Advances in Skeletal Dosimetry through Microimaging: Bone-Specific Assessment of Marrow Cellularity by H-NMR Spectroscopy. Aim 2- Validation of H-MRS Method *In Vivo*

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Introduction

The current gold-standard method for measuring bone marrow (BM) cellularity is histological analysis of a BM biopsy from the iliac crest. Problems with this method are that it is painful and highly invasive—which limits its use for repeated measurements—, it is based on a very small tissue volume, and cellularity measured at the iliac crest is most likely not indicative of cellularity values at other bone sites. Several studies have used proton magnetic resonance imaging (H-MRI) and proton magnetic resonance spectroscopy (H-MRS) to measure marrow cellularity non-invasively in humans. Cellularity is measured as the water fraction (WF)—the ratio of water volume to total volume. Few studies have investigated the accuracy of these methods using water/lipid phantoms and in vivo, comparing MR measurements to iliac crest biopsy measurements. We will compare BM cellularity measured with H-MRS to cellularity measured from histology at the same location on the limbs of fresh large dog cadavers. This study corresponds to aim 2 of a larger study which will conclude with a pilot study of cellularity measurements via H-MRS on human volunteers.

Experimental

Dog limbs will be obtained from the Department of Small Animal Clinical Sciences. Groups of three holes will be drilled at the proximal ends and shaft of each humerus and femur. *Introcan Safety* intra-venous (IV) catheters (B/Braun, Bethlehem, PA) will be used to insert 2 cm silicon tubes (about 1 mm in diameter) into each hole. These catheters define a volume of interest (VOI) at each location. Limbs will be scanned at the McKnight Brain Institute using a Phillips Achieva 3.0T MR scanner. Surface coils will be used to obtain STEAM and PRESS spectra at the physically-marked VOIs. WF will be calculated by dividing the area under the water peak by the area under the entire spectrum. Spectral analysis will be performed using the Java Magnetic Resonance User Interface (jMRUI) version 3.0.

Following spectroscopy scans, bone cubes will be extracted at the locations marked by the fiducials to be processed using standard histology protocols. Four microscope slides corresponding to two upper slices and two lower slices approximately equally spaced to cover the full range of the cube will be selected for imaging. The slides will be stained with hematoxylin and eosin (H&E) and iron stain. High resolution digital images will be produced from each slide. Adobe Photoshop will be used to randomly sample the digital images using square 500 μm regions of interest. Manual image segmentation will be used to remove adipocytes from the image in order to obtain a pixel count for water content in the image. The number of water pixels divided by the total number of pixels in the image will be used to determine WF.

Bland-Altman Plots of spectroscopy-measured WFs and histology-derived WFs from the dog limbs will be generated to characterize the accuracy of each H-MRS method. In addition, the precision of each method will be characterized from the repeated measurements on the same day and consecutive days. STEAM and PRESS will be compared in regards to accuracy and reproducibility. Only the best of the two methods will be used in aim 3. A plot of accuracy versus size of VOI will allow us to determine the effect of VOI size on accuracy of the H-MRS method. This is an important analysis since aim 3 requires measurements on different bone sites on the canine skeleton that may not accommodate large VOIs.

Results and Discussion/Conclusions

This project has not been started. The dog limb study will begin at the end of this month. We received IACUC approval to complete the study on October 30, 2007 (IACUC#F115).