: a) ramp-sampled ultra-short echo time (UTE), and b) zero echo time (ZTE) PETRA, with single-point sampling of k-space center.

The UTE imaging sequence started with a 20 µs rectangular RF pulse followed 40 µs later by FID readout begun simultaneously with gradient ramp-up. The gradient direction was varied for each repetition to acquire center-out k-space half-projections distributed uniformly within a sphere using the method described by Wong and Roos (96). This simultaneous readout and gradient ramp-up allows acquisition of the central region of k-space, and the resulting non-uniform sampling density was corrected for during reconstruction. A flip angle equal to the Ernst angle was used at each field strength: 11.3, 7.9, and 5.0 degrees at 1.5 T, 3 T, and 7 T, respectively. Other acquisition parameters were: TR = 250 ms, number of complex points per readout N = 153, dwell time = 5 µs, number of projections = 5000, field of view (FOV) = 310 mm in all dimensions, gradient ramp time = 250 µs, gradient amplitude = 38.69 mT/m, and number of averages = 1.
The ZTE imaging sequence also begins with a 20 µs rectangular RF pulse, though this pulse is applied after gradients have stabilized at their constant maximum value. The gradient direction was again adjusted for each repetition to acquire the same distribution of center-out k-space half-projections as the UTE sequence. TR, dwell time, number and arrangement of projections, FOV, gradient amplitude, and number of averages were also identical to the UTE sequence described above, and the number of points per projection was N = 128. Because the gradient was already ramped up to full strength before excitation, the same portion of k-space is sampled as the 153-point UTE readout begun simultaneously with gradient ramp-up. Due to receiver dead time, FID readout was begun 40 µs after the end of the RF pulse. During this delay between excitation and beginning of readout, several k-space points are lost. These points were recovered after the main ZTE acquisition using the pointwise encoding time reduction with radial acquisition (PETRA) method (57), which involves single-point acquisition on Cartesian coordinates within a central sphere of k-space. Each single-point acquisition occurs at an echo time of 40 µs. This central Cartesian region was merged with the surrounding radial region during reconstruction.

Data from both UTE and ZTE sequences were re-mapped to a 256 x 256 x 256 point Cartesian coordinate grid. To compensate for non-uniform sampling density, the weight assigned to each k-space point during re-gridding was chosen according to the k-space ‘volume’ occupied by that point (48). Data were Fourier transformed using the non-equispaced fast Fourier transform (NFFT) C subroutine library (97). The reconstructed voxel resolution was 1.21 x 1.21 x 1.21 mm³; however, actual resolution is reduced from its nominal value by point spread function (PSF) blurring due to broad spectral line width.
The signal-to-noise ratio of each image was calculated by dividing the mean signal intensity within a volume of interest (VOI) drawn within the center of the bone sample to the mean signal intensity within a VOI drawn in a region of background noise.

### 2.4. Results

#### 2.4.1. Relaxation Times and SNR

$T_1$ was found to increase monotonically with field strength from $12.8 \pm 0.5$ s to $97.3 \pm 6.4$ s (mean ± standard deviation), and $T_2^*$ to decrease from $220.3 \pm 4.3$ µs to $98.0 \pm 1.4$ µs. These results are shown in Figure 2.5. Fitted parameters $a$ and $b$ in Equation 2.1 were observed to be approximately equal, indicating effective saturation.

![Figure 2.5](image)

**Figure 2.5:** $^{31}$P (a) $T_1$ (squares) and $T_2^*$ (circles) relaxation times and (b) $R_1$ (squares) and $R_2^*$ (circles) relaxation rates of bone mineral phosphorus in fully hydrated solid bone samples at six field strengths. Lines connecting points are a visual guide only, and do not represent data or predictions. Error bars are included, but do not extend beyond markers.

Predicted spectral SNR for both coil- and sample-dominated noise was normalized to their respective values at 1.5 T to facilitate comparison of field strength dependence between noise scenarios (Figure 2.6). Before normalization, predicted coil-dominated
SNR is greater than sample-dominated SNR by a factor of 11.5 at 1.5 T. In each noise scenario, the field dependence is shown for both a realistic receiver dead time of 40 µs in UTE and ZTE imaging sequences reported in literature (in UTE and ZTE, echo time TE is more accurately described as receiver dead time, as no actual echo is created in these sequences). A clear SNR dependence on field strength is lost at $t_{\text{dead}} = 320$ µs for coil-dominated noise, and $t_{\text{dead}} = 130$ µs for sample-dominated noise. For $t_{\text{dead}} = 40$ µs, SNR is predicted to increase as $B_0^{0.4}$ for sample-dominated noise and $B_0^{1.1}$ for coil-dominated noise.

Figure 2.6: Predicted (solid markers) relative bone mineral $^{31}$P SNR based on measured relaxation times at multiple field strengths at $t_{\text{dead}} = 20$ and 40 µs, normalized to the value at 1.5 T for each condition, and power fits (solid lines) at $t_{\text{dead}} = 40$ µs. Relative SNR trends calculated from actual UTE and ZTE imaging acquisitions (open markers), also normalized to the value at 1.5 T for each condition.

2.4.2. Deuterium Exchange and Partial Demineralization

Replacement of exchangeable $^1$H atoms with $^2$H caused a substantial increase in $T_1$, but only a very small, albeit significant, increase in $T_2^*$, as shown in Table 2.1. Equation 2.7
suggests that 78.6 ± 2.0% of $1/T_1 = R_1$ is due to $^1$H-$^{31}$P heteronuclear dipolar interaction at 3 T, and 74.3 ± 2.1% of $R_1$ at 7 T. At each stage of partial demineralization, $T_1$ decreased significantly while $T_2^*$ showed no dependence on level of mineralization (Table 2.2).

<table>
<thead>
<tr>
<th>$B_0$</th>
<th>Condition</th>
<th>$T_1$ (s)</th>
<th>$T_2^*$ (µs)</th>
<th>$R_1$ (s$^{-1} \times 10^3$)</th>
<th>$R_2^*$ (s$^{-1} \times 10^3$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3 T</td>
<td>Unmodified</td>
<td>26.0 ± 1.4</td>
<td>189 ± 2.2</td>
<td>38.5 ± 2.0</td>
<td>5.29 ± 0.06</td>
</tr>
<tr>
<td></td>
<td>D$_2$O-Exchanged</td>
<td>99.0 ± 10.5</td>
<td>203 ± 2.1</td>
<td>10.2 ± 1.0</td>
<td>4.92 ± 0.05</td>
</tr>
<tr>
<td>7 T</td>
<td>Unmodified</td>
<td>66.0 ± 0.8</td>
<td>119 ± 0.4</td>
<td>15.2 ± 0.2</td>
<td>8.40 ± 0.03</td>
</tr>
<tr>
<td></td>
<td>D$_2$O-Exchanged</td>
<td>218 ± 15</td>
<td>121 ± 1.0</td>
<td>4.6 ± 0.3</td>
<td>8.30 ± 0.07</td>
</tr>
</tbody>
</table>

Table 2.1: Bone mineral phosphorus relaxation times in solid bone samples before and after D$_2$O exchange.

<table>
<thead>
<tr>
<th>% Mineralization</th>
<th>$T_1$ (s)</th>
<th>$T_2^*$ (µs)</th>
<th>$R_1$ (s$^{-1} \times 10^3$)</th>
<th>$R_2^*$ (s$^{-1} \times 10^3$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>100.0 ± 0.0</td>
<td>79.9 ± 2.4</td>
<td>91.1 ± 0.3</td>
<td>12.5 ± 0.4</td>
<td>11.0 ± 0.03</td>
</tr>
<tr>
<td>97.4 ± 0.1</td>
<td>71.7 ± 1.6</td>
<td>91.8 ± 0.4</td>
<td>13.9 ± 0.3</td>
<td>10.9 ± 0.05</td>
</tr>
<tr>
<td>94.3 ± 0.4</td>
<td>68.5 ± 0.7</td>
<td>90.3 ± 0.5</td>
<td>14.6 ± 0.1</td>
<td>11.1 ± 0.06</td>
</tr>
<tr>
<td>92.0 ± 0.6</td>
<td>65.5 ± 1.2</td>
<td>91.7 ± 0.2</td>
<td>15.3 ± 0.3</td>
<td>10.9 ± 0.03</td>
</tr>
</tbody>
</table>

Table 2.2: Bone mineral phosphorus relaxation times at 9.4 T in bone powder samples before and after three stages of demineralization.

2.4.3. Imaging

UTE and ZTE images of a cylindrical sample of lamb tibial cortical bone (7 mm diameter, 30 mm length) acquired at 1.5 T, 3 T, and 7 T are shown in Figure 2.7. Quality factor ratios ($Q_{\text{loaded}}/Q_{\text{unloaded}}$), which describe noise dominance, were 0.92, 0.74, and 0.21, respectively. Noise is therefore predominantly coil-dominated at 1.5 T and 3 T, and sample-dominated at 7 T. Image SNR is displayed in each panel of Figure 2.7, and relative SNR trends, normalized to the value at 1.5 T, are displayed within Figure 2.6. These trends roughly follow the coil-dominated SNR predictions for 1.5 T and 3 T, but diverge at 7 T.
2.5. Discussion

The main objective of this study was to evaluate the dependence of SNR in solid-state $^{31}$P imaging on static magnetic field strength, with the expectation that the findings will have implications on the choice of field strength for \textit{in vivo} $^{31}$P MRI studies of bone mineral. Although previous work has cast doubt on the notion that increasing field strength will yield a greater SNR (64,66,98), these prior studies were performed on different sets of bone specimens using disparate hardware and scanning parameters.
and, therefore, are not able to provide conclusive evidence to support or reject this hypothesis. Wu, *et al.* previously reported $^{31}$P $T_1$s of 7 s, 19.8 ± 0.5 s, and 40 s in human cortical bone at 1.5 T (98), lamb cortical bone at 2.0 T (53), and dry rabbit cortical bone at 4.7 T (99), respectively, although the measurement of 51.1 s at 4.7 T by Cao, *et al.* (63) in fully hydrated rat cortical bone is more relevant to the present study. Robson, *et al.* (66) also measured a $T_1$ of 8.6 ± 3.0 s in human cortical bone *in vivo* at 1.5 T. Anumula, *et al.* (64) found a value 54.1 ± 2.7 s for $T_1$ in whole rabbit bone *ex vivo* at 9.4 T. Wu, *et al.* also reported $^{31}$P $T_2^*$ of 199 µs and 143 µs at 1.5 T and 2.0 T, respectively. These values are similar to those found by Robson, *et al.* (207 ± 12 µs at 1.5 T), but are considerably larger than Anumula’s, *et al.* at 9.4 T (92.8 ± 7.5 µs). In addition to field strength, bone mineral $^{31}$P $T_1$ is affected by the concentration of nearby $^1$H nuclei, which in turn could depend on species, age, porosity, and hydration state of the bone. Subtle differences in the composition of bone mineral, which is known to vary with age (20), may also affect measured relaxation times.

The present study employed standardized RF coils, acquisition parameters, and analysis, and measurements were performed on a single set of cortical bone specimens from the same species and anatomical location. Our results generally support the field strength behavior implied in these prior studies, although differences in the state of bone specimens have a considerable effect on relaxation times (for example, $^{31}$P $T_1$s of dry rabbit cortical bone and hydrated rat cortical bone at 4.7 T differ by 28%).

Dipolar coupling involving protons, which is independent of $B_0$, and chemical shift anisotropy, especially of the fraction of $^{31}$P in the form of HPO$_4^{2-}$ (104-128 ppm (21)), as well as induced local gradients arising from the difference in magnetic susceptibility
between bone and water, both being linearly dependent on $B_0$, contribute to the effective transverse relaxation rate ($R_2^*$) (Figure 2.5b). The near-absence of motional averaging of these interactions in bone mineral leads to extremely short $^{31}$P $T_2^*$, although the fact that the line shape fits best to a Lorentzian (rather than exhibiting a powder pattern) likely means that some degree of motional averaging of chemical shift anisotropy does occur. This restricted motion also means that dipolar interactions with nearby $^1$H and $^{31}$P nuclei contain very little power at the $^{31}$P Larmor frequency, leading to a very long $^{31}$P $T_1$.

While a sharp increase in $^{31}$P $T_1$ at high field would not be expected for a small, rapidly tumbling biomolecule such as ATP (1.42 ± 0.21 s, 1.35 s, and 1.24 s at 4 T, 7 T, and 9.4 T, respectively (100-102)), it is reasonable to expect such an increase in a solid due to the long rotational correlation time (103).

Based on the measurements presented in this study, we can conclude that although the relaxation properties of bone phosphorus do become significantly less favorable at higher field strengths, as long as RF pulse duration and receiver dead time are adequately short relative to $T_2^*$ ($\tau_{RF} < 20 \mu s$, $\tau_{dead} < 40 \mu s$), and k-space is traversed rapidly ($t_{dwell} \approx 5 \mu s$, $G > 30$ mT/m), then SNR can be expected to increase with $B_0$ for both sample-dominated and coil-dominated noise. Solid-state projection imaging pulse sequences implemented on clinical hardware with transmit/receive extremity coils already commonly meet such requirements for pulse duration, dead time, and bandwidth. It is important to note that Equations 2.3 and 2.4 predict spectral SNR, and do not include the effect of k-space apodization due to $T_2^*$ decay during readout. Particularly in UTE where central k-space is traversed slowly during gradient ramping, the low-spatial-frequency signals are attenuated resulting in an SNR penalty (104).
Coil sensitivity and therefore SNR varies inversely with solenoid coil diameter, provided the length-to-diameter ratio is constant (105), so a significant decrease in SNR is expected with a larger solenoid receive coil. Birdcage coils, whose sensitivity is less than a solenoid of the same size, would entail a further SNR decrease. The predicted SNR trends should apply for a single RF coil arrangement, provided RF excitation at all field strengths can be accomplished by transmit pulses of equal duration, while still operating within limits on specific absorption rate (SAR) of RF power.

The predicted SNR behavior is supported by SNR calculated from images acquired at three field strengths (Figure 2.6). Mirroring findings by Weiger, et al. (104), the ZTE imaging pulse sequence was found to achieve higher SNR than its UTE counterpart at all field strengths (88%, 126%, and 285% at 1.5 T, 3 T, and 7 T, respectively). A possible explanation for this observation is that ZTE traverses k-space much more quickly than UTE (106) and, with PETRA, uses single-point acquisition to sample the central portion of k-space with a uniform, short delay time after excitation (57). Specifically, in ZTE, the time taken to reach $k = 30 \text{ m}^{-1}$, measured from the center of the RF pulse to arrival at that point, is 40 µs, while in UTE, the time taken to reach the same spatial frequency is much longer, approximately 190 µs, during which much more $T_2^*$ signal decay would occur. This loss in relative SNR efficiency for UTE is exacerbated at elevated $B_0$ due to shortened $T_2^*$. Noise also becomes sample-dominated at 7 T with a quality factor ratio of 0.21, causing image SNR for both sequences to fall below the predicted coil-dominated SNR trend.

While increasing static field strength does offer an SNR advantage, other important factors in MRI may become more problematic at high field. For example, as field...
strength increases and $T_2^*$ decreases (spectral line width increases), PSF blurring will increase, further limiting intrinsic resolution. The FWHM of the PSF, with the gyromagnetic ratio expressed in MHz/T is given by Equation 2.9:

$$FWMH_{PSF} = (\frac{\pi}{2} \gamma G)^{-1}$$  \[2.9\]

At a gradient strength of 40 mT/m, the width of the PSF can be expected to increase from ~2.1 mm at 1.5 T to ~3.9 mm at 7 T. This effect is qualitatively visible in both UTE and ZTE images in Figure 2.7. Lastly, SAR increases quadratically with frequency, and could become a limiting factor at high field strengths.

While this work proposes that $^{31}$P SNR in cortical bone improves with field strength, the SNR of bone phosphorus is still several orders of magnitude lower than for $^1$H imaging of bone water. Assuming a $T_1$ of 223 ms and $T_2^*$ of 390 $\mu$s for bone water at 3 T (80), $TR$ of 250 ms, flip angle equal to the Ernst angle at this $T_1$ and $TR$, pulse duration of 10 $\mu$s, receiver dead time of 30 $\mu$s, and equal spin density, coil-dominated predicted SNR (Equation 2.3) is 55 times higher for $^1$H than for $^{31}$P of equal spin density. Intrinsic resolution, which depends on gyromagnetic ratio as well as $T_2^*$, is also finer in bone $^1$H imaging, achieving a point spread function FWHM of 0.48 mm at 40 mT/m gradient amplitude as opposed to 2.44 mm in $^{31}$P imaging.

Due to the different architecture of RF coils between the 1.5 T – 7 T scanners (solenoids) and the 9.4 T – 11.7 T scanners (saddle), the cylindrical axis of the sample must be orthogonal to the main magnetic field at 1.5 T – 7 T and parallel to the main magnetic field at 9.4 T and 11.7 T. Due to the incoherent macroscopic organization of bone mineral (7), we did not expect relaxation properties to depend on orientation with
respect to $B_0$, as does the collagen water proton signal (8,71). A cursory examination of relaxation times of bone specimens oriented parallel and perpendicular to the main magnetic field did not reveal a significant dependence.

It has been postulated that bone mineralization, and therefore demineralization, proceed under conservation of total bone volume (107). This means that as mineral is lost, it is replaced by water. Deuterium exchange has shown that $^1$H-$^{31}$P dipolar interaction is the principal mechanism for $T_1$ relaxation of phosphorus in bone. Thus, as suggested by Equation 2.10 below, as demineralization progresses, the pool of water protons, $N$, contributing to $^{31}$P relaxation increases:

$$\frac{1}{T_1} = R_1 \propto \gamma_1^2 \gamma_{^{31}P}^2 \sum_i N_i \frac{6}{r_i}$$

Equation 2.10

Although the number of protons, $N$, increases with distance, it is also possible that these additional water protons may be too far from the remaining $^{31}$P nuclei to contribute appreciably to relaxation; however, because the remaining mineral crystals are smaller after partial demineralization, the proportion of $^{31}$P nuclei at the surface of these crystals is increased (i.e. the surface-to-volume ratio of the crystals is greater after demineralization), also leading to an increase in longitudinal relaxation via a decrease in the average distance, $r$, between $^{31}$P and $^1$H nuclei. Because of the significant shortening of $T_1$ at relatively modest levels of demineralization, use of a single, general value for $T_1$ of bone mineral $^{31}$P across different subjects for eventual bone mineral density quantification may affect the accuracy of such an examination.
While the experimentally observed gains in SNR with field strength are in fair agreement with predictions, the gains in larger objects such as the human extremities are likely more modest. Even using an extremity coil, noise will presumably be sample-dominated, and so the in vivo SNR gain, illustrated in the sample-dominated SNR predictions in Figure 2.6, will likely not exceed a factor of two across the range of clinical field strengths. Lastly, besides diminishing SNR returns with increasing field strength, other criteria, including increased PSF blurring, must be taken into consideration when choosing a field strength for $^{31}$P imaging of mineralized tissues.

2.6. Conclusions

In spite of the steep increase in bone mineral $^{31}$P $T_1$ and decrease in $T_2^*$, SNR is predicted to increase modestly with field strength. These predictions are supported as well by solid-state $^{31}$P imaging. This work also shows that $^1$H-$^{31}$P dipolar interaction is the dominant longitudinal relaxation mechanism and that $T_1$ is reduced with decreasing level of mineralization of bone. The results have implications on MRI-based studies of bone mineralization.
CHAPTER 3: BI-COMPONENT $T_2^*$ ANALYSIS OF BOUND AND PORE BONE WATER FRACTIONS FAILS AT HIGH FIELD STRENGTHS

3.1. Abstract

Osteoporosis involves degradation of bone’s trabecular architecture, cortical thinning, and enlargement of cortical pores. Increased cortical porosity is a major cause of the decreased strength of osteoporotic bone. The majority of cortical pores, however, are below the resolution limit of MRI.

Recent work has shown that porosity can be evaluated by MRI-based quantification of bone water. Bi-exponential $T_2^*$ fitting and adiabatic inversion-recovery preparation are the two most common methods purported to distinguish bound and pore water in order to quantify matrix density and porosity.

To assess the viability of $T_2^*$ bi-component analysis as a method for quantifying bound and pore water fractions, we have applied this method to human cortical bone at 1.5 T, 3 T, 7 T, and 9.4 T, and validated the resulting pool fractions against µCT-derived porosity and gravimetrically-determined bone densities. We also investigated alternative methods: 2D $T_1$-$T_2^*$ bi-component fitting by incorporating saturation-recovery, 1D and 2D fitting of CPMG echo amplitudes, and deuterium inversion recovery.

The short-$T_2^*$ pool fraction was moderately correlated with porosity ($R^2 = 0.70$) and matrix density ($R^2 = 0.63$) at 1.5 T, but the strengths of these associations were found to diminish rapidly as field strength increased, falling below $R^2 = 0.5$ at 3 T. Addition of the
$T_1$ dimension to bi-component analysis only slightly improved the strengths of these correlations. $T_2^*$-based bi-component analysis should therefore be used with caution.

The performance of deuterium inversion-recovery at 9.4 T was also poor ($R^2 = 0.50$ versus porosity and $R^2 = 0.46$ versus matrix density). The CPMG-derived short-$T_2$ fraction at 9.4 T, however, is highly correlated with porosity ($R^2 = 0.87$) and matrix density ($R^2 = 0.88$), confirming the utility of this method for independent validation of bone water pools.

### 3.2. Introduction

As explained in **Chapter 1**, osteoporosis is a common bone disease which involves deterioration of trabecular bone architecture (30) and enlargement of pores and thinning of cortical bone (29). This increased cortical porosity is a major cause of the impaired strength of osteoporotic bone (108,109). Measurement of cortical porosity is, therefore, of great interest for assessment of bone health.

NMR and MRI methods have recently been used to study bone density and porosity. Unfortunately, the majority of cortical pores are smaller than the spatial resolution achievable by *in vivo* MRI, necessitating other approaches that do not rely on resolution of pore spaces.

The NMR $^1$H signal in bone arises from three major pools: pore water with relatively long $T_2$ (1 ms – 1 s) (70), bound water with short $T_2$ (300 – 400 µs) (69,71,72), and macromolecular and mineral water signal with extremely short $T_2$ (< 100 µs) (73). These components have been illustrated previously in a schematic $T_2$ spectrum (**Figure 1.9**).
As bone substance is lost and pore spaces expand, pore water concentration increases (71,72,74,75,81,84,110,111). Bound water concentration, which should parallel collagen density (69), is proportional to bone density and, therefore, is inversely proportional to porosity (60,71,72,75,76,78,79,81,84,112). Total water concentration, the sum of bound and pore water concentrations, is also weakly associated with porosity (33,71,74,81,113-115).

Due to the specific absorption rate (SAR) limitations of clinical MRI scanners, it is not possible to apply the refocusing RF pulses necessary to acquire Carr-Purcell-Meiboom-Gill (CPMG) echo amplitude data reflecting the $T_2$ components of bone $^1$H signal. $T_2^*$ bi-component fitting of free induction decay (FID) data or a series of images at different $TE$s has been investigated as an alternative, due to its relative ease of implementation.

$T_2^*$ of pore water, however, is shortened due to strong internal magnetic field gradients arising from the difference in magnetic susceptibility between water and bone tissue ($\Delta \chi \approx 2.5$ ppm SI) (116). The reduced separation between bound and pore water $T_2^*$ values, illustrated in Figure 3.1, complicates separation via bi-component fitting, which, as a form of inverse Laplace transformation, is an ill-posed problem (117). Because the strength of the induced magnetic fields increases linearly with field strength, this effect becomes more severe at higher field strengths.
To assess the viability of $T_2^*$ bi-component analysis as a method for quantifying bound and pore water fractions in humans, we have scanned a set of human cortical bone specimens at 1.5 T, 3 T, 7 T, and 9.4 T, and validated bi-exponential fitting of the resulting FIDs against µCT-derived porosity and gravimetrically-determined bone densities. These specimens are expected to vary widely in bone density and porosity. We also compared $T_2^*$ bi-component analysis at these four field strengths to $T_2$ bi-exponential fitting of CPMG echo amplitudes (69) and deuterium inversion-recovery NMR (71) at 9.4 T.

3.3. Materials and Methods

3.3.1. Specimen Source and Preparation
The tissue examined consisted of 15 specimens of cortical bone taken from the previously frozen tibial mid-shafts 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 was expected. A 4-mm slice was cut from each thawed tibia with a rotating blade at the region of maximum
cortical bone thickness, 38% of the length of the tibia from the medial malleolus to the medial condyle. A rectangular beam was cut from the longest of the three faces of the roughly triangular bone slice, and trimmed to fit into a 5-mm NMR tube. The direction of the long axis of the bone was indicated on the end of each beam by cutting a notch parallel to the bone’s axis. Specimens were stored individually in phosphate-buffered saline.

3.3.2. $^1$H NMR Spectroscopy

1.5 T, 3 T, and 7 T experiments were performed on whole-body human MRI scanners (Siemens, Erlangen, Germany) using custom-built $^1$H-free solenoidal radiofrequency (RF) coils (to eliminate signal contamination from the coil), 10 mm in diameter and 25 mm in length. Each coil was constructed of two parallel six-turn windings of copper wire on a polytetrafluoroethylene (PTFE) tube, mounted to a PTFE board, and tuned and matched capacitively. Coils were connected to the transmit/receive interface box (Stark Contrast, Erlangen, Germany) using PTFE-dielectric and insulated coaxial cable. At 9.4 T, experiments were performed on a vertical-bore NMR spectrometer and micro-imaging system (Avance III, Bruker, Billerica, MA) using a standard commercially-available 5-mm broadband inverse (BBI) probe with a 1-axis gradient.

Each bone was removed from its storage solution, gently blotted dry, placed quickly into a small, air-tight NMR tube with minimum interior air volume to prevent evaporation of free water, and scanned with a saturation-recovery (SR) pulse sequence, shown in Figure 3.2a. Longitudinal magnetization was saturated by a train of 90° pulses followed by spoiler gradients, and the partially-recovered magnetization was measured after each
of 12 saturation recovery times ($T_{SR}$) arrayed logarithmically from 3 ms to 6 s. 32 signal acquisitions were averaged, and the scan time for this sequence was 6 min.

Figure 3.2: $^1$H SR-FID (a), SR-CPMG (b), SIR-FID (c), and $^2$H IR-FID (d) NMR pulse sequences. In (a) and (b), the saturation-recovery time, $T_{SR}$, is arrayed logarithmically from 3 ms to 6 s in 12 steps. In (b), the number of refocusing pulses, $N$, is arrayed logarithmically from 0 to 5000 in 20 steps.
An equation consisting of the sum of two decaying exponentials plus a noise offset term,

\[ f(t) = M_S \exp\left(-\frac{t}{T_{2S}}\right) + M_L \exp\left(-\frac{t}{T_{2L}}\right) + n, \]  

[3.1]

was fitted using non-linear least squares (NLLS) to the magnitude free induction decay (FID) data after the longest \( T_{SR} \). Short-\( T_2^* \) fraction is given by \( M_S/(M_S+M_L) \), and the corresponding short \( T_2^* \) relaxation time by \( T_{2S}^* \). Similarly, long-\( T_2^* \) fraction is given by \( M_L/(M_S+M_L) \) and the long \( T_2^* \) relaxation time by \( T_{2L}^* \).

Two-dimensional bi-component \( T_1\)-\( T_2^* \) fitting, which has been shown to improve accuracy (118), was also performed by fitting a similar equation,

\[ f(T_{SR}, t) = M_S \left(1 - \exp\left(-\frac{T_{SR}}{T_{1S}}\right)\right) \exp\left(-\frac{t}{T_{2S}}\right) + M_L \left(1 - \exp\left(-\frac{T_{SR}}{T_{1L}}\right)\right) \exp\left(-\frac{t}{T_{2L}}\right) + n, \]  

[3.2]

to the set of saturation recovery-prepared magnitude FIDs. Here, the short-\( T_2^* \) fraction is given by \( M_S/(M_S+M_L) \), the short \( T_2^* \) relaxation time by \( T_{2S}^* \), and the short-\( T_2^* \) pool’s \( T_1 \) relaxation time by \( T_{1S} \); fractions and relaxation times are analogous for the long-\( T_2^* \) pool.

All reconstruction and fitting was performed in Matlab (Mathworks, Natick, MA).

Additionally, taking advantage of the lack of specific absorption rate (SAR) limitations and the availability of high-powered hardware at 9.4 T, each bone was scanned using a SR-prepared Carr-Purcell-Meiboom-Gill (SR-CPMG) pulse sequence (119), shown in **Figure 3.2b**. \( T_{SRs} \) were arrayed identically to the SR-FID sequence, and the number of refocusing pulses, \( N \), was arrayed logarithmically from 0 to 5000 in 20 steps, and one signal acquisition was performed. Scan time for this sequence was 29 min. Analysis
was performed similarly to the 1D and 2D bi-exponential fitting of FIDs, with FIDs simply substituted for arrays of echo amplitudes, using the following equations:

\[ f(TE) = M_S \exp\left(\frac{-TE}{T_{2S}}\right) + M_L \exp\left(\frac{-TE}{T_{2L}}\right) + n \]  

[3.3]

and

\[ f(T_{SR}, TE) = M_S \left(1 - \exp\left(\frac{-T_{SR}}{T_{1S}}\right)\right) \exp\left(\frac{-TE}{T_{2S}}\right) + M_L \left(1 - \exp\left(\frac{-T_{SR}}{T_{1L}}\right)\right) \exp\left(\frac{-TE}{T_{2L}}\right) + n \]  

[3.4]

Finally, each bone was scanned with a single adiabatic inversion recovery-prepared FID (SIR-FID) pulse sequence, shown in Figure 3.2c, at all fields. Adiabatic inversion pulses may be designed with both long duration and broad bandwidth, and are thus \( T_2 \)-selective, rather than \( T_2^* \)-selective. Inversion time \( (TI) \) was stepped from 10 to 270 ms at \( TR = 300 \text{ ms} \). All other parameters were identical to SR-FID. The magnitude FID from each \( TI \) was processed by fitting a sum of two exponentials in a similar manner as 1D FID data, but with the relaxation times of the two pools instead set as fixed constants equal to the fitted values from 2D \( T_1-T_2^* \) bi-component analysis. The \( TI \) at which the long-\( T_2^* \) pool fraction was minimized was selected for each bone as the optimal inversion-nulling time for pore water signal.

3.3.3. \( ^2 \text{H} \) NMR Spectroscopy

Labile protons, consisting predominantly of bound and pore water, were exchanged with \( ^2 \text{H} \) by immersion in deuterium oxide (D\(_2\)O). Bones were blotted dry and placed in a 20-fold volume excess of 99.9\% purity D\(_2\)O-saline for six days, and were removed and transferred to a container with the same volume of fresh D\(_2\)O-saline on days two and four to ensure full exchange.
Following full deuterium exchange, specimens were scanned using $^2$H inversion-recovery (IR) (71). A $^2$H spectrum of bone at 9.4 T consists of a narrow central peak with $T_1 = 200\pm40$ ms flanked by a doublet with $T_1 = 11\pm2$ ms. The narrow single peak corresponds to bone water residing in the pore system of bone and whose motion is unimpeded by interaction with bone collagen. The doublet peak with splitting of 4.8 kHz results from quadrupole interaction of the deuteron with the electric field gradient along the O-$^2$H bond in bone water that is hydrogen-bonded to matrix collagen (120-122).

The pulse sequence and relevant parameters are shown in Figure 3.2d. $T_I$ was stepped in 10 ms increments in order to capture the null point of the narrow pore water peak, the post-acquisition delay was 1 s to ensure return to equilibrium longitudinal magnetization, and 48 signal acquisitions were averaged. Scan time for this sequence was 21 minutes. A fully relaxed spectrum was also acquired. The integral of the fully-relaxed spectrum represents total bone water, the integral of the spectrum with the narrow pore water signal nulled represents bound water only, and the difference between these two represents pore water only. Bound and pore water fractions were calculated by dividing the integral of the pore water-nulled spectrum or the difference spectrum, respectively, by the integral of the fully relaxed spectrum.

3.3.4. µCT Imaging

Bone specimens were scanned on a Scanco µCT35 scanner (Scanco, Brüttisellen, Switzerland) at 18.5-µm isotropic resolution. Bone exteriors were masked by 3D active snakes using the ITK-SNAP software package (123), and pores were segmented from this masked 3D image by thresholding. Porosity was calculated as pore (segmented) volume divided by total (masked) volume.
3.3.5. Gravimetry

Fully hydrated bone specimens were removed from liquid, gently blotted dry, and weighed to establish their initial mass. The bones were then placed in tared crucibles and dried at 105° C for 110 hr to remove all bound and pore bone water. Completion of drying was verified by no change in mass over a 24-hour period. Bones were again weighed and the dry mass was recorded. The bones were then incinerated at 600° C for 30 hr to burn off all organic matrix, and the residual ash was weighed again. Total water mass was then obtained as the difference between initial and dry mass, total matrix mass was the difference between dry and ash mass, and total mineral mass was equal to the ash mass. These masses, divided by total bone volume obtained from the µCT bone exterior mask, yield total water, matrix, and mineral densities.

3.4. Results

3.4.1. ¹H NMR Spectroscopy

The measured NMR pool fractions by bi-exponential fitting, along with validation measurements by µCT, ²H IR NMR, and gravimetry, are shown in Table 3.1. The short-$T_2^*$ fraction at 1.5 T was 69.6±12.7% (37.7-82.6%) (mean ± standard deviation and range (min-max)). At 3 T, the mean short-$T_2^*$ fraction was similar to 1.5 T at 68.1%, but the standard deviation and range were larger, at 21.9% and 17.0-98.6%, respectively. Short-$T_2^*$ fractions at 7 T and 9.4 T were 82.3±14.0% (49.8-99.9%) and 55.1±28.7% (18.6-98.3%).
Table 3.1: Bone properties measured by µCT, 2H IR, gravimetry, SR-CPMG NMR at 9.4 T, SR-FID NMR at four field strengths, and SIR-FID NMR at three field strengths. Bone labels are composed of the two-digit age and one-letter gender of the donor.

Short-$T_2^*$ pool relaxation times were 401±119 (301-762) µs, 389±116 (249-768) µs, 368±76 (302-613) µs, and 302±150 (125-615) µs at 1.5 T, 3 T, 7 T, and 9.4 T, respectively. Long-$T_2^*$ relaxation times decreased more dramatically at higher field strength: 4110±1230 (1840-7170) µs, 4350±8570 (980-35300) µs, 1300±420 (380-1860) µs, and 886±525 (410-2160) µs at 1.5 T, 3 T, 7 T, and 9.4 T, respectively. Short- and long-$T_2^*$ relaxation times and short-$T_2^*$ pool sizes are plotted in **Figure 3.3**. If one outlier, 35300 µs, is excluded from this mean and standard deviation at 3 T, the long-$T_2^*$ relaxation time becomes 2150±540 (980-2940) µs. This outlier was a result of poor fitting due to oscillations in the magnitude FID; this phenomenon, which appeared in several specimens at multiple fields, will be discussed in detail in the discussion section.
Inclusion of the $T_1$ dimension reduced the standard deviations of 2D short-$T_2^*$ fractions relative to 1D, particularly at 3 T, but average pool fractions and $T_2^*$ relaxation times were unchanged. The $T_1$ relaxation times of the short-$T_2^*$ fractions were 82.6±10.4 (62.2-97.3) ms, 145±25 (103-186) ms, 400±68 (206-496) ms, and 358±240 (93-565) ms at 1.5 T, 3 T, 7 T, and 9.4 T, respectively, and $T_1$s of the long-$T_2^*$ fractions were 651±273 (379-1210) ms, 880±281 (465-1470) ms, 1790±470 (898-2470) ms, and 1300±370 (751-1940) ms.

Short-$T_2$ fractions by bi-component $T_2$ fitting of CPMG echo amplitudes at 9.4 T were generally larger than short-$T_2^*$ fractions by FID fitting: the 1D short-$T_2$ fraction was 78.1±8.5% (59.0-87.4%), and the 2D short-$T_2$ fraction was 77.0±9.3% (55.4-86.6%). Relaxation times for short- and long-$T_2$ fractions were 540±150 (430-960) µs and 77±53 (22-220) ms, respectively, for 1D fitting. Short-$T_2$ relaxation time for 2D fitting was unchanged at 540±150 (430-980) µs, while long-$T_2$ relaxation time was 55±38 (17-161) ms. The corresponding $T_1$ relaxation times of short- and long-$T_2$ fractions obtained by 2D fitting were 480±80 (320-560) ms and 1210±300 (880-1910) ms, respectively.
Average signal to noise ratios (SNRs), defined as the magnitude of the first data point of the FID signal divided by the standard deviation of the magnitude of the final 25 data points, were 4130, 3040, 15600, and 34300 at 1.5 T, 3 T, 7 T, and 9.4 T, respectively. The noise terms, $n$, in Equations 3.1 and 3.2 for 1D and 2D bi-component $T_2^*$ fitting were on the order of 0.1% of total signal ($M_S+M_L$) or less at all field strengths, and were therefore inconsequential. In Equations 3.3 and 3.4 for 1D and 2D bi-component $T_2$ fitting of CPMG data, the noise terms, $n$, were 2-3% of total signal. This larger value of $n$ is a result of the overly simple assumption of two discrete pools; the presence of a small fraction of signal that has $T_2$ much longer than the fitted long-$T_2$ value appears to the bi-component fitting method as a non-zero noise level.

Three example 2D relaxation spectra of a bone specimen taken from a 37 year old male donor are shown in Figure 3.4: $T_2^*-T_2$ and $T_1-T_2$ spectra at 9.4 T, and a $T_1-T_2^*$ spectrum at 3 T. Relaxation times given in the labels next to each pool represent the centroid of each pool. $T_2$ values were distributed across three orders of magnitude (horizontal axis in Figure 3.4a and 3.4b), while $T_2^*$ values spanned less than two (vertical axis in Figure 3.4a and horizontal axis in Figure 3.4c).
Figure 3.4: 2D $T_2^*-T_2$ and $T_1-T_2^*$ relaxation spectra at 9.4 T, and 2D $T_1-T_2^*$ relaxation spectrum at 3 T, generated using the MERA software package (124). Spectra are from a bone specimen taken from a 37 year old male donor. The $T_2^*-T_2$ spectrum is generated from CPMG data, the $T_1-T_2$ spectrum from SR-CPMG data, and the $T_1-T_2^*$ spectrum from SR-FID data.
Optimal inversion times were 91.3±20.3 (60-130) ms at 1.5 T, 81.3±23.3 (50-130) ms at 3 T, and 174±30 (130-240) ms at 7 T. These TIs resulted in short-\(T_2^*\) signal fractions of 99.3±0.6% (97.7-100%) at 1.5 T, 99.2±0.6% (98.1-100%) at 3 T, and 99.7±0.2% (99.3-100%) at 7 T.

3.4.2. \(^2\)H NMR Spectroscopy

The deuterium-exchanged \(^2\)H IR bound water fraction was 62.6±9.6% (48.7-77.9%). Mean \(T_1\) of the bound water pool was 11.2±1.7 (9.8-15.9) ms, and mean \(T_1\) of the pore water pool was 197±42 (129-282) ms. The mean inversion-recovery time to achieve nulling of the central pore water peak was 142±31 (92-206) ms. An example plot in Figure 3.5 shows the fully relaxed, pore water-nulled, and subtracted pore water-only spectra taken from the specimen from a 27 year old female donor.

![Figure 3.5: \(^2\)H spectra showing the bound and pore D\(_2\)O components (inset is magnified vertically and truncated). Pore water (narrow central peak in green) is calculated by subtracting the bound water spectrum obtained by inversion-](image-url)
recovery nulling of pore water (the quadrupolar coupled split peaks shown in red) from the fully relaxed spectrum (shown in blue). This spectrum is taken from a specimen from a 27 year old female donor with the osteonal axis orthogonal to $B_0$. A splitting of 4.8 kHz is observed, consistent with the orientation-dependent quadrupolar splitting observed by Ong et al. (71).

### 3.4.3. µCT and Gravimetry

Porosity measured from µCT image segmentation was 8.96±8.61% (3.06-33.53%). Gravimetric mineral density was 1118±130 (751-1219) mg/cc and organic matrix density was 503.7±24.3 (437.0-527.5) mg/cc, consistent with previous observations in porcine femoral cortical bone by Cao et al. (63,79). Total water density, including both bound and pore water, was 326.2±48.4 (281.4-435.6) mg/cc. A matrix of $R^2$ values for inter-parameter correlations is given in Table 3.2. Porosity and matrix density were highly negatively correlated ($R^2 = 0.91$), supporting the notion that any increase in porosity occurs at the expense of a loss of matrix volume.
Table 3.2: Inter-parameter correlations ($R^2$) of measured bone properties. All correlations are statistically significant ($p < 0.05$) unless italicized.

1D short-$T_2^*$ pool fraction was moderately negatively correlated with porosity and positively with matrix density at 1.5 T ($R^2 = 0.70$ and 0.63, respectively), but the strengths of these associations diminished rapidly as field strength increased. In fact, at 9.4 T, no statistically significant correlation was observed. Scatter plots displaying 1D
short-$T_2^*$ pool fractions versus matrix density are shown in Figure 3.6. In general, addition of the $T_1$ dimension improved the strengths of these correlations (except at 7 T), but this improvement still did not raise the correlations at 9.4 T to the level of statistical significance.

Fitted pool fractions by bi-component $T_2$ fitting of CPMG echo amplitudes at 9.4 T (Figure 3.7a) were generally better correlated with porosity and matrix density than were those derived from $T_2^*$ fitting of FIDs (Figure 3.7b) and $^2$H IR. Coefficients of determination ($R^2$) of short-$T_2$ fraction by 1D fitting of CPMG echo amplitudes to porosity
and matrix density were 0.87 and 0.88, respectively; with the addition of the $T_1$ dimension, these increased slightly to 0.90 and 0.89, respectively.

Figure 3.7: Scatter plots displaying the correlations of 1D bi-component (a) short-$T_2^*$ $^1$H signal fraction by fitting of FID data and (b) short-$T_2^*$ $^1$H signal fraction by fitting of CPMG data at 9.4 T. Short-$T_2$ fraction is very strongly associated with organic matrix density, while short-$T_2^*$ has no association with matrix density.

Bound water fraction by $^2$H IR, however, was only moderately correlated with porosity ($R^2 = 0.50$) and matrix density ($R^2 = 0.46$), but correlations of $^2$H IR bound water fraction with short-$T_2^*$ fraction by 1D FID and 2D SR-FID fitting showed the same trend of reduced association as field strength increases.

### 3.5. Discussion

Bi-exponential fitting is applicable as long as the time constants representative of the two pools are sufficiently separated from one another. Due to the ill-posed nature of the inverse Laplace transform, significant errors may arise in the fitted pool fractions and time constants once the two time constants become similar. Because $T_2^*$ of pore water is substantially shortened by dephasing due to internal magnetic field gradients arising from the large susceptibility difference between water and bone tissue ($\Delta \chi_v \sim 2.5$ ppm SI) (116), resulting in decreased separation of bound and pore water relaxation times,
$T_2^*$ bi-component fitting of FIDs is inferior to $T_2$ fitting of CPMG echo amplitudes (69). As field strength increases, these internal magnetic field gradients increase proportionally, and pore water $T_2^*$ further decreases, more severely impacting the ability of bi-component fitting of FIDs to distinguish bound and pore water. This is reflected in the reduced strength of the correlations of short-$T_2^*$ fractions versus porosity and matrix density as field strength increases. While the results are promising at 1.5 T, less than half the variance in matrix density is explained by 1D short-$T_2^*$ fraction at 3 T. The phase dispersion resulting from the static internal field inhomogeneity is refocused in the CPMG sequence, yielding greater separation in $T_2$ and improved fitting performance.

The measured short-$T_2^*$ component fractions of 69.6% and 68.1% at 1.5 T and 3 T, respectively, are nearly identical to the 68.5% and 69% measured by Li et al. (125) in bovine bone, but are slightly lower than the 74.4% and 75.9% measured in human bone at these same field strengths. The ages and pore volume fractions of the human bones studied by Li et al., however, are unknown. While Li et al. observed decreases in short $T_2^*$ relaxation time from 450 µs to 320 µs, and in long $T_2^*$ relaxation time from 7.17 ms to 3.02 ms, at 1.5 T compared to 3 T, the relaxation times presented here are relatively consistent at these two field strengths. Most importantly, however, no significant differences between 1.5 T and 3 T short-$T_2^*$ fraction are observed in the present results or in either of Li’s measurements.

At 3 T and higher fields, oscillations appear in the magnitude FIDs of several bone specimens, presumably resulting from non-water off-resonant spins. Similar oscillations have previously been observed in bone (81) and tendon (78). In many cases, the amplitude of these oscillations is sufficient to disrupt the monotonic decrease of the FID,
a condition that is not able to be fitted by a sum of monotonically decaying exponentials. An example of such an oscillatory FID at 7 T of a bone specimen from a 53-year-old female donor, with attempted mono-exponential and bi-exponential fits, is shown in Figure 3.8a. These oscillations appear most regularly in bone from old female donors, who have greater porosity. Although care was taken in the preparation of these samples to remove marrow fat, some may remain trapped in the enlarged pore spaces of osteoporotic cortical bone. A small amount of lipid also exists within cortical bone matrix at the cement line surrounding each osteon (126).

![Figure 3.8](image)

Figure 3.8: Log-magnitude FID at 7 T of a cortical bone specimen from a 53 y/o female donor (a). Note the irregular oscillation of the signal, which causes failure of bi-exponential fitting (red line, \( R^2 = 0.999592 \)): 97.4% short-\( T_2^* \) signal fraction, versus 86.6% by 2D \( T_1-T_2 \) bi-exponential fitting at 9.4 T. Fat at 7 T is 1040 Hz off-resonance. A FID from a 53 y/o male donor (b) not exhibiting these oscillations is also shown for comparison (\( R^2 = 0.999901 \)). Similar plots of fitted CPMG echo amplitudes for the same 53 y/o female (c) and male (d) donors are also shown.
Note also that the oscillation in Figure 3.8a does not appear to have a fixed period; rather, its period varies. If this signal were purely from the methylene resonance of fatty acid triglycerides, a frequency of 1040 Hz would be expected at 7 T. After Fourier transform, only the main water peak is visible. Fourier transformation of the fitting residual also yields no insight into the source of this signal; the spectrum consists only of residual signal distributed symmetrically around the main water peak. It is possible that this oscillation arises from fat that exists in a spatially heterogeneous magnetic field due to susceptibility effects, from other non-fat sources within the same environment, or some combination of these effects (all of which would be refocused by the 180° pulses in CPMG), but further experimentation would be necessary to support any of these speculative hypotheses.

If bone specimens exhibiting these problematic oscillations are retrospectively excluded from correlation analyses (four females ages 53-97 years, one male age 83 years), the $R^2$ values describing the association of short-$T_2^*$ fraction with μCT porosity and matrix density in the remaining 10 specimens are substantially improved. At 1.5 T, 3 T, and 7 T, $R^2 = 0.87$, 0.93, and 0.86, respectively, for correlations with μCT porosity, and 0.83, 0.78, and 0.61 for correlations with matrix density. At 9.4 T, none of the samples yielded data judged suitable for fitting. It must be noted that the specimens removed from this analysis were predominantly from the population to which an MRI study of bone composition would be of greatest benefit, and it is not possible to know in advance of such a study whether the resulting data will be usable.

Short-$T_2$ fraction by 1D bi-component fitting of CPMG echo amplitudes outperforms bi-component fitting of FIDs at all field strengths. This is likely due to the much greater
separation of bound and pore water $T_2$ relaxation times. Whereas the two pools are separated by only one order of magnitude in $T_2^*$, the difference in $T_2$ is two orders of magnitude. This $T_2$-based method, however, is not applicable to clinical scanners due to SAR limitations and the requirement of extremely short, high-amplitude 180° refocusing pulses. Nevertheless, the method is suitable for independent determination of bound and pore water fractions.

Addition of a second dimension generally improves the stability and accuracy of bi-component fitting (118). In the present case, addition of $T_1$ to $T_2^*$ by incorporation of saturation-recovery into the sequence improves the strengths of correlations between fitted short-$T_2^*$ fraction and validation methods, except at 7 T. Though it would be time-prohibitive to incorporate saturation-recovery into a spatially-resolved in vivo bi-component $T_2^*$ scanning protocol, this result suggests that methods which take advantage of differences in both $T_1$ and $T_2$ (or $T_2^*$) may be superior to those relying solely on $T_2^*$. An example of such an approach is single adiabatic inversion recovery (80,81,83,84,127), which selectively inverts long-$T_2$ pore water while saturating short-$T_2$ bound water. As the longitudinal magnetization of pore water (which was also found to have longer $T_1$ than bound water) passes through its null point, a solid-state imaging readout is performed to selectively image bound water.

The optimal inversion times calculated in this study from SIR-FID experiments are consistent with previous work at 3 T by Li et al. (128), but calculation of these results utilizes the same 1D bi-component $T_2^*$ fitting method that is the main subject of this work. These $TI$ results, therefore, should be interpreted with the same caution as 1D bi-component $T_2^*$ fitting for calculation of bound and pore water fractions in general.
Specifically, the value at 1.5 T is well supported by strong correlations between 1D short-\textit{T}_2^* fraction and both \textmu CT porosity and gravimetric density, while the value at 3 T is somewhat less well supported. Also consistent with Li et al., the long-\textit{T}_2^* fraction is very well-suppressed (to less than ~5\%) in a range of approximately ±20 ms surrounding the optimal value for each bone, suggesting that the effectiveness of adiabatic inversion nulling of long-\textit{T}_2^* signal is not significantly decreased by even moderate deviations from the optimal \textit{TI}.

In addition, note that the optimal inversion time of 174 ms at 7 T is greater than one half of the \textit{TR} = 300 ms used in this experiment. The spin dynamics, as predicted by the Bloch equations, preclude the possibility of a signal pool being nulled by inversion in steady-state at a \textit{TI} greater than half the \textit{TR}. Primarily on the basis of this observation, and secondarily due to the weak correlations between 7 T 1D short-\textit{T}_2^* fraction and the two confirmatory measurements, we have no confidence in this calculated optimal \textit{TI} at 7 T.

\textsuperscript{2}H IR was found to be less effective in separating the two water (i.e. deuterium oxide) pools than bi-component \textit{T}_2 fitting of CPMG data. Bound water fractions obtained by this \textsuperscript{2}H IR method were considerably less strongly correlated with \textmu CT porosity and matrix density than was short-\textit{T}_2 fraction by fitting of CPMG amplitudes. Correlations of short-\textit{T}_2^* fraction at 1.5 T with these same validation methods were also stronger than those of \textsuperscript{2}H IR bound water fraction. This unexpectedly inferior performance may be due to a distribution of \textit{T}_1 values within pore D\textsubscript{2}O, thereby preventing complete nulling of the central pore water peak. These results suggest that bi-component fitting of CPMG data
is a more reliable NMR-based method for quantification of bound and pore water fractions than $^2$H IR.

It is important to keep in mind that bi-component analysis, whether based on $T_2^*$ or $T_2$, and whether performed in one or two dimensions, involves one major, and incorrect, assumption: the existence of two pools with discrete relaxation times. $T_2$ values are instead distributed continuously over several orders of magnitude. While deviations from this assumption may not have severe consequences at low field, where relaxation times of bound and pore water are well-separated and, thus, well-approximated by this two-pool model, the results are more severely impacted as the relaxation times converge. Non-negative least squares methods, such as the MERA software package (124), do not require an assumption of the number of pools, but demand careful regularization to generate a valid relaxation spectrum, which may have any number of peaks. As bound and pore water relaxation times converge, this method often fails to distinguish multiple pools, instead returning a single peak containing 100% of $^1$H signal.

The $T_1$ values obtained for the long-$T_2^*$ fraction by 2D $T_1$-$T_2^*$ fitting at 7 T and 9.4 T (1790 ms and 1300 ms, respectively) are greater than 1200 ms and, therefore, greater than $T_{SR}/5$. If these values are accurate, this may cause the magnetization after $T_{SR} = 6$ s to be slightly below the equilibrium magnetization. As a consequence, long-$T_2^*$ longitudinal magnetization at 7 T measured by 1D $T_2^*$ fitting may be attenuated by 3.5%, and the resulting 1D short-$T_2^*$ fraction may be higher than the actual fraction by approximately 1%. It is unlikely that this effect is responsible for the large (>10%) difference in 1D $T_2^*$ pool sizes at 7 T compared to other field strengths. Also, the more
reliable $T_1-T_2$ results at 9.4 T show a long-$T_2$ fraction $T_1$ value of 1210 ms, which is sufficient for assumption of full longitudinal relaxation ($T_{SR} = 5T_1$).

This study has several limitations. First, we focus only on cortical bone, where in vivo examinations will likely target both cortical and trabecular bone. Though we expect our results to be generalizable (the important difference between trabecular and cortical bone being the predominance of pore versus bound water, respectively), this may benefit from further investigation into the effects of marrow fat. Also, we examine bi-component $T_2$ fitting of CPMG echo amplitudes at only one field strength, 9.4 T. Although this is a widely available field strength for spectroscopic hardware, it would be instructive to examine whether short-$T_2$ fraction is also sensitive to field strength. Finally, the source of the oscillations in magnitude FIDs remains unclear. Further investigation, possibly by chemical removal of fat or a complementary method to quantify lipid and protein content, would likely yield additional insight into this phenomenon.

3.6. Conclusion

$T_2^*$ bi-component fitting for quantification of bound and pore water fractions performs moderately well at 1.5 T, but becomes less reliable as field strength increases. It should therefore be used with caution, and other methods for distinguishing between bone water fractions, namely those based on adiabatic inversion-recovery or dual-band saturation should be considered for use in in vivo examinations. For validation of bound and pore water fractions, bi-component fitting of CPMG echo amplitudes is superior to $^2\text{H}$ IR, and is on par with $\mu$CT as a method of investigating bone porosity within a single specimen.
CHAPTER 4: SINGLE ADIABATIC INVERSION RECOVERY ZERO ECHO TIME MRI IS A SURROGATE MEASURE OF BONE MATRIX DENSITY

4.1. Abstract

Magnetic resonance has the potential to image and quantify two pools of water within bone: free water within the Haversian pore system (transverse relaxation time, $T_2 > 1$ ms), and water hydrogen-bonded to matrix collagen ($T_2 \sim 300-400 \mu$s). While total bone water concentration quantified by MRI has been shown to scale with porosity, greater insight into bone matrix density and porosity may be gained by relaxation-based separation of bound and pore water fractions. The objective of this study was to evaluate a recently developed surrogate measurement for matrix density, single adiabatic inversion recovery (SIR) zero echo-time (ZTE) MRI, in human bone.

Specimens of tibial cortical bone from 15 donors (27-97 y/o, eight female and seven male) were examined at 9.4 T field strength using three methods: (1) $^1$H ZTE MRI, to capture total $^1$H signal; (2) $^1$H SIR-ZTE MRI, to selectively image matrix-associated $^1$H signal; and (3) $^1$H ZTE MRI after deuterium exchange, to image only non-labile $^1$H signal. Total water, bone matrix, and bone mineral densities were also quantified gravimetrically, and porosity was measured by µCT.

ZTE $^1$H concentration was $32.7\pm3.2$ M (range: 28.5-40.3 M), and was correlated positively with porosity ($R^2 = 0.80$) and negatively with matrix and mineral densities ($R^2 = 0.90$ and 0.82, respectively). SIR-ZTE $^1$H concentration was $32.9\pm3.9$ M (range: 24.4-
39.8 M), and its correlations were opposite in direction to those of total water: negative with porosity ($R^2 = 0.73$) and positive with matrix density ($R^2 = 0.74$) and mineral density ($R^2 = 0.72$). Porosity was strongly correlated with gravimetric matrix density ($R^2 = 0.91$, negative) and total water density ($R^2 = 0.92$, positive). The strong correlations of SIR-ZTE $^1$H concentration with ground-truth measurements suggest that this quantitative solid-state MRI method provides a nondestructive surrogate measure of bone matrix density.

4.2. Introduction

Bone water exists in two major pools that are visible to solid-state MRI: water diffusing freely within the Haversian pore system (70), and water that is hydrogen-bonded to matrix collagen (69,71,72). These two pools differ significantly in their $^1$H nuclear magnetic resonance (NMR) relaxation properties, and are diagrammed in a schematic $T_2$ spectrum shown in Figure 4.1.
Figure 4.1: Schematic $T_2$ relaxation spectrum diagramming the three major $^1$H NMR signal pools in bone. Pore water has $T_2 > 1$ ms and is broadly distributed, while bound water has $T_2 \sim 300-500$ µs. Collagen signal, at $T_2 \sim 40-60$ µs, is below the detection limit at clinical field strengths, but becomes visible using micro-imaging and spectroscopic hardware. As porosity increases, as shown in the inset µCT images of bone specimens from 27 y/o and 83 y/o female donors (dense and porous bone, respectively) collagen and bound water content decrease while pore water content increases and shifts to longer $T_2$ values due to the smaller surface-to-volume ratio of enlarged pores.

MRI of matrix-bound water has been studied by several groups in recent years as a possible surrogate for collagen bone matrix (60,63,74,75,77-81,84,129). There are two general approaches to long-$T_2$ suppression taken in prior work: bi-component effective transverse relaxation time ($T_2^*$) fitting of a free-induction decay (FID) or a series of images obtained at multiple echo times ($TE$) (75,77,78), and magnetization preparation using either a long, low-amplitude $T_2^*$-selective radiofrequency (RF) saturation pulse (60,63,79) or a $T_2$-selective adiabatic inversion pulse followed by an inversion-recovery delay time ($TI$) (74,80,81,83,84,129).
The single adiabatic inversion-recovery (SIR) method has the potential to outperform other methods of long-$T_2^*$ suppression. While duration and bandwidth are inversely proportional in non-adiabatic pulses, they are less strictly linked in adiabatic RF pulses. Such a pulse can therefore simultaneously possess a long duration and broad bandwidth. The long duration allows it to saturate short-$T_2$ signal while inverting long-$T_2$ signal, and the broad bandwidth encompasses the broad frequency distribution of pore water within the complex internal magnetic field environment of bone pores due to the large difference in volume magnetic susceptibility ($\chi_v$) between water ($\chi_v = -8.9$ ppm) and bone ($\chi_v = -11.3$ ppm (116)).

The objective of the present study was to measure zero echo time (ZTE), SIR-ZTE, and non-exchangeable ZTE $^1$H concentrations in human cortical bone, and validate the proportionality of SIR-ZTE $^1$H concentration to bone matrix density and its inverse correlation to porosity. The results of this work will establish the surrogacy of SIR-ZTE $^1$H concentration for matrix density. This would lay a foundation upon which to build a two-part non-invasive in vivo examination of bone matrix and mineral densities, and, therefore, true bone tissue mineralization density (also referred to as ‘degree of mineralization of bone’ (130)).

4.3. Materials and Methods

4.3.1. Specimens and Scanners

The tissue examined consisted of cortical bone specimens taken from the tibial mid-shaft of seven male and eight female human donors, aged 27-97 years (National Disease Research Interchange, NDRI). This set ranges from young, dense bone to severely
porous bone due to age-related bone loss. Donors with bone-demineralizing disorders were excluded. A 4-mm slice was sectioned from each thawed tibia with a rotating blade at the region of maximum cortical bone thickness, 38% of the length of the tibia from the medial malleolus to the medial condyle. Then, a rectangular beam with its long axis perpendicular to the osteonal axis was cut from each slice and trimmed to fit inside a 5-mm NMR tube. Specimens ranged from 15 to 35 mm in length. The direction of the osteonal axis of the bone was indicated on the end of each beam by cutting a notch parallel to the bone’s osteonal axis, and specimens were stored individually in phosphate-buffered saline.

All NMR and MRI scanning was performed in a 9.4 T vertical-bone NMR spectrometer and micro-imaging scanner (Avance III, Bruker, Billerica, MA). For spectroscopy, a broad-band inverse (BBI) probe with a one-axis z-gradient was used, and for imaging, a 20-mm quadrature birdcage probe in a three-axis microimaging gradient set was used. Bones were imaged in the presence of an intensity reference sample consisting of a 20-mm column of 10 mM MnCl₂ in 90% D₂O/10% H₂O in a 5-mm NMR tube. This sample had a ¹H concentration of 11.1 M, T₂ = 530 µs, and T₁ = 12.7 ms.

4.3.2. NMR Spectroscopy

Prior to imaging, all bones were scanned using a saturation recovery (SR)-prepared Carr-Purcell-Meiboom-Gill (CPMG) pulse sequence. Saturation recovery times (Tᵣ) were arrayed logarithmically in 12 steps from 3 ms to 6 s, and the number of refocusing pulses, N, was arrayed logarithmically from 0 to 5000 in 20 steps, resulting in TEs ranging from 60 µs to 1 s. One signal acquisition was performed, and scan time for this
sequence was 29 minutes. All other relevant pulse sequence parameters are given within the pulse sequence diagram in Figure 4.2.

![Pulse sequence diagram](image)

Figure 4.2: SR-CPMG pulse sequence. Saturation-recovery times ($T_{SR}$) were arrayed logarithmically in 12 steps from 3 ms to 6 s, the number of refocusing pulses, $N$, was arrayed logarithmically from 0 to 5000 in 20 steps, and one signal acquisition was performed.

Two-dimensional bi-component $T_1$-$T_2$ fitting was performed by fitting a sum of two exponential functions in two dimensions,

$$f(T_{SR}, TE) = M_S \left(1 - \exp \left(\frac{-T_{SR}}{T_{1S}}\right)\right) \exp \left(\frac{-TE}{T_{2S}}\right) + M_L \left(1 - \exp \left(\frac{-T_{SR}}{T_{1L}}\right)\right) \exp \left(\frac{-TE}{T_{2L}}\right) + n,$$

[4.1]

to the array of SR-CPMG echo amplitudes. Here, the magnetizations of the short- and long-$T_2$ components are given by $M_S$ and $M_L$, respectively. These terms are converted to short- and long-$T_2$ fractions by dividing by their sum, i.e. $M_S/(M_S+M_L)$ and $M_L/(M_S+M_L)$. Longitudinal ($T_1$) relaxation times are given by $T_{1S}$ and $T_{1L}$, $T_2$ relaxation times by $T_{2S}$ and $T_{2L}$, and the noise level by $n$. This fitting process yields the sizes of each of the two pools and their characteristic $T_1$ and $T_2$ relaxation times.

$T_2^*$ values for the pools were also obtained by fitting a similar equation,
to the two-dimensional array of FIDs beginning at the center of each CPMG echo. Here, $t$ is the time within the FID after each echo. These data were taken from the SR-CPMG data set after the longest $T_{SR}$ of 6 seconds, which satisfies the condition of full longitudinal relaxation. This process of fitting Equation 4.2 yields two pools characterized by $T_2$ and $T_2^*$ relaxation times, rather than $T_1$ and $T_2$. Fitting was performed in two dimensions, rather than one, for improved accuracy and stability (118). All fitting was performed in Matlab (Mathworks, Natick, MA). Example data from bone from a 53-year-old male donor, along with bi-component fits, are shown in Figure 4.3.

$$f(TE, t) = M_S \exp \left( - \frac{TE}{T_{2S}} \right) \exp \left( - \frac{t}{T_{2S}} \right) + M_L \exp \left( - \frac{TE}{T_{2L}} \right) \exp \left( - \frac{t}{T_{2L}} \right) + n,$$ [4.2]
Figure 4.3: NMR data (points) from a bone specimen from a 53-year-old male with bi-component fits (curves). Panel (a) shows a $T_1$ fit of saturation-recovery data, (b) shows a $T_2$ fit of CPMG echo amplitudes, and (c) shows a $T_2^*$ fit of a FID. Although only one-dimensional data are shown, fits were performed using the two-dimensional methods given in the methods section (a,b: $T_1$-$T_2$; c: $T_2$-$T_2^*$).

4.3.3. Zero Echo-Time Imaging

Imaging and reconstruction were performed using the standard Bruker ZTE pulse sequence (Figure 4.4a), and a modified form of this sequence incorporating a single adiabatic inversion preparation and delay time (SIR-ZTE, Figure 4.4b). ZTE was chosen due to its superior SNR performance to UTE in samples with extremely short $T_2^*$ (131). Gradient amplitude was limited to 73.4 mT/m by two simultaneous requirements
of the reconstruction method (59): (1) that the field of view (FOV) fully enclose all sources of signal, including the plastic support structure of the NMR probe, and (2) that the readout bandwidth be low enough (i.e., that the dwell time be long enough) so as not to lose more than ~2 readout points during the hardware-dependent 6.4 μs transmit/receive switching dead time.

Figure 4.4: ZTE (a) and SIR-ZTE (b) imaging pulse sequences. ZTE parameters: 51896 projections, TR = 2 ms, 1 min 43 sec scan time. SIR-ZTE parameters: 6588 projections, TR = 200 ms, 21 min 58 sec scan time. FOV = 64 mm isotropic, resolution = 500 μm isotropic, and 1 signal acquisition for both.

First, to image total bone \(^1^H\) signal, each specimen was scanned with the reference sample using ZTE without adiabatic inversion recovery. Then, each specimen was immediately scanned again using SIR-ZTE. The adiabatic inversion pulse, with
bandwidth of 5 kHz and duration of 5 ms, selectively saturates the longitudinal magnetization, \( M_z \), of the short-\( T_2 \) protons \( (M_z = 0) \) while inverting long-\( T_2 \) signal \( (M_z < 0) \) \( (81-83,132,133) \). The \( T_2 \) response of \( M_z \) to this pulse was calculated by Bloch equation simulation. Following an appropriately chosen inversion-recovery time, the long-\( T_2 \) magnetization will be nulled as it passes through zero, and the short-\( T_2 \) magnetization will have recovered by longitudinal relaxation to \( M_z > 0 \). A ZTE imaging module applied at this time will thus selectively image short-\( T_2 \) signal. SIR-ZTE was performed with a repetition time \( (TR) \) of 200 ms and inversion time \( (TI) \) of 50 ms, and in both of these sequences, \( FOV \) was 64 mm and resolution was 500 \( \mu m \) (both isotropic). RF pulse duration was increased in order to allow for a larger excitation flip angle in SIR-ZTE. This modification also necessitated decreases in gradient magnitude and readout bandwidth.

4.3.4. Deuterium Exchange

Finally, all labile protons in the bone specimens, which consist predominantly of bound and pore water, were rendered invisible to \(^1H\) NMR by exchange with heavy water (\(^2H_2O\)). Bones were gently blotted dry and individually placed in a 20-fold volume excess of 99.9% \(^2H_2O\)-saline. At two and four days of immersion, the specimens were removed, blotted dry, and placed into fresh volumes of \( D_2O \)-saline in order to ensure full exchange.

Following exchange, the relaxation times of the remaining \(^1H\) signal in the bone specimens were determined by mono-component exponential fitting of SR-FID and CPMG data sets: \( T_1 = 1650 \) ms, \( T_2 = 87 \) \( \mu s \), and \( T_2^* = 36 \) \( \mu s \). Several scan parameters were changed to optimize the subsequent ZTE scans (Figure 4.4a) for these altered
relaxation times. The dwell time for this sequence was decreased to 3.2 μs, excitation flip angle to 2.8°, and pulse duration to 1 μs. FOV was changed to 32x32x80 mm and resolution to 250x250x625 μm (with the FOV and voxels’ long axes parallel to the long axis of the bone specimen), and 16 signal averages, resulting in a total scan time of 28 minutes. Gradient strength varied from 229 mT/m in the axial plane to 92 mT/m along the long axis of the specimen. The resulting images include only signal from non-exchangeable protons; bound and pore water no longer contribute to image intensity.

Bones were also scanned using ²H inversion-recovery (IR) spectroscopy (71). A ²H spectrum of bone at 9.4 T consists of a narrow central peak with long $T_1 \sim 200$ ms pertaining to pore water, flanked by a doublet with short $T_1 \sim 10$ ms and 4.3 kHz splitting, consistent with prior observations by Ong et al. (71). This splitting results from un-averaged quadrupolar interactions between anisotropically-restricted ²H nuclei of ²H₂O molecules bonded to collagen (121,134). Two spectra were acquired: one equilibrium spectrum containing both the central pore water peak and the bound water doublet, and one spectrum in which the pore water peak was nulled by inversion-recovery (individually for each specimen), leaving only the bound water doublet. Bound water fraction was calculated as the ratio of the integral of the pore water-nulled spectrum to that of the equilibrium spectrum.

### 4.3.5. Density Quantification

To convert raw image intensity to ¹H concentration, each image was corrected for longitudinal and transverse relaxation, including transverse relaxation during RF pulses. The steady-state signal acquired in the ZTE sequence (Figure 4.4a) is given in Equation 4.3:
where $\rho$ is $^1$H concentration. Because this pulse sequence has an echo time of zero, $\exp(-TE/T_2^*) = 1$. The $f_{xy}$ and $f_z$ terms represent the response of the transverse and longitudinal magnetization, respectively, to rectangular RF pulses in the general case, where the pulse is not infinitesimally short relative to $T_2^*$ (93):

$$f_{xy} = \exp\left(-\frac{\tau}{2T_2}\right)\alpha \text{sinc}\left(\sqrt{\alpha^2 - \left(\frac{\tau}{2T_2}\right)^2}\right)$$

and

$$f_z = \exp\left(-\frac{\tau}{2T_2}\right)\left(\cos\left(\sqrt{\alpha^2 - \left(\frac{\tau}{2T_2}\right)^2}\right) + \frac{\tau}{2T_2} \text{sinc}\left(\sqrt{\alpha^2 - \left(\frac{\tau}{2T_2}\right)^2}\right)\right),$$

where $\tau$ is RF pulse duration and $\alpha = \gamma B_1\tau$ is the nominal flip angle.

Each image was imported into Matlab, and volumes of interest (VOIs) were drawn to fully enclose the bone and the reference sample. Then, within each VOI, the image intensity was corrected by solving Equation 4.3 for $\rho$, using the average relaxation times for the set of bone specimens and the measured relaxation times of the reference sample. Next, the bone and reference VOIs were refined by automatic thresholding (135), and, finally, the $^1$H concentration ($[^1\text{H}]$) within the bone was calculated as the ratio of the mean corrected intensity within the bone to that of the reference, multiplied by the known $^1$H concentration, $[^1\text{H}] = 11.1$ M, within the reference sample.
Quantification of $^1\text{H}$ concentration in the SIR-ZTE images is similar, except for two important differences. First, the contribution of the adiabatic inversion pulse was added to Equation 4.3:

$$S(\vec{r}) \propto \rho(\vec{r}) \frac{1 + (f_{\text{HS}} - 1) \exp\left(-\frac{\tau}{T_1}\right) - f_{\text{HS}} \exp\left(-\frac{\tau}{T_1}\right)}{1 - f_{\text{HS}} \exp\left(-\frac{\tau}{T_1}\right)} f_{sv} \exp\left(-\frac{TE}{T_2}\right),$$

where $f_{\text{HS}}$, the response of the longitudinal magnetization to the adiabatic inversion pulse, was calculated for the bone and reference by Bloch equation simulation based on their respective relaxation times. Also, to maintain consistency, the VOIs obtained by automatic thresholding in the ZTE images were carried over for SIR-ZTE correction. Apart from these two changes, quantification is performed in the same manner as in the ZTE images.

4.3.6. Micro-CT Imaging

Bone specimens were imaged using a Scanco µCT35 scanner (Scanco, Brüttisellen, Switzerland) at 18.5-µm isotropic voxel resolution. Bone boundaries were masked by the 3D active snakes method using ITK-SNAP (123). Pores were then segmented within these bone masks by automated thresholding, and porosity was calculated as pore volume divided by total bone volume.

4.3.7. Gravimetry

Bone specimens were removed from phosphate-buffered saline, blotted dry, and weighed to determine their fully hydrated mass. The bones were then placed in tared crucibles and dried at 105°C for 110 hr to remove all bound and pore water. Completion of drying was verified by observing no change in mass over a 24-hour period. The
bones were again weighed, and their dry mass was recorded. Finally, the bones were incinerated at 600°C for 30 hr to burn off all organic material, and the residue was weighed. Total water mass was calculated as the difference between hydrated and dry masses, matrix mass was the difference between dry and ash masses, and mineral mass was equal to the residual ash mass. These masses were divided by total bone volume (the volume of the µCT bone boundary mask) to yield total water, matrix, and mineral densities (63,90).

4.4. Results

4.4.1. Gravimetry and µCT

All bone measurement results, both from MRI and validation methods, are given in Table 4.1. Mean porosity across the 15 donors was 8.96±8.61% (3.06-33.5%); all data expressed in this format are mean ± standard deviation (min-max). Volume renderings of the pore spaces of four representative bone specimens are shown in Figure 4.5. Gravimetric mineral density was 1118±130 mg/cc (751-1219 mg/cc), matrix density was 503.7±24.3 mg/cc (437.0-527.5 mg/cc), and total water density was 326.2±48.4 mg/cc (281.4-345.6 mg/cc) bone tissue (i.e. 32.6% v/v or 36.1 M). The mineralization mass ratio, which is the unitless ratio of gravimetric mineral density to matrix density, was 2.212±0.173 (1.719±2.367), and bone mineralization, which is the bone mineral density normalized by bone volume fraction (1 - porosity), was 1225±36 mg/cc (1130-1286 mg/cc).
<table>
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<th>SIR-ZTE $^1$H (M)</th>
<th>Non-Exch. ZTE $^1$H (M)</th>
<th>µCT Porosity (%)</th>
<th>Mineral Density (mg/cc)</th>
<th>Matrix Density (mg/cc)</th>
<th>Water Density (mg/cc)</th>
<th>$T_1-T_2$ Bound Fraction (%)</th>
<th>$^2$H IR Bound Fraction (%)</th>
<th>Mineralization Mass Ratio</th>
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<td>9.3</td>
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Table 4.1: ZTE, SIR-ZTE, and non-exchangeable (i.e., not removed by $^2$H$_2$O exchange) $^1$H concentration measurements by MRI, and reference measurements by µCT, gravimetry, and $^1$H and $^2$H NMR. Sample labels indicate age and gender.
Porosity was strongly correlated with gravimetric mineral density ($R^2 = 0.98$, negative), matrix density ($R^2 = 0.91$, negative), and total water density ($R^2 = 0.92$, positive). Matrix and mineral densities were also strongly positively correlated ($R^2 = 0.91$). The mineralization mass ratio, however, was strongly positively correlated with mineral density ($R^2 = 0.96$) and negatively correlated with porosity ($R^2 = 0.94$).

4.4.2. MRI-Derived Density

ZTE $^1$H concentration was $32.7 \pm 3.2$ M (28.5-40.3 M), SIR-ZTE $^1$H concentration was $32.9 \pm 3.9$ M (24.4-39.8 M), and non-exchangeable $^1$H ZTE concentration was $12.1 \pm 1.0$ M (9.7-13.4 M). MRI quantifies the electromagnetic signal emitted by $^1$H nuclei within a
voxel, so these measurements are properly expressed in molar concentrations of $^1$H nuclei, rather than mass densities in mg/cc.

ZTE, SIR-ZTE, and non-exchangeable ZTE $^1$H concentration maps of four representative bone specimens are given in Figure 4.6. As age and porosity increase, ZTE $^1$H concentration increases, and SIR-ZTE $^1$H concentration (in which pore water is suppressed) decreases. Note especially the region of extreme structural degradation (indicated by white arrows), with high ZTE $^1$H concentration and commensurately lower SIR-ZTE $^1$H concentration.
Figure 4.6: Maps of ZTE, SIR-ZTE, and non-exchangeable ZTE $^1$H concentrations, in mol/L, in bone specimens from four representative donors. Age and gender of the donors are given within each quadrant, and the endosteal surface of each specimen faces left. Arrows indicate a region of high porosity, which has elevated total water and reduced matrix densities, and correspondingly increased ZTE and decreased SIR-ZTE $^1$H concentrations.

ZTE $^1$H concentration was correlated positively with porosity and gravimetric water density ($R^2 = 0.80$ and $0.79$, respectively), and negatively with matrix density and mineral density ($R^2 = 0.90$ and $0.82$, respectively). SIR-ZTE correlations were opposite those of ZTE; SIR-ZTE $^1$H concentration was correlated negatively with porosity and total water density ($R^2 = 0.73$ and $0.76$, respectively), and positively with matrix density.
and mineral density ($R^2 = 0.74$ and 0.72, respectively). Scatter plots of ZTE and SiR-ZTE $^1$H concentration versus porosity, water density, and organic matrix density are given in Figure 4.7. No correlations involving non-exchangeable ZTE $^1$H concentration reached the level of statistical significance. All relevant correlation coefficients between parameters are given in Table 4.2.
Figure 4.7: Scatter plots displaying the correlations of MRI-derived ZTE (a,c,e) and SIR-ZTE (b,d,f) $^1$H concentrations versus µCT porosity (a,b), gravimetric water density (c,d), and gravimetric organic matrix density (e,f). ZTE $^1$H concentration is positively correlated with porosity and gravimetric water density and negatively with matrix density, while SIR-ZTE correlations show the opposite behavior. Clustering of data is due to severe bone loss being present in a small subset of bones from post-menopausal female donors.)
<table>
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<th>µCT Porosity (%)</th>
<th>Matrix Density (mg/cc)</th>
<th>Mineral Density (mg/cc)</th>
<th>Water Density (mg/cc)</th>
<th>Mineralization Mass Ratio</th>
<th>Bone Mineralization (mg/cc)</th>
<th>T$_1$-T$_2$ Bound Fraction (%)</th>
<th>$^2$H-IR Bound Fraction (%)</th>
<th>Non-Exch. ZTE [$^1$H] (M)</th>
<th>SIR-ZTE [$^1$H] (M)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ZTE [$^1$H] (M)</td>
<td>0.80± 0.90± 0.82± 0.79± 0.69± 0.71± 0.86± 0.52± 0.04 0.77†</td>
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<tr>
<td>SIR-ZTE [$^1$H] (M)</td>
<td>0.73± 0.74± 0.72± 0.76± 0.64± 0.54† 0.86± 0.66± 0.09</td>
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<tr>
<td>Non-Exch. ZTE [$^1$H] (M)</td>
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<tr>
<td>$^2$H-IR Bound Fraction (%)</td>
<td>0.50† 0.46† 0.49† 0.65† 0.44* 0.34* 0.66†</td>
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<tr>
<td>T$_1$-T$_2$ Bound Fraction (%)</td>
<td>0.90† 0.89† 0.88† 0.96† 0.79† 0.63‡</td>
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<tr>
<td>Bone Mineralization (mg/cc)</td>
<td>0.73† 0.70† 0.84† 0.62‡ 0.84†</td>
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<tr>
<td>Mineralization Mass Ratio</td>
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<td>Water Density (mg/cc)</td>
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<td>Mineral Density (mg/cc)</td>
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<td>Matrix Density (mg/cc)</td>
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Table 4.2: Inter-parameter correlations ($R^2$) of measured bone properties. All correlations are statistically significant unless italicized (*p<0.05, †p<0.005, ‡p<0.0005).

4.4.3. NMR Spectroscopy

The short-$T_2$ $^1$H fraction had $T_1 = 480±80$ ms (320-560 ms), $T_2 = 540±150$ µs (430-980 µs), $T_2^* = 400±50$ µs (330-520 µs), and accounted for 77.0±9.3% (55.4-86.6%) of total signal by 2D bi-component $T_1$-$T_2$ fitting. The long-$T_2$ fraction had $T_1 = 1210±300$ ms.
(880-1910 ms), $T_2 = 55000\pm38000 \, \mu\text{s} \, (17000-161000 \, \mu\text{s})$, $T_2^* = 940\pm230 \, \mu\text{s} \, (600-1540 \, \mu\text{s})$, and accounted for 23.0±9.3\% (13.4-44.6\%) of the signal. Short-$T_2$ fraction by 2D $T_1$-$T_2$ bi-component fitting was strongly negatively correlated with ZTE $^1\text{H}$ concentration ($R^2 = 0.86$) and strongly positively correlated with SIR-ZTE $^1\text{H}$ concentration ($R^2 = 0.86$). Short-$T_2$ fraction was also very strongly correlated with porosity ($R^2 = 0.90$, negative) and gravimetric matrix density ($R^2 = 0.89$, positive).

Bound water fraction by $^2\text{H}$ IR was 62.6±9.6\% (48.7-77.9\%), with $^2\text{H} \, T_1 = 11.2\pm1.7 \, \text{ms}$ (9.8-15.9 ms). Pore water had $^2\text{H} \, T_1 = 197\pm42 \, \text{ms} \, (129-282 \, \text{ms})$, and was nulled at $142\pm31 \, \text{ms} \, (92-206 \, \text{ms})$. $^2\text{H}$ IR bound water fraction was less well correlated with ZTE $^1\text{H}$ concentration ($R^2 = 0.52$, negative), SIR-ZTE $^1\text{H}$ concentration ($R^2 = 0.66$, positive), porosity ($R^2 = 0.50$, negative), and matrix density ($R^2 = 0.46$, positive) than was short-$T_2$ $^1\text{H}$ fraction.

4.5. Discussion

In this study, we presented non-invasive, non-destructive MRI-based surrogate measurements for total bone water (ZTE), bound water (SIR-ZTE), and non-exchangeable $^1\text{H}$ concentrations in human cortical bone, and compared these results to gravimetric bone density measurements and µCT porosity. The strong correlations of SIR-ZTE $^1\text{H}$ concentration with both gravimetric matrix density and µCT porosity support the applicability of this method as a surrogate measurement of bone matrix density. This measurement may be combined with a previously established $^{31}\text{P}$ MRI-based examination of bone mineral density (63,129) to investigate bone tissue mineralization.
Although ZTE $^1$H concentration is a surrogate for total bone water concentration and bone porosity (74,92,114), it is not directly related to the density of bone’s collagen matrix; rather, it is more strongly related to the voids within that matrix (i.e. pore volume fraction). As such, ZTE $^1$H concentration is not a surrogate for bone matrix density. Although the present results do not show a measurable advantage of $T_2$-selective SIR-ZTE over ZTE for prediction of porosity, previous work (74) suggests that $T_2$-selective methods correlate more strongly with bone matrix density than do non-selective methods.

ZTE $^1$H concentration (32.7±3.2 M, 28.5-40.3 M) in this work is greater than the 24 M (115) and 19.3-31.8 M (114) found in previous $^1$H UTE work at 3 T, but the results are more consistent with the 29-41 M measured more recently by Horch et al. (81) using a 4.7 T micro-imaging system. This may be due to the improved abilities of ZTE and experimental (i.e. non-clinical) hardware to image the shortest-$T_2$ components in bone. The $^1$H ZTE image likely contains signal not only from water, but also the extremely short-$T_2$ signal fraction that remains after deuterium exchange. If non-exchangeable $^1$H density (12.1±1.0 M, 9.7-13.4 M) is subtracted from total water density measured by $^1$H ZTE, the result, 20.6±3.2 M (16.4-28.2 M), is closer to measurements using UTE on a 3 T clinical scanner.

Likewise, the SIR-ZTE $^1$H concentration of 32.9±3.9 M (24.4-39.8 M) found in the present study is also higher than the 12-24 M found by Horch et al. (81) or 12-23 M by Manhard et al. (84) using SIR-UTE, but these differences may also be explained by the greater ability of ZTE to capture the shortest-$T_2$ signal present in bone. The strengths of the correlations of SIR-ZTE $^1$H concentration to porosity and matrix density in the
present work, however, are broadly consistent with Horch’s reported correlations versus peak stress (81) and CPMG-derived short-$T_2$ pool fraction (81,84).

4.5.1. $T_2^*$-Related Image Blurring

In MRI, signal acquisition occurs in the Fourier domain; that is, the scanner acquires signals that correspond to the prevalence of signal variations at certain spatial frequencies, and the image is reconstructed by taking the inverse Fourier transform of these frequency-domain data. Low spatial frequencies are responsible for variation in image intensity over large distances, while high spatial frequencies represent fine details and sharp edges. In all forms of ZTE, these spatial frequencies are acquired along radial spokes beginning at zero spatial frequency and ending at high spatial frequency, and traversal from zero frequency to high frequency takes a finite amount of time.

In the case of short $T_2^*$, the signal decays significantly while sampling each spoke, causing attenuation of higher spatial frequencies to an extent depending on the $T_2^*$ of the signal being imaged and the particular scan parameters used. This attenuation is described by the modulation transfer function (MTF); mono-exponential examples of MTFs are shown in Figure 4.8a for ZTE, SIR-ZTE, and non-exchangeable ZTE imaging. This attenuation causes sharp image features to be blurred. This is represented by the point-spread function (PSF, Figure 4.8b), the Fourier transform of the MTF, which describes how a single infinitesimally small point source of signal is blurred due to attenuation of high spatial frequencies. Shorter $T_2^*$ results in a more rapid decay of the MTF and a broader PSF.
Figure 4.8: a) The modulation transfer function (MTF) describes the $T_2^*$ decay of the MRI signal over the course of signal acquisition. b) The Fourier transform of the MTF is the point spread function (PSF), which describes how a single infinitesimally small point source of signal is blurred due to attenuation of higher spatial frequencies. Shorter $T_2^*$ results in a more rapid decay of the MTF and a broader PSF. c) A rectangular profile with several gaps, representing a 1D cross-section through a porous bone, is convolved with the PSFs of ZTE, SIR-ZTE, and non-exchangeable ZTE. These gaps are more severely blurred in cases of shorter $T_2$.

Blurring in an image can be thought of as the convolution of a perfectly un-blurred image by the point-spread function. In Figure 4.8c, a rectangular profile with several gaps,
representing a 1D cross-section through a porous bone, is convolved with the PSFs of ZTE, SIR-ZTE, and non-exchangeable ZTE. These gaps are less visible in cases of shorter $T_2^*$ and the corresponding rapid decay in MTF and broad PSF. In Figure 4.6, due to the suppression of long-$T_2$ signal from pore water, the shorter $T_2^*$ of the residual signal in the SIR-ZTE images ($T_2^* \sim 300 \mu s$) causes slightly greater point-spread function blurring than in ZTE images. ZTE images of non-exchangeable $^1$H are even more severely blurred due to the chemical removal of all exchangeable protons (primarily bone water), leaving only extremely short-$T_2^*$ ($< 60 \mu s$) signal.

4.5.2. Non-Exchangeable $^1$H Signal
The general categorization of bone $^1$H signal into long-, short-, and extremely short-$T_2$ pools, corresponding to pore water, bound water, and matrix collagen, respectively, implies that if bone water is rendered invisible to $^1$H MRI by exchange with $^2$H$_2$O, the remaining extremely short-$T_2$ signal should represent matrix collagen. The weak and statistically insignificant associations of non-exchangeable ZTE $^1$H concentration with gravimetric and µCT measurements do not support this simple hypothesis; the true composition of this short-$T_2$ pool and its exchangeability with $^2$H$_2$O appears to be more complicated.

In 2010, Horch et al. (69) examined the effects of $^2$H$_2$O exchange on bone NMR spectra. These spectra contained three peaks: an on-resonance long-$T_2^*$ peak consistent with water protons, containing 57.7% of total signal; an off-resonance long-$T_2^*$ peak centered at approximately -4.0 ppm, containing 4.6% of total signal and close to the methylene proton resonance (-3.5 ppm); and a broad peak consistent with the short-$T_2^*$ signal from collagen, containing the remaining 33.7% of total signal. Following $^2$H$_2$O exchange, the
water peak was completely absent, but the methylene and collagen signal components were relatively unaffected. Complementary results from relaxation spectroscopy showed that only signal with $T_2 \sim 400 \mu s$ was completely removed by exchange, but only 58% of long-$T_2$ signal was removed, and extremely short-$T_2$ signal ($T_2 \sim 60 \mu s$) was unaffected. These observations suggest that only a small portion the residual 42% of long-$T_2$ signal corresponds to the off-resonance long-$T_2^+$ signal and, therefore, may arise from lipids, whose protons are not labile.

In our work, further spectroscopic experiments were performed on the present set of 15 specimens to partition the non-exchangeable $^1$H signal into on-resonance and off-resonance fractions. Correlations of the on-resonance portion of non-exchangeable ZTE $^1$H concentration with porosity and all three gravimetric densities yielded nominally stronger correlations, but still did not reach statistical significance. Furthermore, since the specimens were carefully prepared to exclude bone marrow, it is unlikely that such a large fraction of non-exchangeable $^1$H signal arises from marrow fat. A small amount of lipid exists surrounding each osteon at the cement line (126), but this is also unlikely to account for such a large amount of non-exchangeable signal. In light of these results, it appears that the non-exchangeable $^1$H signal component in cortical bone does not arise predominantly from bone matrix collagen, or, if it does, its measured density is affected by a property other than simply the density of collagen in bone.

4.5.3. Sensitivity to Relaxation Times

All conversions of image intensity to density in this work were performed using average relaxation times for the set of 15 bone specimens, rather than individually measured relaxation times for each specimen. This ensures that the strengths of the correlations
observed between the measured MRI $^1$H concentrations and reference measurements are translatable to eventual *in vivo* use, where measurement of relaxation times in each subject would not be practical. Under this constraint, the correlations of SIR-ZTE $^1$H concentration with porosity and matrix density remain strong ($R^2 > 0.7$, $p < 0.00005$).

If the $T_1$ value used in **Equations 4.3-4.6** is longer than the true $T_1$ in the specimen, $^1$H concentration will be overestimated. A ±5% deviation in $T_1$ results in ±3.0% error in calculated ZTE $^1$H concentration and ±5.2% error in SIR-ZTE $^1$H concentration. Variation in $T_2^*$ will have very little effect on the calculated ZTE $^1$H concentration: the same ±5% error in $T_2^*$ results in error of approximately ±0.022%. However, due to the $T_2$-selectivity of the adiabatic inversion pulse, a ±5% change in $T_2^*$ will cause a noticeable error of ±3.8% in SIR-ZTE $^1$H concentration.

In **Equations 4.3-4.5**, such errors may artificially amplify the true differences in total water $^1$H concentration; greater porosity is associated not only with higher total water content, but also longer total water $T_1$ and $T_2^*$ due to decreased surface interaction and susceptibility effects in larger pores (115). **Equations 4.4-4.6**, however, suggest that these same errors may slightly reduce the sensitivity of SIR-ZTE $^1$H concentration to true bound water content in the case of perfect long-$T_2$ suppression, but this possible effect is alleviated by the fact that, while pore water relaxation properties are very strongly affected by bone porosity, bound water relaxation times are relatively constant (69). Only the stable bound water $T_1$ and $T_2^*$ values are used in quantification.

The assumption of perfect long-$T_2$ nulling by SIR-ZTE in all specimens, however, is not realistic for a single $TI$ applied to both dense and porous bones. In porous bone,
although bound water relaxation times are stable, pore water $T_1$ and $T_2$ are longer than in dense bone. $T_1$ of long-$T_2$ pore water ($T_{1L}$) by bi-component 2D $T_1$-$T_2$ fitting (see Equation 4.1) ranges from 880 to 1910 ms, and is positively correlated with porosity ($R^2 = 0.70$, $p < 0.0001$), and the long $T_2$ relaxation time ($T_{2L}$) ranges from 17 ms to 161 ms and is strongly positively correlated with porosity ($R^2 = 0.91$, $p < 10^{-7}$). In empirically choosing $TI$ for a set of specimens such that pore water is nulled, $TI$ is biased toward optimal nulling of pore water in more porous bones; these bones have more pore water to be nulled, so proper inversion is more important. In dense bones, with shorter $T_{1L}$ and $T_{2L}$, pore water magnetization may be incompletely inverted during the adiabatic inversion pulse (i.e., $M_z > -1$) due to transverse relaxation during the pulse, and will undergo significantly faster longitudinal relaxation after inversion, thus overshooting the null point ($M_z > 0$) during the inversion-recovery delay. Some of this pore water magnetization in dense bone will therefore also be imaged, along with bound water magnetization, by SIR-ZTE. This additional contribution of pore water in dense bones is responsible for the overestimation of bound water density (which is 60-80% of total bone water (69,71)) by SIR-ZTE, causing average SIR-ZTE $^1$H concentration (32.9±3.9 M, 24.4-39.8 M) to be slightly greater than average ZTE $^1$H concentration (32.7±3.2 M, 28.5-40.3 M). This overestimation, however, does not diminish the strength of the positive correlation of SIR-ZTE $^1$H concentration with bone matrix density, or the negative correlation with porosity. In fact, this phenomenon may enhance the sensitivity of SIR-ZTE to changes in matrix density and porosity.
4.5.4. Bone Mineralization

The strong positive correlation ($R^2 = 0.91$) between matrix and mineral densities measured by gravimetry confirms that the degree of mineralization in these bones is not the primary determinant of bulk bone mineral density; changes in mineral density are primarily a result of structural degradation rather than a deficit of mineralization. The mineralization mass ratio, which is defined as gravimetric mineral density divided by matrix density, however, was strongly negatively correlated with porosity ($R^2 = 0.94$), consistent with the notion that bone mineralization is decreased due to rapid bone turnover in age-related bone loss.

Tissue mineralization density calculated in this work, $1225\pm36\ \mathrm{mg/cc\ matrix\ (1130-1286\ mg/cc\ matrix)}$, was similar to previous microradiographic measurements by Boivin et al. (136) ($1082\pm17\ \mathrm{mg/cc}$). Gravimetric densities are also consistent with gravimetric measurements performed by Cao et al. (63) in rat bone.

4.5.5. Translatability to the Clinic

This work has established SIR-ZTE $^1\mathrm{H}$ concentration as a surrogate for matrix density. This examination, in combination with a $^{31}\mathrm{P}$ ZTE examination of bone mineral density (129), could be developed into a non-invasive in vivo MRI assessment of bone mineral and matrix densities, and their ratio, the degree of mineralization of bone. If reduced to practice, this MRI method would allow clinicians to discriminate between age-related macroscopic bone loss and impairment of bone mineralization. Such an examination is not possible using standard x-ray-based screening methods.
This work, however, does not support the proportionality of non-exchangeable ZTE $^1$H concentration to bone matrix density. The presence of both on-resonance and off-resonance components suggests the presence of several sources of signal in ZTE images after $^2$H exchange. Further experiments, such as chemical manipulations to dissolve and remove lipid, would provide additional insight into the nature of this extremely short-$T_2$ component.

This study benefitted from the enhanced performance of experimental versus clinical hardware. The 9.4 T scanner used in this work is equipped with much stronger gradients than clinical scanners, narrowing the point spread function and, thus, reducing blurring. This allows for better delineation of bone margins, and even enables visualization of individual pore spaces in severely porous bones (see Figure 4.5, panel 83F). The use of a 20-mm RF probe and very high field strength also yields higher SNR than is achievable using clinical hardware.

4.6. Conclusion

Based on the strong correlations of SIR-ZTE $^1$H concentration with gravimetric matrix density and porosity, long $T_2$-suppressed solid-state MRI is a promising surrogate for bone matrix density.
CHAPTER 5: BONE MINERAL $^{31}$P AND MATRIX-BOUND WATER DENSITIES MEASURED BY SOLID-STATE $^1$H AND $^{31}$P MRI

5.1. 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 of bone mineral and collagen-bound water densities on clinical scanners, 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 $^1$H MRI on whole body 7 T and 3 T scanners, respectively. Images were corrected for relaxation and RF inhomogeneity to obtain density maps. Cortical porosity was measured by μCT, 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 $^1$H 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: $R^2 = 0.67$, $p < 0.0005$; BW: $R^2 = 0.81$, $p < 0.0001$) 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.

5.2. Introduction

As explained in Chapter 1, osteoporosis is a structural bone disorder in which 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. The differentiating factor between these disorders is bone mineralization: the mass of mineral in a volume of bone matrix only, excluding pore spaces (see Figure 5.1).

![Figure 5.1](image)

Figure 5.1: 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.

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/cm². However, because the mechanical properties of bone depend on both mineral and matrix, bone mineral density alone cannot fully describe bone health.

Recent advances in solid-state $^{31}$P and $^1$H magnetic resonance imaging (MRI) have led to the possibility of quantitative measurement of bone mineral $^{31}$P and collagen-bound water $^1$H densities (55,60,63-68,75-84,137,138). These two measurements, taken individually, reflect the apparent densities of bone mineral and matrix, but their ratio can provide insight into bone mineralization. Only Cao et al. (63) have quantified both $^{31}$P and bound water in a single set of specimens. 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 µCT-derived porosity, pQCT-derived apparent BMD, and donor age.
5.3. Materials and Methods

5.3.1. 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]P = 12 mM; signal contribution is negligible compared to bone[^31]P ~ 7 M) inside plastic tubes (Figure 5.2), 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
x 15 x 5 mm, served as a short-$T_2$ $^1$H reference signal sample ($^1$H $T_1 = 284$ ms, $T_2^* = 156$ µs). The $^{31}$P and $^1$H 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 trioctyl phosphate ($C_{24}H_{51}O_4P$, $T_1 = 709$ ms, $T_2^* = 4560$ µs, $[^{31}\text{P}] = 2.124$ mol/L) and a solution of 90% D$_2$O/10% H$_2$O doped with 28.9 mM MnCl$_2$ ($T_1 = 7.3$ ms, $T_2^* = 272$ µs, $[^1\text{H}] = 11.079$ mol/L) were used for $^{31}$P and $^1$H calibration, respectively.

5.3.2. Hardware
All $^{31}$P scanning was performed on a 7 T Magnetom whole-body MRI scanner (Siemens, Erlangen, Germany), and all $^1$H scanning on a 3 T 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 7 T (120.3 MHz) and $^1$H at 3 T (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 Figure 5.2 with a bone specimen. In addition to the previously explained intensity reference phantoms, which are not visible in Figure 5.2, three samples containing trioctyl phosphate were rigidly affixed to the exterior of the coil to serve as fiducial markers in both $^{31}$P and $^1$H images. These landmarks were used to register each image to an RF field map.

5.3.3. MR Imaging
Due to its excellent SNR performance in samples with extremely short $T_2^*$ (131), ZTE with PETRA (57), shown in Figures 5.3a and 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.
Figure 5.3: Imaging pulse sequences used for bone $^{31}$P and bound water density quantification: $^{31}$P ZTE sequence (a) with PETRA module (b), and $^1$H Single Adiabatic Inversion Recovery Rapid ZTE (SIR-rZTE) sequence (c) with PETRA module (d). Relevant pulse sequence parameters are shown.

Each sample was imaged using three sequences, shown in Figure 5.3: $^{31}$P ZTE with PETRA (57) (Figures 5.3a,b), $^1$H ZTE with PETRA (similar to Figures 5.3a,b), and $^1$H Single Adiabatic Inversion-Recovery Rapid ZTE with PETRA (SIR-rZTE, Figures 5.3c,d) (81,83,84,112).

$^{31}$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 \(= 12 \mu s\), \(TR = 20\) 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.

\(^1\)H ZTE images were acquired with the following parameters: \(FOV = 80\) mm isotropic, pulse duration = 32 \(\mu s\), flip angle = 8.7 degrees, gradient amplitude = 18.3 mT/m, \(N = 40\), dwell time = 16 \(\mu s\), \(TR = 7\) 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.

\(^1\)H 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 \(\mu s\), 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 $^1$H 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 (61). 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 $^1$H), and Fourier transformed using the NFFT C subroutine library (97). Reconstructed isotropic voxel resolutions were 2 mm$^3$ and 1 mm$^3$ in $^{31}$P and $^1$H images, respectively.

5.3.4. $B_1$ Mapping and Registration

A map of RF amplitude and, by reciprocity, receive sensitivity was acquired by Bloch-Siegert $B_1$ mapping (139). This sequence was applied on the $^1$H nucleus at 3 T 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 trioctyl phosphate landmarks were obtained using a $^1$H ZTE image with voxel resolution and FOV size and location identical
to the bone specimen scans, and were used to register the $B_1$ map to each $^1$H and $^{31}$P bone specimen image (140).

5.3.5. 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 5.3a,b is given in Equation 5.1:

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

[5.1]

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 $T_2$ (93):

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

[5.2]

and
\[ I_c(\vec{r}) = \exp \left( -\frac{\tau}{2T_2} \right) \left( \cos \left( \sqrt{\left( \gamma B_1(\vec{r}) \tau \right)^2 - \left( \frac{\tau}{2T_2} \right)^2} \right) + \frac{\tau}{2T_2} \text{sinc} \left( \sqrt{\left( \gamma B_1(\vec{r}) \tau \right)^2 - \left( \frac{\tau}{2T_2} \right)^2} \right) \right), \] [5.3]

where \( \tau \) 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 Equation 5.1 for \( \rho(\vec{r}) \), and density can be quantified relative to a similarly corrected reference sample in the same image field of view (FOV) (64).

\(^{31}\text{P} \) \( T_1 \) of bone mineral is strongly dependent on the level of mineralization and may vary significantly among donors (131). To accurately perform this correction for \(^{31}\text{P} \) density quantification, \(^{31}\text{P} \) relaxation was measured in each individual bone using saturation recovery. \(^1\text{H} \) bound water relaxation times chosen for density computation were population averages from the literature: \( T_1 = 290 \text{ ms} \) (84) and \( T_2^* = 350 \mu\text{s} \) (112).

Unlike bone mineral \(^{31}\text{P} \), \(^1\text{H} \) NMR signal in bone at 3 T 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 \( T_2 \sim 300-400 \mu\text{s} \), corresponding to motionally restricted water bound to bone matrix collagen (‘bound water’); and extremely short \( T_2 \leq 50 \mu\text{s} \), corresponding to \(^1\text{H} \) nuclei in bone matrix collagen itself (‘collagen’) (69). In practice, the collagen signal is beyond the reach of clinical hardware, even with solid-state pulse sequences. However, bound water and collagen \(^1\text{H} \) signal both are proportional to bone matrix density (60,63,69,71,72,75,76,78,79,81,84,112), while pore water is inversely proportional to...
bone matrix density (71,72,75,81,84,110), and total bone water density is only weakly correlated with bone matrix density (71,81). It is therefore necessary to isolate and image only the $^1$H 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 $^1$H signal while also being able to invert the broad band of long-$T_2$ pore water spins resonating over a range of frequencies (81,82,141). The response of the equilibrium longitudinal magnetization, $f_{HS} = M_z/M_0$, to a 5-kHz bandwidth, 5-ms duration hyperbolic secant adiabatic RF pulse is shown for a range of $T_2$s in Figure 5.4. After an appropriate inversion time delay ($T_I$), pore water longitudinal magnetization will be nulled ($M_z ≈ 0$) as a consequence of partial longitudinal ($T_1$) recovery of the magnetization, while bound water longitudinal magnetization will have recovered from $M_z = 0$ 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:

$$S(\vec{r}) \propto \rho(\vec{r}) \frac{1 + (f_{\text{HS}} - 1) \exp\left(-\frac{TE}{T_2}\right) - f_{\text{HS}} \exp\left(-\frac{TR}{T_2}\right)}{1 - f_{\text{HS}} f_{xy}(\vec{r}) \exp\left(-\frac{TR}{T_2}\right)} f_{xy}(\vec{r}) \exp\left(-\frac{TE}{T_2}\right) B_1(\vec{r}).$$  \[5.4\]

In Equation 5.4, $f_x$ includes the cumulative effect of all seven excitation pulses, and $f_{xy}$ is equal for each excitation.

$^31$P ZTE and $^1$H 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 5.1-5.4.
The reference samples and bone specimens were further masked by Otsu automatic
thresholding (135) 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 $^{31}\text{P}$ or $^1\text{H}$ density of the
reference. This yields a measurement of bone $^{31}\text{P}$ 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 $^{31}\text{P}$ density to
bound water density is a surrogate of bone mineralization.

5.3.6. X-Ray-Based Porosity and Densitometry

For comparison to MRI-derived densities, cortical porosity was measured by micro-
computed tomography ($\mu$CT). A 3D $\mu$CT image of each cortical bone specimen was
acquired with 9-µm isotropic resolution using a Bruker SkyScan $\mu$CT 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 mm x 0.4 mm x 2.3 mm using a Stratec XCT
was quantified in this single slice.
5.3.7. 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 \( \mu \)CT 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.

5.4. 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 Figure 5.5, and maps of \( ^{31}P \) and bound water densities are shown in Figure 5.6. 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.
Figure 5.5: 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 5.6: 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 $^1$H 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.
Cortical porosity is known to increase with age, particularly in post-menopausal women (29). Bone porosity was correlated positively with age ($R^2 = 0.65$, $p < 0.0005$) and negatively with pQCT density ($R^2 = 0.64$, $p < 0.0005$), confirming that age-related bone mineral loss is present in this set of donors. The data also suggest a negative correlation between pQCT density and age ($R^2 = 0.29$, $p < 0.05$).

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 (Figure 5.6).
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<td>1361</td>
<td>109.8</td>
<td>6.47</td>
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<td>27.2</td>
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<td>95.9</td>
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<td>870</td>
<td>28.8</td>
<td>26.0</td>
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Table 5.1: Measured bone parameters with means and standard deviations. $[^{31}\text{P}]$ density in mg of hydroxyapatite per cc and bound water volume fraction are inferred from MRI-derived densities based on certain assumptions described in the discussion. Abbreviations: BVF, bone volume fraction; HAp, hydroxyapatite; BMR, bone mineral ratio.

Various measures for MRI-derived densities and x-ray-based measurements are given in Table 5.1. Mean bone mineral $[^{31}\text{P}]$ density was 6.7±1.2 mol/L (corresponding to 1129±204 mg/cc mineral), and mean bound water $[^{1}\text{H}]$ density was 31.4±4.2 mol/L (corresponding to 28.3±3.8 %v/v). Coefficients of determination for correlations between measured parameters are given in Table 5.2. Both $[^{31}\text{P}]$ and bound water (BW) densities were correlated negatively with porosity ($[^{31}\text{P}]$: $R^2 = 0.67$, $p < 0.0005$; BW: $R^2 = 0.81$, $p < 0.0001$) and age ($[^{31}\text{P}]$: $R^2 = 0.39$, $p < 0.05$; BW: $R^2 = 0.70$, $p < 0.0001$), and positively with pQCT density ($[^{31}\text{P}]$: $R^2 = 0.46$, $p < 0.05$; 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’ (12), can be inferred from apparent pQCT bone mineral density divided by the bone volume fraction (BVF), expressed as (1 – 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. (142). As expected in fully mineralized bone, the MRI-measured $^{31}\text{P}$ and bound water densities (the latter scaling with matrix density) were highly correlated ($R^2 = 0.59$, $p < 0.005$, Figure 5.7), and the density ratio was not correlated with age, porosity, or pQCT density.

Table 5.2: Correlation matrix of $R^2$ values. All correlations are statistically significant unless italicized (*$p<0.05$, †$p<0.005$, ‡$p<0.0005$). Abbreviations: BMR, bone mineralization ratio; BWD, bound water density; $^{31}$PD, $^{31}$P density; BVF, bone volume fraction = 1 - porosity.

<table>
<thead>
<tr>
<th></th>
<th>Age</th>
<th>Porosity</th>
<th>pQCT</th>
<th>pQCT/BVF</th>
<th>$^{31}$P $T_1$</th>
<th>$^{31}$PD</th>
<th>$^{31}$PD/BVF</th>
<th>BWD</th>
<th>BWD/BVF</th>
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<td>BMR</td>
<td>2.5x10^{-3}</td>
<td>0.08</td>
<td>0.09</td>
<td>0.06</td>
<td>0.33†</td>
<td>0.50†</td>
<td>0.53†</td>
<td>8.8x10^{-3}</td>
<td>0.19</td>
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<tr>
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<td>0.48†</td>
<td>0.35*</td>
<td>0.48†</td>
<td>0.27*</td>
<td>0.25*</td>
<td>0.09</td>
<td>0.09</td>
<td>0.10</td>
</tr>
<tr>
<td>BWD</td>
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<td>0.81‡</td>
<td>0.50†</td>
<td>0.77‡</td>
<td>0.19</td>
<td>0.59†</td>
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<td>$^{31}$PD/BVF</td>
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<td>0.06</td>
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<tr>
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<td>0.67‡</td>
<td>0.46†</td>
<td>0.62‡</td>
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<tr>
<td>$^{31}$P $T_1$</td>
<td>0.02</td>
<td>0.30*</td>
<td>0.51†</td>
<td>0.27*</td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>pQCT/BVF</td>
<td>0.59‡</td>
<td>0.95‡</td>
<td>0.47†</td>
<td></td>
<td></td>
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<td>0.64‡</td>
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<tr>
<td>Porosity</td>
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</tbody>
</table>
Figure 5.7: Correlation plot of bone mineral $^{31}\text{P}$ density to bound water $^1\text{H}$ density. The two MRI-derived densities are highly correlated, as expected in a set of equally mineralized bones.

Point‐spread function (PSF) blurring is more severe in the $^{31}\text{P}$ maps than $^1\text{H}$ (Figure 5.6), due to the lower gyromagnetic ratio ($\gamma = 17.24$ MHz/T) and shorter $T_2^*$ of bone mineral $^{31}\text{P}$. Average image SNR was 12.5 in $^{31}\text{P}$ ZTE images, and 20.2 in $^1\text{H}$ SIR-ZTE images. Finally, bone mineral $^{31}\text{P}$ $T_1$ relaxation time was positively correlated with pQCT density ($R^2 = 0.51$, $p < 0.005$) and negatively with porosity ($R^2 = 0.30$, $p < 0.05$). $^{31}\text{P}$ $T_2^*$ relaxation time was $130.8 \pm 1.2$ µs, and was not correlated with pQCT density or porosity.

5.5. Discussion

The adiabatic inversion recovery method takes advantage of differences in transverse relaxation between bound and pore water spins to isolate bound water signal (81). 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 $1/T_2^* \approx 1/T_2 >> 1/T_2'$. 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) (116). This causes inhomogeneous broadening resulting in reduced pore water $T_2^*$, such that $1/T_2^* \approx 1/T_2'$ >> $1/T_2$, 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) (75-78,110) or suppression of long-$T_2^*$ signal using long, low-amplitude pulses (60,63,79,92), this work uses adiabatic inversion recovery (76,81,83,84,112), a $T_2$ (and partially $T_1$) selective method shown in Figures 5.2c,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 (64)) 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 $^1$H 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 $^{31}$P/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 5.1.

The stoichiometry of bone mineral approaches that of calcium hydroxyapatite, but significant deviations from this composition do exist (21,22). The Ca/P ratio of bone mineral is also known to vary somewhat in bone disease (23), 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 $^1$H signal. Under this assumption, however, it is straightforward to convert the density in mol $^1$H/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$_2$O. The average value of the inferred bound water volume fraction is 28±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. (114). 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 (131). The present work finds decreased $^{31}\text{P} T_1$ with increased bone porosity as well. Bone mineral $^{31}\text{P}$ longitudinal relaxation is primarily due to dipolar interactions with nearby protons. As bone is mineralized, mineral crystals displace water (107), causing the $^{31}\text{P}$ 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 $^{31}\text{P}$ nucleus interacts with a greater number of protons (33), 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 $^{31}\text{P}$ 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 $^1\text{H}$ nucleus to a $^{31}\text{P}$ nucleus vanishingly small. However, the number of these $^1\text{H}$ nuclei at a given distance increases as the square of distance; therefore, the aggregate contribution of distant $^1\text{H}$ dipolar interactions to $^{31}\text{P}$ longitudinal relaxation is not negligible, and the increase in the number of distant pore water $^1\text{H}$ nuclei may contribute to the observed decrease in $^{31}\text{P} T_1$.

In addition, 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 (31), 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 $^1$H 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 $^1$H 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 (64). 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 $^1$H 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.

### 5.6. Conclusions

In summary, solid-state $^{31}$P and bound water-selective $^1$H 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.
6.1. Conclusions

The main results of this thesis research are summarized as follows:

$^{31}$P NMR Relaxation of Cortical Bone Mineral at Multiple Magnetic Field Strengths and Levels of Demineralization: The $T_1$ and $T_2^*$ relaxation times of bone mineral $^{31}$P were systematically measured in lamb cortical bone at six magnetic field strengths ranging from 1.5 T to 11.7 T. Although $T_1$ increases from $12.8 \pm 0.5$ s to $97.3 \pm 6.4$ s, and $T_2^*$ decreases from $220.3 \pm 4.3$ µs to $98.0 \pm 1.4$ µs, predicted SNR under both coil-dominated and sample-dominated noise conditions increases with field strength. SNR trends in UTE and ZTE images at 1.5 T, 3 T, and 7 T using a standardized set of custom solenoidal RF coils parallel these predictions. Despite the predicted and observed SNR advantage of imaging at high field, other issues must be considered in an ultimate choice of field strength. PSF blurring becomes more severe as field strength increases due to the decrease in $T_2^*$, and SAR is greater at higher Larmor frequencies. These tradeoffs can, and we believe do, justify the choice of 3 T as the optimal field strength for an examination of bone mineral $^{31}$P and matrix-associated $^1$H.

Bi-Component $T_2^*$ Analysis of Bound and Pore Bone Water Fractions Fails at High Field Strengths: The viability of bi-component $T_2^*$ fitting for quantification of bound and pore bone water fractions was assessed at 1.5 T, 3 T, 7 T, and 9.4 T against bone mineral, matrix, and water densities obtained gravimetrically, and bone volume fraction calculated from high-resolution µCT images. Short-$T_2^*$ pool fraction is moderately correlated with porosity ($R^2 = 0.70$) and matrix density ($R^2 = 0.63$) at 1.5 T, but the
strengths of these associations diminish rapidly as field strength increases. In contrast, CPMG-derived short-$T_2$ fraction at 9.4 T is highly correlated with porosity ($R^2 = 0.87$) and matrix density ($R^2 = 0.88$), confirming the utility of this method for experimental validation of bone water pools. Based on these results, we advise caution in using $T_2^*$-based relaxometry methods to analyze bone $^1$H signal, and instead suggest $T_2$-based methods.

**Single Adiabatic Inversion Recovery Zero Echo Time MRI is a Surrogate Measure of Bone Matrix Density:** $^1$H single adiabatic inversion recovery (SIR) zero echo-time (ZTE) MRI was evaluated as a surrogate measure of matrix density in human cortical bone. SIR-ZTE $^1$H density was correlated negatively with porosity ($R^2 = 0.73$) and positively with matrix density ($R^2 = 0.74$) and mineral density ($R^2 = 0.72$). These strong correlations with ground-truth measurements suggest that this quantitative solid-state MRI method provides a nondestructive surrogate measure of bone matrix density, a property that is not measurable using standard x-ray-based techniques.

**Bone Mineral $^{31}$P and Matrix-Bound Water Densities Measured by Solid-State $^1$H and $^{31}$P MRI:** Bone mineral $^{31}$P and matrix-associated $^1$H densities were acquired by $^{31}$P ZTE and $^1$H SIR-rZTE MRI at 7 T and 3 T, respectively. These measurements were compared to cortical porosity measured by µCT and apparent mineral density by pQCT. Both $^{31}$P and SIR-rZTE $^1$H densities were correlated negatively with porosity ($^{31}$P: $R^2 = 0.67, p < 0.0005$; $^1$H: $R^2 = 0.81, p < 0.0001$) and age ($^{31}$P: $R^2 = 0.39, p < 0.05$; $^1$H: $R^2 = 0.70, p < 0.0001$), and positively with pQCT density ($^{31}$P: $R^2 = 0.46, p < 0.05$; $^1$H: $R^2 = 0.50, p < 0.005$). As expected in the absence of bone-demineralizing disorders, the bone mineralization ratio (the ratio of $^{31}$P density to SIR-rZTE $^1$H 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.

6.2. Future Work

Based on the results presented in this dissertation, several issues could benefit from further investigation:

6.2.1. Technical Development

**Rapid \( T_1 \) measurement of bone mineral \(^{31}\text{P} \):** As explained in Chapter 2, the \( T_1 \) of bone mineral \(^{31}\text{P} \) is strongly dependent on the level of demineralization. This means that, for best performance, an eventual *in vivo* examination of bone mineral density must include measurement of \(^{31}\text{P} \ T_1 \). Many methods exist for \( T_1 \) quantification, but not all are suitable for the extremely long \( T_1 \) values, up to 100 seconds, of bone mineral \(^{31}\text{P} \). A systematic comparison of multiple methods, including dual-\( TR \), dual-flip angle, saturation-recovery and inversion-recovery Look-Locker, and other methods is necessary to choose the best candidate for an *in vivo* examination of bone mineral density.

**Theoretical investigation of the performance of the inverse Laplace transform using synthetic data and noise:** Chapter 3 experimentally evaluates the performance of \( T_2^* \) bi-component fitting for quantification of bound and pore bone water fractions. A further theoretical evaluation of this topic based on synthetic data and noise would complement this work. Specific questions to be answered would include:

1. At what point does the separation of time constants become too small to be recovered by bi-component fitting?
2. What is the SNR required for accurate bi-component fitting of data with time constants separated by a given factor?

3. How does the number of fitted components (two, three, or unconstrained) affect requirements on separation of time constants and SNR?

4. What methods could feasibly handle fitting of components with both different relaxation times and frequency offsets?

Comparison of $^1$H bi-component $T_2^*$ fitting at 1.5 T to SiR-rZTE at 3 T: Most prior work by other groups on bi-component $T_2^*$ fitting in bone was performed at 3 T. As shown in Chapter 3, this method performs poorly at high field, but may be suitable at lower field. Though the SNR of $^{31}$P ZTE of bone mineral at 1.5 T is not optimal, the greater separation in $^1$H $T_2^*$ values at low field will certainly allow for better quantification of bound and pore water fractions by bi-component fitting at 1.5 T than at 3 T, but further investigation is needed to determine if this method can outperform $^1$H SiR-rZTE at 3 T.

Examination of the source of off-resonance $^1$H signal: The signal oscillations visible in Figure 3.8 complicate bi-component $T_2^*$ fitting of $^1$H signal, but their origin is unknown. They do not have a fixed period, so it is not possible to assign a chemical shift to this component. It has been hypothesized that this signal may arise from marrow fat. This may be tested by acquiring and analyzing spectroscopic data from specimens before and after chemical removal of fat by immersion in chloroform.

6.2.2. Translation to the Clinic

Anisotropic FOV and resolution for ZTE SNR enhancement: As mentioned briefly in Chapter 5, bones in the appendicular skeleton are long and narrow. Voxel size can
therefore be sacrificed along the long axis of the bone in favor of higher SNR, and the alias-free FOV can be reduced orthogonal to this axis to reduce scan time. This method has already been developed (143) and implemented on phantoms in our lab, and could easily be incorporated into an in vivo scan protocol.

**Scaling of the combined $^{31}$P and $^1$H method to human subjects:** The work outlined in Chapter 5 was performed on clinical scanners, but used a small solenoidal RF coil. Scaling this method to a larger volume coil, sized to fit the human leg, would entail an SNR penalty. This loss of SNR must be quantified, particularly in $^{31}$P, and appropriate pulse sequence modifications should be implemented to regain this lost SNR.

**Tracking of response of mineral density, matrix density, and DMB to anti-resorptive treatment in post-menopausal osteoporotic women:** The ultimate test of this method for paired measurement of bone mineral and matrix densities is its ability to measure a response to treatment of post-menopausal osteoporotic female subjects with standard anti-resorptive therapy. Accuracy and reproducibility should first be assessed in healthy control subjects, and then this method should be applied to a group of post-menopausal osteoporotic women undergoing treatment with bisphosphonates, and a group of matched healthy control subjects, to track the recovery of bone mineral and matrix densities and DMB.
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