ESRF	**Experiment title:** Evaluation of Bone Structure and Mineralization in IGF-I Deficient Fetal Mice	**Experiment number**: MD25
Beamline: ID19	**Date of experiment**: from: 21-JUN-03 to: 24-JUN-03	**Date of report**: 16-FEB-05
Shifts:	**Local contact(s)**: X. Thibault	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

Andrew Burghardt [1*], Yongmei Wang [2], Hashem Elalieh [2], Xavier Thibault [3*], Daniel Bikle [2], Francoise Peyrin [3*], Sharmila Majumdar [1]

[1] Department of Radiology, University of California, San Francisco
[2] Department of Medicine, University of California/Endocrine Unit VAMC
[3] ESRF and CREATIS

Report:

Introduction

Insulin-like growth factor I (IGF-I) has previously been established as an important regulator of skeletal metabolism. Our previous work in IGF-I deficient adult mice has demonstrated the importance of IGF-I action on both bone formation and resorption [1]. However, the role of IGF-I during prenatal development has not been evaluated in detail. The commercial micro-computed tomography (μCT) systems used to study adult animals, lack the resolution and image quality required to adequately capture the small, low density trabecular tissue seen during prenatal development in mice. The unique advantages of synchrotron based micro-tomography (high flux, monochromaticity, parallel beam geometry) could make it possible to image such tissue with enough detail to visualize and quantify the 3D micro-structure. In this preliminary study (MD25) synchrotron radiation micro-tomography (SR-μCT) has been used to evaluate the structural and material effects of IGF-I deficiency in fetal mice at the 18th day of gestation.

Methods

Mice heterozygous for the IGF-I gene were bred and the females sacrificed at the 18th day of gestation. Each fetus was genotyped to identify IGF-I deficient (IGF-I -/-) and wildtype (WT) animals. The Lumbar spine and tibia were excised from 6 IGF-I -/- and 6 WT controls. Samples were fixed in 70% ethanol and air dried prior to imaging.

Each tibia and spine sample was attached to the end of a small pin with wax and mounted on a motorized stage designed for micro-tomography experiments on the ID 19 beamline [2]. Monochromatic 10keV SR x-rays were directed at the sample/detector assembly and 1000 projection images were acquired over 180 degrees. A 3D filtered back-projection algorithm was employed to reconstruct tomographic images across a 1024x1024x1024 matrix spanning a 1 mm^3 field of view and resulting in an isotropic voxel size 0.97μm.

All image datasets were processed with software provided by a commercial μCT manufacturer (Scanco Medical AG, Bassersdorf, Switzerland). Semi-automatically drawn contours were generated to define a

region of interest (ROI) for histomorphometric and densitometric analysis. The ROI for the tibia consisted of 300 slices (~300μm) in the proximal metaphysis starting at the end of the growth plate and extending distally. The full cancellous compartment of a lumbar ossification center was selected for each spine. A light Gaussian filter ($\sigma = 0.5$, kernel = 3) to remove high frequency noise, followed by a fixed threshold was used to segment the images into a bone and marrow phase.

Structural indices (BV/TV, Tb.Th, Tb.N, Tb.Sp, DA, and SMI) were calculated as detailed in our previous μCT study with adult animals [1]. The degree of mineralization of the bone phase (DMB) was determined from the original greyscale images. The binary image map from the segmentation step was eroded by two pixels to remove partial volume components and then used to mask the greyscale data. The mean linear attenuation was calculated as the sum of the masked image divided by the number of bone voxels in the mask. This value was converted to mineral density (mg HA/cm3) using a theoretical model of x-ray attenuation in bone described by Nuzzo et al [3].

Results

Representative images for the spine and tibia of IGF-I -/- and WT animals are shown in Figure 1. Structure and densitometric analysis showed genotypic and anatomic differences. While there was no significant difference in bone volume between anatomic sites for the WT animals, the proximal tibia was found to be more plate-like (47%, $p < 0.05$), more anisotropic (20%, $p < 0.0001$), with slightly thicker trabeculae (5%, $p < 0.05$), larger Tb.Sp (30%, $p < 0.05$), and a lower Tb.N (21%, $p < 0.05$). DMB, derived from the mean bone greyscale value, was significantly higher (38%, $p < 0.0001$) in the spinal ossification center compared to the proximal tibia.

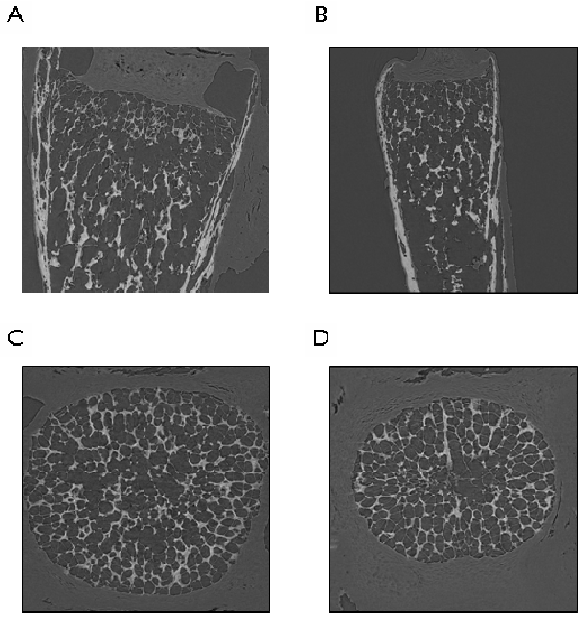

Figure 1: (A) WT tibia proximal metaphysis (B) IGF-I -/- tibia proximal metaphysis (C) WT spinal ossification center (D) IGF-I -/- spinal ossification center.

Bone volume was systematically lower in IGF-I -/- animals although this difference was only significant in the proximal tibia (-15%, $p < 0.05$). The spine and tibia of IGF-I deficient animals were also found to have a more rod-like architecture ($p < 0.05$, $p < 0.01$), and larger trabecular separation ($p < 0.05$, $p < 0.05$). Trabecular thickness tended to be lower but statistical significance was only found in the spine (-16%, $p < 0.001$). Tb.N was significantly higher in the Spine (17%, $p < 0.05$) while DA was significantly lower in the tibia (-10%, $p < 0.0001$). DMB was found to have only a minor reduction (-6.8%, $p < 0.0001$) in the knockout lumbar spine. In the tibia, DMB was slightly higher (8.2%, $p < 0.001$) in IGF-I -/- animals.

Conclusions

To our knowledge, this study represents the first time that the cancellous bone of pre-natal mice has been imaged in 3D with a resolution sufficient to resolve individual trabeculae. At 18 days, IGF-I deficient embryos were found to have significant material and structural differences from their controls in both the lumbar spine and proximal tibia. However, the degree of differentiation was dependent on anatomic site. Furthermore, the structural differences did not always follow the same patterns seen in adult IGF-I deficient mice. Interestingly, the density results for the spine were not in agreement with our previous histological findings. Experiments with different techniques (FTIR, SEM) are in progress to address this discrepancy. Finally, follow-up SR-μCT experiments at different gestational time points could shed further light on the role of IGF-I during pre-natal development.

References

[1] D. Bikle, S. Majumdar, A. Laib, L. Powell-Braxton, C. Rosen, W. Beamer, E. Nauman, C. Leary, and B. Halloran, "The skeletal structure of insulin-like growth factor I-deficient mice," *J Bone Miner Res*, vol. 16, pp. 2320-9, 2001.

[2] M. Salome, F. Peyrin, P. Cloetens, C. Odet, A. M. Laval-Jeantet, J. Baruchel, and P. Spanne, "A synchrotron radiation microtomography system for the analysis of trabecular bone samples," *Med Phys*, vol. 26, pp. 2194-204, 1999.

[3] S. Nuzzo, F. Peyrin, P. Cloetens, J. Baruchel, and G. Boivin, "Quantification of the degree of mineralization of bone in three dimensions using synchrotron radiation microtomography," *Med Phys*, vol. 29, pp. 2672-81, 2002.