AGE-RELATED EFFECT OF BONE REMODELING ON COLLAGEN CROSSLINKS IN HUMAN CORTICAL BONE

INTRODUCTION: Age-related bone fractures are a major concern of health care of elderly populations. Although bone mass loss has been considered a major cause of such fractures, recent studies have shown that the quality of the collagen network also plays a significant role in reduced toughness of bone.1,2 However, the underlying mechanisms are still not well understood. To address this issue, the objective of the present study was to investigate the age-related effect of bone remodeling on the collagen molecule integrity in human bone. First, novel methodologies were developed to collect and test bone specimens directly acquired from newly formed secondary osteons and interstitial bone regions, but not in the interstitial bone regions. In this study, the newly formed secondary osteons are considered to be new bone tissues formed in the bone remodeling process, whereas bone tissues in the interstitial bone regions are deemed to be old bone tissues and are not affected by bone remodeling process.

MATERIALS & METHODS: To collect bone specimens from both secondary osteons and interstitial bone regions, a novel approach using a miniature specimen puncher (Figure 1) was developed. Briefly, a cross-section of femurs was first cut and polished to a 1.0-mm thick slice. Then, the assembly of the specimen holder and the base block was placed on the stage of a light microscope and adjusted until the center of the puncher assembly coincided with the central axis of the microscope (Figure 2 (a)]. After the puncher is positioned, it was fixed there and the bone slice prepared was placed in the specimen-mounting chamber. By adjusting position of the bone slice in the chamber, the center of the area of interest (e.g., secondary osteons or interstitial bone regions) was aligned with the viewing center so that it coincides with the central axis of the puncher (Figure 2 (b)]. After the bone specimen was aligned, the guiding block of the puncher is placed on top of the specimen holder to hold the specimen in place. The puncher tip then is carefully inserted through the guiding hole until touching the bone specimen. The bone specimen from the area of interest was punched out by tapping the back of the puncher tip using a hammer (Figure 2 (c)]. Finally, the bone specimen was collected in the specimen-collecting chamber of the base block of the puncher assembly. Finally, the specimens collected using this approach were stored in buffered saline solution for collagen analysis. To investigate age-related changes in collagen, twelve femurs were collected from Musculoskeletal Transplant Foundation and the Willed Body program in the state of Texas and divided into two age groups (n=6): young (< 45 years of age) and elderly (> 70 years of age), respectively. Using the punching approach aforementioned, six bone specimens (about 0.25 mm x 1.0 mm each) were collected from cross sections of anterior aspects of femoral diaphysis of each femur and pooled for later measurements. The collected bone specimens from the osteonal and interstitial bone regions were analyzed using high performance liquid chromatography (HPLC) techniques described elsewhere.3 In this study, the concentrations of two mature enzymatic collagen crosslinks (LP-lysylpyridinoline and HP-hydroxylysylpyridinoline) and a marker of non-enzymatic glycation induced collagen crosslinks (PE-pentosidine) were measured for the bone specimens from the two distinct anatomical regions. A two-factor ANOVA analysis was performed to determine the effect of age and anatomical sites on the concentration of the collagen crosslinks measured. post hoc multiple comparisons were also performed to detect the differences between the two age groups. The significant differences were considered only if p < 0.05.

RESULTS: ANOVA analyses demonstrated that both age and anatomic sites (e.g., secondary osteons and interstitial bone regions) have significant effects on the concentration of non enzymatic crosslinks (PE as a marker) (p < 0.05), but have little effects on the enzymatic collagen crosslinks (HP and LP) in the collagen network (p > 0.05). Table 1 shows the experimental results. In addition, little changes in the collagen crosslinks were observed in the interstitial bone regions between the two age groups, except for HP, which showed a significant difference

DISCUSSION: The results of the present study suggest that age-related changes in collagen crosslinks mainly occur in the newly formed secondary osteons. Since the newly formed secondary osteons are the products of bone remodeling process, this result provides direct evidence that bone remodeling is involved in the age-related changes in collagen. In addition, enzymatic crosslinks of collagen also appear to vary with age and anatomical sites (interstitial and osteonal regions), suggesting that bone formation induced by bone remodeling process is age-dependent.

ACKNOWLEDGEMENT: The present study was supported by a Whitaker Foundation grant. We thank Mr. K. Hricisak for his technical assistance in fabricating the punching device.

References: