Abstract 80: Orthopaedic Research Society; Vol. 35. 2010.

Microdamage Morphology and Distribution in the OVX Fatigue-Loaded Rat Ulna: Effects of Absolute Estrogen Status and Strain Mode on Microdamage Accumulation and Repair

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INTRODUCTION: Osteoporotic fragility fractures and stress fractures are thought to be attributed to the accumulation and coalescence of bone microdamage (mdx) (1, 2). These fractures are usually prevented by bone remodeling processes that are targeted to areas of mdx (3, 4). Osteocyte apoptosis that occurs in areas of damaged bone may act as a localizing signal for osteoclastic resorption and subsequent repair (5, 6). Factors affecting how and where this mdx accumulates and is repaired include age, sex, load strain-mode (compression, tension, shear), and the amount of previous strain-mode-specific adaptation (7-9). Hormones such as estrogen (est.) also play a significant role by regulating apoptosis of osteocytes, osteoblasts, and osteoclasts (10, 11). To better understand the events that proceed fragility fractures and the impact of est. on this process, we utilized the rat ovariectomy (ovx) fatigue loading model (12) to test the hypothesis that the lack of est. would lead to a higher amount of mdx accumulation following a single bout of fatigue loading and 18 days of healing time. In addition, we hypothesized that there would be stronger strain-mode correlations with specific mdx types in est. deficient animals due to less repair of fatigue damage.

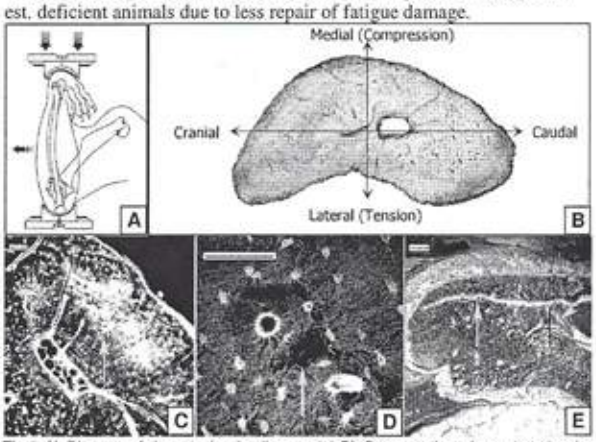
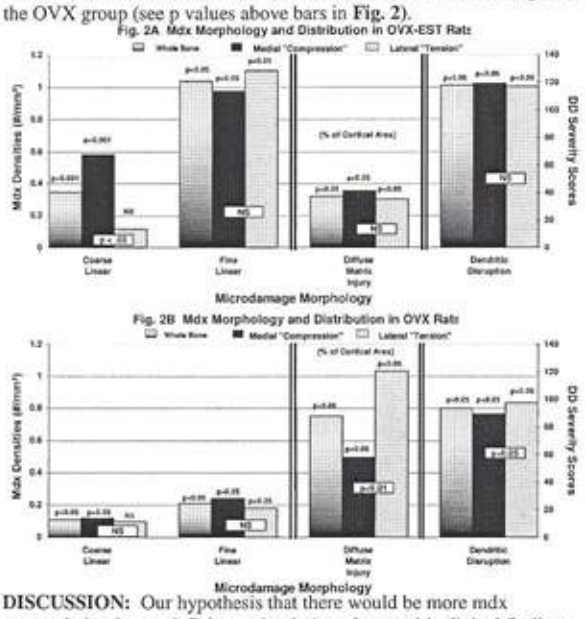


Fig.1 A) Diagram of the rat ulna loading model B) Cross-section of rat ulna showing medial 'compression' and lateral 'tension' cortices C) Diffuse matrix damage D) Dendritic disruption E) Coarse linear mdx (arrow) and fine linear mdx (arrowhead); scale bars=50μ

METHODS: Following IACUC approval, 36 five m.o. female Fischer 344 rats were obtained from the Natl. Inst. on Aging (Bethesda, MD). Six rats were used for load/strain calibration and the remaining 30 were divided into two experimental groups (OVX and OVX-EST). To control the absolute est. status of the animals, both groups underwent ovx and only the OVX-EST group was repleted with daily β-Estradiol (0.05 mg/kg) injections, while the OVX group remained est. deficient. The animals were given 7 days to recover from surgery before mechanical loading of their forelimbs (**Fig. 1A**) was performed at 2 Hz and 3000 μstrain (20-25 N). This produced ~ 85% of fracture displacement which has been shown to cause a "high" level of mdx (13). All animals ambulated normally within 24 hours. During fatigue loading two animals suffered fractures in each experimental group leaving N=13. The contralateral ulnae served as controls. All animals were sacrificed 18 days following fatigue loading by CO_2 inhalation. 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 (**Fig. 1 B**) using a PCM-2000 confocal microscope (Nikon, Melville, NY). The specific mdx types quantified included: coarse linear microcracks (> 100 microns in length), fine linear microcracks (< 100 microns in length), diffuse matrix injury (DMI), and dendritic disruption (DD) (**Fig. 1C-E**). DMI was calculated as a percentage of cortical bone area while DD was quantified using a severity score wherein each entity was given a score of 5 points then divided by cortical area. Two investigators independently quantified DD and the results were averaged. Kruskal-Wallis Z tests were employed for comparisons with significance set at $p<0.05$.

RESULTS: **Fig. 2** highlights the results of the mdx quantification. Intra-group medial ("C") vs. lateral ("T") comparison showed a significant difference in the coarse linear mdx in the OVX-EST group with a higher crack density in the medial cortex. In the OVX group, there was a significant med./lat. difference in the DMI and DD with the lat. cortices having more mdx (see p values superimposed on bars in **Fig. 2**). The inter-group comparison showed that the OVX-EST group had statistically significantly more mdx in all subtypes except for coarse linear microcracks in the lat. ("T") cortex, and DMI which was higher in the OVX group (see p values above bars in **Fig. 2**).



DISCUSSION: Our hypothesis that there would be more mdx accumulation in est. deficient animals (consistent with clinical findings in postmenopausal women who suffer fragility fractures) was only true for the DMI. This is an interesting finding in light of previous studies including our own work showing no association between DMI and repair-directed remodeling (14, 15). Therefore it is possible that the reduction in osteoclast apoptosis by est. deficiency (11) and the increase in osteocyte apoptosis due to damage (6) and est. withdrawal (10) that early est. deficiency leads to a paradoxical *increase* in osteoclastic repair of damage in our 5 m.o. rats. If the rats were older and/or est. deficient for a longer period of time prior to fatigue loading (> 7 days as in the present study) it is feasible that other mechanisms such as increased porosity and decreased robusticity and bone mineral density could lead to a greater mdx burden that would overwhelm repairing capacity such as seen in long term osteoporosis. It is also notable that DMI was not only larger in the est. deficient animals but there was also a significant med/lat strain-mode difference found as well consistent with previous reports of this being a tension specific form of mdx (1, 7). These findings could mean that est. may protect against this subtle form of mdx, and its accumulation in est. deficiency may lead to a continuing decline in bone tissue mechanical properties contributing to fragility fractures.

REFERENCES: **1)** Norman and Wang. *Bone* 1997; 20(4):375-379. **2)** Danova et al. *Bone* 2003;33:197-205. **3)** Frost. *Bone Microdamage: Factors that impair its repair*. Berlin: Springer-Verlag; 1985;123-148. **4)** Burr. *Calcif Tissue Int.* 1993;53(Suppl 1):S75-S81. **5)** Noble et al. *Am J Physiol Cell Physiol* 2003;284:C934-C943. **6)** Cardoso et al. *J Bone Miner Res.* 2009;24(4):597-605. **7)** Boyce et al.: *J Ortho Research* 1998;16:322-329. **8)** Reilly and Currey. *J Experi Biol* 1999;202:543-552. **9)** Norman et al. *Trans Orth Res Soc.* 2009:674. **10)** Tomkinson et al. *J Clin Endocrin and Metabol.* 1997;82(9):3128-3135. **11)** Raisz and Seeman. *J Bone and Mineral Research* 2001;16(11)1948-1952. **12)** Hagino et al. *J Bone Miner Res.* 1993;8(3):347-357. **13)** Uthgenannt and Silva: *J Biomech* 2007;40(2):317-24. **14)** Bentolila et al. *Bone* 1998;23(3):275-281. **15)** Mendenhall et al. *Trans Orth Res Soc.* 2007:1404

[Supported by OREF 01-024]