INTRODUCTION  It has long been known that variations in the microstructure of bone have a profound effect on the mechanical properties of the tissue and that these properties vary significantly between and within the cortices of bones. However, the microstructural basis and the functional significance of such variation remains poorly understood. One reason for this is the difficulty in acquiring large enough samples of a material comprised only of that particular structural type of interest for mechanical testing [1]. The availability of depth-sensing nanoindentation instruments with capabilities for measuring displacements on the order of nanometers makes it possible to measure the properties of the small microstructural features in bone. The primary aim of this research was to investigate the relationship between microstructure and nanoindentation properties of intramuscular herring bones.

MATERIALS AND METHODS  Intramuscular herring bones, which have a simple microstructure: collagen fibrils that are believed to have a single orientation (longitudinal) with a variation in mineralization along the length, have been used [2]. The apex region represents bone tissue at the earliest stages of calcification. The end nearest the backbone is fully mineralized. Nanoindentation properties were measured both at the longitudinal direction in the transverse section and at the transverse direction in the longitudinal section along the length.

The crystal orientation was examined with high-intensity synchrotron x-ray radiation at the National Synchrotron Light Source (Brookhaven National Laboratory). The coherence length and angle spread were measured to confirm that the crystals were aligned in the same orientation along the entire length of the bone so that the mechanical data could be ascribed to structural features. The coherence length (CL) represents the average distance between imperfections in specific crystallographic directions. The angle spread (AS) characterizes the degree of misalignment between perfectly coherent domains [3]. To confirm the correlation between nanoindentation properties, the coherence length, and the angular spreads, linear regressions were performed.

RESULTS  The elastic modulus in the fully mineralized regions was approximately 20 GPa and 13 GPa in the longitudinal and transverse directions, respectively. The elastic modulus decreased as moving toward the apex region of the earliest stages of mineralization in the both longitudinal and transverse directions was approximately 5 GPa (Figure 1). The anisotropic ratio \( E_{\text{longitudinal}}/E_{\text{transverse}} \) was decreased from 1.5 to 1.0 as moving toward the apex region of
the earliest stages of calcification. The properties at the early stages of mineralization demonstrated isotropic.

The variation in elastic modulus with orientation of the specimen axis to the long axis of the bone at the fully mineralized regions was between 20 GPa and 13 GPa and decreased montonically. The coherence length in the longitudinal direction progressively increased with full mineralization from 28 nm to 32 nm. However, crystal size could not be measured in the other directions because angular spread was wide. Nanoindentation properties was positively correlated with coherence length (r=0.85, p=0.03).

Figure 1. Elastic modulus of intramuscular herring bones in the both longitudinal and transverse direction along the length (degree of mineralization).

DISCUSSION In lamellar bone, a broad distribution of the fiber axis for collagen has been observed due to the local meandering, kink, and twisting of collagen fibers in the bone matrix. However, it has been shown that the ultrastructure of intramuscular herring bones was relatively simple microstructure, having collagen fibril aligned in the long axis [2]. Because it has long been assumed that collagen orientation directly influences the orientation of the minerals nucleated within the hole zone of the collagen, it is interesting to see if crystal orientation is aligned with that of collagen fibril. Indeed, the crystal and collagen were all aligned in the same orientation along the entire length of the bone.

At the earliest stages of calcification, the angular spreads were isotropic in different crystallographic directions. In the course of maturation, the angular spreads showed a tendency toward anisotropy. The higher degree of mineralization was due to the larger crystal size, which enhanced the stiffness of the tissue. Both the size and orientation of the bone mineral crystals were measured and identified as determinant factors for the mechanical properties of bone.

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