Imaging Collagen-associated Water In Bone By Magnetic Resonance Imaging And Near Infrared Spectroscopy

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Introduction: Cortical bone loss is responsible for most fractures that occur after 65 years of age (1) and for a 13-fold increase in femoral neck fracture risk between 60 to 80 years of age (2). Unfortunately, even the latest in-vivo bone imaging modalities such as high-resolution peripheral quantitative computed tomography (HR-pQCT) can resolve only relatively larger cortical bone pore spaces (>130 µm) typically residing near the medullary cavity, and thus more subtle bone changes may not be detected. Ultra-short echo time (UTE) magnetic resonance imaging (MRI) has recently been proposed as a mean to assess changes in the entire cross-section of the cortical bone in human subjects without being limited by imaging resolution (3). It is hypothesized that UTE MRI can obtain quantitative measurements of bone water which resides either in pore spaces (i.e., pore water) or bound to collagen matrix (i.e., bound water). Other than gravimetric water measurements (wet weight - dry weight), which are limited by the lack of spatial resolution, there have been no studies for correlation to the UTE MRI measurements. Here, we investigate the use of near infrared (NIR) spectroscopy, a technique sensitive to molecular structure and composition changes in tissues, for assessment of the spatial distribution of molecular components in bone, including collagen, fat and water. In cartilage, it has been shown that the NIR absorbance peak at 5200 cm⁻¹ arises from the combination of free and bound water whereas 6890 cm⁻¹ arises from free water (4). Although the frequency regions for water absorbance in bone are similar to those in cartilage, it has not yet been determined whether there are specific collagen-associated water absorbances present. The goal of this study is to compare the MRI-derived cortical bone water measurements to NIR-derived water measurements obtained from human mid-tibia cortical bone samples to confirm the association of measured water with the collagen component.

Methods: Material and Methods: Tissues: Whole human tibias (n=10) were obtained from donors (age range 27 - 97, NDRI, Philadelphia, PA) and were stored frozen at -30º C until processing. Cross-sectional specimens 750 µm thick were cut from the region of maximum cortical bone thickness (i.e., 38% distance proximal to distal endplate) from the thawed tibia for NIR spectral imaging. The specimens were stored in phosphate buffered saline (12 mM) and protease inhibitor solution at 4º C prior to imaging. Two samples were decalcified by soaking in 25ml of 50mM Tris and 5mM EDTA chelating solution for 1 month, with solution changes every 2 days. MRI data collection and processing: 3D UTE imaging was performed on cortical diaphyseal bone segments approximately 5 cm in length using a 4-channel surface coil on a 3T whole-body clinical MRI scanner. Bound and pore water fractions were assessed by bi-exponential fitting of 23-echo UTE MRI data (3). NIR spectral imaging data collection: NIR spectral images of bone slices were collected using a Perkin Elmer Spotlight 400 spectrometer from bone samples. Data were collected in the frequency
The range of spectral resolution is 4000-7800 cm\(^{-1}\) at 64 cm\(^{-1}\) and 50\(\mu\)m pixel resolution using 2 co-added scans. The imaging time was approximately 22 minutes for each sample. Care was taken to minimize the water loss during imaging by covering the sample tightly between a glass slide and a coverslip. Data processing: NIR images of bone were analyzed using ISYS 5.0 software (Malvern Instruments, Columbia, MD). The inner trabecular regions were removed from the bone images prior to data processing by masking based on the fat absorbance (primarily from marrow) at 5792 cm\(^{-1}\). Second derivative (Savitzky Golay, 3rd order polymer and 7 points of smoothing) images of NIR spectra were analyzed to obtain mean intensities of NIR absorbance peaks at 7008, 4608 and 5792 cm\(^{-1}\) which represent the average content of bound water, collagen and fat, respectively, in the entire cortical bone section. The mean NIR peak intensity at 7008 cm\(^{-1}\) was correlated to the MRI bound water content and to the NIR-determined collagen and fat absorbances.

**Results:** NIR spectra from bone were dominated by water peaks at 5150 and 7008 cm\(^{-1}\), a collagen peak at 4608 cm\(^{-1}\) (5) and a shoulder at 4900 cm\(^{-1}\) (Figure 1). The spectral absorbances from fat were seen in the trabecular bone at 4384, 5664 and 5792 cm\(^{-1}\) (6). The IR images in Figure 2 show the distribution of collagen, fat and water content in the entire bone section. The bound water content from MRI data ranged from 61 to 90%. Our results showed that there was a significant correlation \((r=0.78)\) between the IR water parameter, 7008 cm\(^{-1}\), and the MRI derived bound water in cortical bone. There was also strong correlation \((r=0.72)\) between NIR water intensities at 7008 cm\(^{-1}\) and intensities of collagen absorbance at 4608 cm\(^{-1}\). There was not a significant correlation \((r=0.21)\) between the IR intensities of water absorbances at 7008 cm\(^{-1}\) and fat absorbance at 4608 cm\(^{-1}\).

**Discussion:** The significant correlation between the NIR derived water measurement and MRI derived water measurement showed that the results obtained with both methods were comparable. Similarly, the strong correlation between the collagen absorbance at 4608 cm\(^{-1}\) and water absorbance at 7008 cm\(^{-1}\) indicates that the water absorbance at 7008 cm\(^{-1}\) arise from the water that is bound to collagen component. In our decalcified bone data, we found that the water absorbance at 7008 cm\(^{-1}\) was related to collagen, as opposed to mineral, confirming this hypothesis. In summary, our study confirms the feasibility to assessing various constituents of cortical bone in a spatially resolved manner using UTE MRI and NIR. Further studies are required to find water compartments associated with fat and mineral components.

**Significance:** Since the UTE MRI experiment described in our study can be performed in human subjects, it provides a means to assess cortical bone composition in vivo.
Inverted second derivative spectrum of bone