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### FOR MORE INFORMATION

Lisa M. Miller, National Synchrotron Light Source, Brookhaven National Laboratory  
lmiller@bnl.gov

## Accretion of Bone Quantity and Quality in the Developing Mouse Skeleton

L.M. Miller,<sup>1</sup> W. Little,<sup>2</sup> A. Schirmer,<sup>1</sup> F. Sheik,<sup>2</sup> B. Busa,<sup>2</sup> and S. Judex<sup>2</sup>

<sup>1</sup>National Synchrotron Light Source, Brookhaven National Laboratory; <sup>2</sup>Department of Biomedical Engineering, Stony Brook University

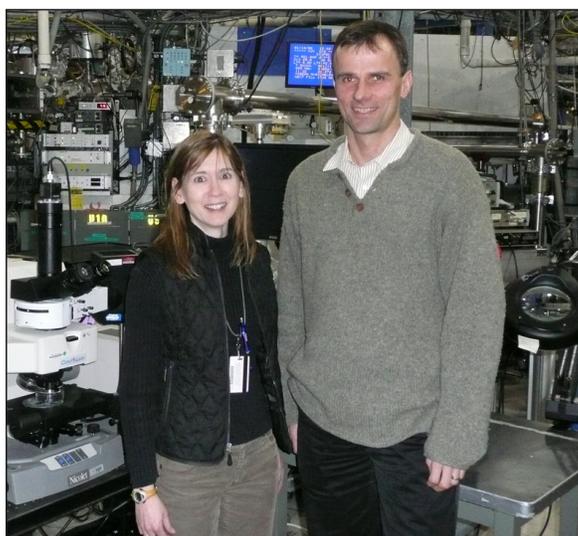
*In this work, synchrotron infrared microspectroscopy was used to image the chemical composition of young mouse bone and these results were directly correlated with nanomechanical testing and x-ray micro-computed tomography (micro-CT) of the same sample. We found that bone mineral formation proceeded very rapidly in mice by one day of age, where the degree of mineralization, the tissue mineral density, and the mineral crystallinity reached 36%, 51%, and 87% of the adult values, respectively. However, even though significant mineralization had occurred, the elastic modulus of one-day-old bone was only 14% of its adult value, indicating that the intrinsic stiffening of the bone lags considerably behind the initial mineral formation.*

Bone mineral density (BMD) measurements are the most common way to assess osteoporosis fracture susceptibility in the clinical setting. However, it has become increasingly clear that there is a substantial overlap in the BMD of normal individuals and patients who sustain fractures. These findings suggest that the quality of bone, in addition to bone quantity, is important for defining fracture risk. An important contributor to bone quality is the chemical makeup of bone. It is well established that the chemical composition and mechanical properties of bone change with age. However, the interdependence between bone's specific chemical makeup (e.g. mineral content, composition, and crystallinity; collagen content, structure, and cross-linking) and its corresponding mechanical properties (e.g. strength, toughness, stiffness) is still poorly understood. In this work, we evaluated the chemical, structural, and mechanical properties of the mouse tibia dur-

ing the first 40 days of life using synchrotron IR microspectroscopy, microCT, and nanoindentation, respectively. All techniques were performed on identical samples so that direct correlations could be made. The focus of this investigation was at the microscopic level, where we tested the hypothesis that specific compositional properties of bone determine the stiffness of the tissue.

Results showed that there was considerable bone quantity and density present in the mouse tibia at birth. At 1 day of age, the degree of mineralization (phosphate/protein ratio), the density of mineralized bone (TMD), and mineral crystallinity had reached 36%, 51%, and 87% of the adult values, respectively (**Figure 1**). Spatially, the variability in mineralization across the mid-tibia was very high for the early time points and declined over time (**Figure 2**). In contrast to the notable changes in mineralization, carbonate substitution into the mineral lattice (carbonate/phosphate ratio) and collagen cross-linking did not show any significant changes over this time period.

Despite the fast accretion of bone quantity during early development, the process of bone stiffening lagged behind. The elastic modulus of 1-day-old bone was only 14% of the adult value and increased to



Authors (from left) Lisa Miller and Stefan Judex

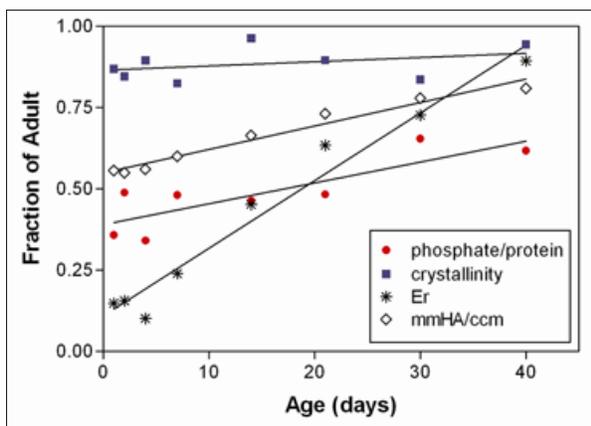
89% after 40d. Between samples of different time points, significant positive correlations were observed between the elastic modulus and TMD ( $R^2=0.84$ ), phosphate/protein ratio ( $R^2=0.59$ ), and crystallinity ( $R^2=0.23$ ), whereas collagen cross-linking showed a small but significant negative correlation ( $R^2=0.15$ ).

This initial lag in elastic modulus may be associated with the specific locations of the mineral crystals within the collagen fibrils. Electron micrographs have shown that, in early mineralization, the overall accumulation of mineral mass is predominant in the collagen "hole

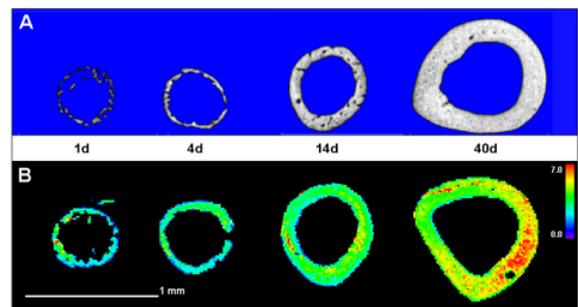
zones" compared to "overlap zones," where 64% of the crystals were located in the collagen hole zones. Since the elastic modulus of the mouse bone remained low until the tissue mineral density reached ~65% of its adult value, it is possible that mineral accumulation in the hole zones has little effect on bone's intrinsic stiffness. However, once the mineral content increased to where the overlap zones become mineralized, the elastic modulus increased rapidly as well.

In summary, these results indicate that specific chemical and structural properties modulate bone's stiffness during early growth and

suggest that changes in bone may be co-regulated by similar genes during this development period. The intrinsic stiffening of the bone, however, lags considerably behind the initial mineral formation, emphasizing the importance of bone mineral quality for optimizing matrix integrity. With clear evidence that bone's chemical properties and micro-structure play an important role in defining the micro-mechanical properties of the skeleton during growth, a better mechanistic understanding of the underlying processes may enable the diagnosis, prevention, and treatment of poor bone quality.



**Figure 1.** In this study, tibiae of female BALB mice were harvested at 8 time points ( $n=4$  each) distributed between 1d and 40d of age. Tibiae of 450d old mice served as fully mineralized control specimens. **(A)** Micro-CT and **(B)** FTIRM phosphate/protein images of the mouse mid-diaphysis at 1d, 4d, 14d, and 40d of age. All images for each technique are plotted on the same intensity scale for direct comparison. Results showed that the spatial variability in mineralization across the mid-tibia was very high for the early time points and declined over time.



**Figure 2.** Phosphate/protein, crystallinity, elastic modulus, and TMD as a function of age. In order to compare all parameters on a common scale, the y-axis values represent the fraction of the adult (15mos) value. Results showed that bone mineralization (phosphate/protein ratio, crystallinity, and TMD) proceeded quickly at an early age, whereas bone stiffness (elastic modulus) lagged behind. This initial lag in elastic modulus may be associated with the specific locations of the mineral crystals within the collagen fibrils.