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Diffuse Damage Accumulation with Age is Cortex and Gender-Dependent in Human Cortical Bone

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Diffuse Damage Accumulation with Age is Cortex and Gender-Dependent in Human Femoral Cortical Bone

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Introduction
Bone damage (i.e. linear microcrack and diffuse damage) accumulation is believed to play a significant role in stiffness loss and bone fragility [1-3]. The accumulation of linear microcracks with age has been well established [4,5]. However, the accumulation of diffuse damage with age is less well understood. Previous reports have shown no change in diffuse damage area with age in human femoral cortical bone [6] and in vertebral trabecular bone [7]. A recent study [8] however suggests that old and new bone accumulate diffuse damage differently in the anterior cortex of cortical bone from the tibia. The objective of this study was to determine if diffuse damage accumulation in human femoral cortical bone depends on cortical bone site and if it is age related. Results of this study may help identify biological features responsible for diffuse damage accumulation.

Methods
Forty-six fresh human femurs were harvested from 24 male (22 to 91 years; average age = 61.8 ± 19.9 yrs.) and 22 female cadavers (24 to 94 years; average age = 61.0 ± 20.0 yr) and 22 female cadavers (24 to 94 years; average age = 61.0 ± 20.0 yr) and 22 female cadavers (24 to 94 years; average age = 61.0 ± 20.0 yrs.). Parallel cross-sectional cuts were made 1 cm apart at the proximal right femur. The sections were stained with basic fuchsin (BF), cut into four quadrants (anterior, posterior, medial and lateral) with a metallurgical saw and embedded in plastic. An 80μm thick transverse slice was removed from the center of each quadrant with a wire saw and mounted for examination using a fluorescence microscope at a magnification of 125X. Five fields were randomly chosen from each slide (quadrant). Diffuse damage areas were identified as focal areas of diffuse staining with or without damage apparent within them. Diffuse damage area density parameter (Df.Dm.Ar) was defined as the ratio of the total damaged area (Dm.Ar) and bone area (B.Ar) (Df.Dm.Ar=Dm.Ar/B.Ar, mm2/mm2). Analysis started with examination of interactions using a full-factorial mixed ANOVA model of diffuse damage with age, gender and quadrant effects. Groups were separated in ANOVA models when significant interactions were found. Significant relationships were examined using regression analysis. JMP™ (SAS Institute, Cary, NC) was used for all analyses. Significance was set at p<0.05.

Results
A significant three-way interaction indicated that the relationship of diffuse damage with age depends on which gender and which quadrant are considered (p<0.03). The change in diffuse damage in the lateral cortex was significant for both males (R²=0.22, p<0.02) and females (R²=0.21, p<0.03) but the trends were opposite between genders, increasing with age in females (Figure 1) but decreasing with age in males (Figure 2). The relationship of diffuse damage with age was not significant in other cortices for separate male and female groups (p=0.10 to p=0.62) but a marginally significant positive trend was found for the anterior cortex in the pooled male and female groups (p=0.0519).

Df.Dm.Ar was not significantly different between the anterior (mean = 2.49% ± 2.05%), posterior (mean = 2.46% ± 1.74%), medial (mean = 2.65% ± 2.15%) and lateral (mean = 2.30% ± 2.11%) cortices (p=0.69) or between males (2.65% ± 1.80%) and females (2.29% ± 2.19%) (p=0.37). The results also revealed that Df.Dm.Ar averaged over cortices did not change with age (p=0.81).

Discussion
Our results show a significant linear relationship between diffuse damage area and age in the lateral cortex of the femur that increased with age for females and decreased with age for males. Previous work also showed a significant difference between diffuse damage area in young and old bone taken from the anterior cortex of the tibia, with young bone displaying significantly more diffuse damage than old bone suggesting a decrease in diffuse damage with age [8]. This finding in the anterior cortex of the tibia has been associated with loading in tension. The relationship of diffuse damage with age in the lateral cortex of the femur in the current study is consistent with the results from the tibia in that the anterior and lateral cortices of the proximal femoral diaphysis are predicted to experience tensile rather than compressive in vivo strains [9]. However, the difference in trends between genders in the current study suggests that loading mode alone is not sufficient to explain the modulation of diffuse damage in the tissue but an interaction between loading mode and gender-specific hormones may be necessary. Differences in bone composition and morphology also exist between cortical bone of the femur and tibia which may explain different relationships and trends between the two bones. Previous work has shown that the occurrence of diffuse damage area is higher in secondary osteons [8] and significantly decreases with increasing mineral content [6]. Previous studies have also demonstrated differences in the age-related relationships of osteonal area and mineral content in the femur and tibia [10] with the femur developing more osteonal area and mineral percentage with age. Together, these data suggest that what appears to be opposite trends in the accumulation of diffuse damage area with age in the femur and the tibia is likely due to age-related differences in bone composition and morphology in the femur and the tibia. It is hypothesized that these differences are due to remodeling rates or other processes adaptive to mechanical factors that are different between males and females and affect the femur and tibia differently.

References