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MICRODAMAGE MORPHOLOGY AND DISTRIBUTION IN THE FATIGUE-LOADED RAT ULNA: EFFECTS OF AGE AND STRAIN MODE ON OSTEON-RELATED REPAIR

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INTRODUCTION: Osteon and hemi-osteonal formation is integral in maintaining skeletal homeostasis. Deterioration of homeostasis is not only associated with the reduction of bone mass, but also with the degradation of bone tissue mechanical properties (i.e. bone "quality"), both which lead to fractures. In cortical bone, bone quality is greatly enhanced by regional prevalence of specific osteon 'morphotypes' that are distinguishable by their collagen organization (e.g. parallel-fibered vs. alternating 2nd osteons) [1,2]. These morphotypes differentially affect energy absorption by influencing osteon pullout and microdamage (mdx) propagation [3,4]. Studies of human and non-human bones have shown that spatial distributions of morphotypes are associated with a prevalent/ predominant habitual strain mode (i.e. tension vs. compression) between regions of the same bone [5,6]. These structure/function relationships are also clinically important because they directly correlate with healthy tissue. The idea of preventing the degradation of this relationship that occurs with age poses a compelling challenge — can the mechanisms involved in spatial/temporal formation of osteon 'morphotypes' be controlled and ultimately used in attenuating bone senescence? This investigation employs an animal model as a means toward the end of controlling 2nd osteon construction. Regional differences in osteon morphotypes might form in response to differences in mdx morphologies or strain environments (e.g. tension vs. compression) [7]. Therefore, using the rat ulna loading model (**Fig.1A**) we examined the affects of age and strain mode on mdx and resorption formation. We tested the hypothesis that location (medial 'compression' vs. lateral 'tension' **Fig.1B**) and age correlate with differential mdx/resorption production that, in turn, correlates with the formation of distinctive osteon 'morphotypes' in the compression/tension cortices.

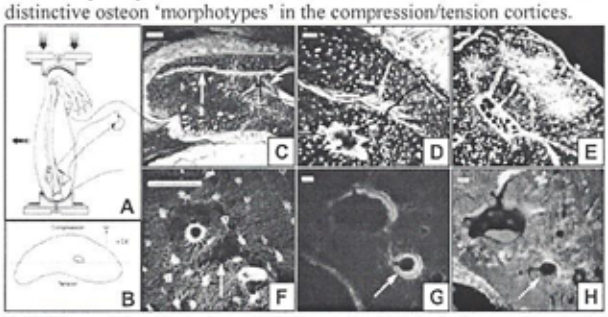
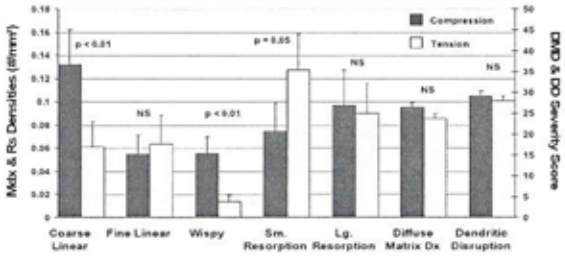


Fig.1 A) Diagram of the rat ulna loading model B) Cross-section of rat ulna showing medial 'compression' and lateral 'tension' cortices C) Coarse linear mdx (arrow) and fine linear mdx (arrowhead) D) Wispy mdx E) Diffuse matrix damage F) Dendritic disruption G) FITC image showing tetracycline-labeled infilling of a resorption space H) CPL image of the same section as in G; All scale bars = 50 microns

METHODS: Twenty-four male Fischer rats (12, 5 month-old, 351g ± 22g; 12, 15 month-old, 430g ± 18g) were obtained from the National Institute on Aging (Bethesda, MD). With IACUC approval, 8 rats of each age formed the experimental groups and the remaining animals were used for load/strain calibration. The right forearms were subjected to cyclic axial compressive loading from the flexed carpus to the olecranon using a pneumatic-driven loading apparatus (AMTI, Watertown, MA). The load strain calibration experiment showed that 3000 µstrain was sufficient to produce ~ 85% of fracture displacement which has been shown to cause a "high" level of mdx [8]. Fatigue loading was performed at 2 Hz with a load force of 20-25 N for 5 and 15 month-old rats respectively. The contralateral ulnae served as non-loaded controls. In each group one ulna fractured during loading leaving them with n=7. All animals ambulated normally within 24 hours. After 18 days the animals were sacrificed by CO_2 inhalation, and the ulnae were dissected free of soft tissue, fixed in EtOH, and bulk stained en bloc in 1% basic fuchsin (Mallinckrodt Baker, Inc.). Ten transverse sections were cut from the mid-third diaphysis, mounted on slides and ground to ~50µm. Mdx entities were quantified in medial ('compression') and lateral ('tension') regions using a PCM-2000 confocal microscope (Nikon, Melville, NY). Bright-field and circularly polarized light (CPL) microscopy was also used to examine each section for damage and osteon 'morphotypes.' The specific mdx entities quantified included: coarse linear, fine linear, wispy, diffuse matrix damage (DMD), and dendritic disruption (DD) (**Fig.1**). Among the mdx entities, DD could only be quantified using a visual analogue scale derived severity score. Resorption space densities (Rs.N/Ct.Ar) were quantified in two groups: ≤50µm and >50µm diameter. Kruskal-Wallis ANOVA was employed for comparisons with significance set at $p<0.05$.

RESULTS: In support of the initial hypothesis, there were significant differences in mdx densities, including DD mdx, between young and old animals, with the young having significantly greater mdx (2.1/mm² vs. 1.1/mm², $p < 0.01$). However, the older animals showed significantly greater Rs.N/Ct.Ar. Significant 'compression' vs. 'tension' differences in Rs.N/Ct.Ar were found only with Rs ≤50 µm diameter and only in the older rats (**Fig.2**). There were significant compression vs. tension differences in the coarse linear and wispy mdx types, which occurred in both groups (**Fig.2**). Considering all mdx entities (excluding DD) in each group, there was greater mdx density in the medial 'compression' cortex vs. lateral 'tension' cortex in young (1.4/mm² vs. 0.5/mm², $p < 0.001$) but not in older animals (0.6/mm² vs. 0.5/mm², $p = 0.6$). The hypothesis that distinctive mdx entities would correlate with tension- and compression-specific osteon 'morphotypes' could not be adequately tested, because only a few of the resorption spaces filled in by 18 days post-loading (**Fig.1G**). Consequently, our CPL examination for osteon 'morphotypes' only revealed a few completed 2nd osteons (**Fig.1H**). Nevertheless, 63% of resorption spaces were found to be associated with mdx (within 50µm of a mdx entity) supporting the possibility that they were repairing mdx.

Fig. 2 Mdx and Resorption Morphology and Distribution in 15 MO Rats



DISCUSSION: Results of this study showed age- and strain-mode-related differences in mdx and resorption densities between the medial 'compression' and lateral 'tension' regions. However, our protocol did not allow sufficient time for osteon infilling even though previous studies noted that intracortical remodeling in the rat ulna is well underway at 10 days post fatigue [9]. For this reason we could not adequately test the hypothesis that specific 2nd osteon 'morphotypes' correlate with specific mdx forms or densities. Although 2nd osteons are not known to occur in laboratory rats under typical circumstances, they do form after metabolic stress (calcium deprivation) [10] and as a consequence of fatigue loading [11]. Therefore, if more time is allotted for osteon infilling (up to 28 days) [11], then the rat ulna loading model still appears to be viable for investigating the mechanisms regulating osteon construction. Additional studies are warranted to determine if specific forms of mdx correlate with the formation of specific osteon 'morphotypes' that have been described in higher mammals. The significant medial-lateral mdx differences might reflect the strain-mode-related differences in thresholds for mdx formation. Our data are paradoxical in view of previous studies suggesting that mdx forms more readily in tension because ambient strains in tension are typically lower than those in compression. An additional important finding of the present study is that some forms of mdx quantified were not closely spatially associated (i.e., not within 50µm) with remodeling activity. For example, our findings are consistent with findings of Bentolila et al. [9] showing no obvious association of diffuse matrix mdx with the activation of repair-directed remodeling. [Supported by OREF 01-024]

REFERENCES: **1)** Martin et al.: *Bone* 1996; 19:165. **2)** Skedros et al. *Tran Orthop Res Soc* 2006; 1600. **3)** Hiller et al.: *J Orthop Res* 2003; 21:481. **4)** Bigley et al.: *J Biomech* 2006; 39:1629. **5)** Carando et al.: *Bone* 1989; 10:139. **6)** Kalmey and Lovejoy: *Bone* 2002; 31(2):327. **7)** Skedros et al.: *J Morphol* 2004; 259(3):281. **8)** Uthgenannt and Silva: *J Biomech* 2006 in press. **9)** Bentolila et al.: *Bone* 1998; 23(3):275. **10)** Ruth: *Am J Anat* 1956; 93:429. **11)** Noble et al.: *Am J Physiol Cell Physiol* 2003; 284:C934.