

Estrogen Deficiency Produces Strain-Mode-Specific Differences in Bone Resorption and Microdamage in the Rat Ulna: Implications for the Etiology of Stress Fractures in the Female Athlete Triad

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INTRODUCTION:

Healthy young adult females can become oligomenorrheic within 60-80 days of starting an aerobic exercise program [1]. Perturbations in hormone levels that result from this intensive exercise have been implicated in the development of stress fractures in the setting of the female athlete triad — amenorrhea, osteopenia, and eating disorder. A similar sequence of events might contribute to the increased incidence of stress fractures in female military recruits when compared to their male peers. Because stress fractures are typically more common in women [2,3], a gender-specific mechanism could compound the natural microdamage (mdx) produced by exercise-related loading. In previous studies [4] we have speculated that this effect might be more important than structural differences between female and male bones (e.g., female tibiae are typically less robust than those of men [5]). While decreased estrogen (EST) levels are associated with the female triad, increased levels of EST regulate apoptosis of osteocytes, osteoclasts and osteoblasts, which are important in the microscopic repair/remodeling process [6,7]. Therefore, the decreased EST along with osteocytic damage due to loading may ultimately result in increased accumulation and/or impaired repair of the mdx associated with stress fractures. In order to understand the effect of EST status on this accumulation of fatigue mdx, we utilized the rat ovariectomy (OVX) fatigue loading model [8] to isolate the effects of EST depletion on resorption. We hypothesized that EST deficiency would lead to a higher amount of resorption following simulated acute onset of fatigue. Our second hypothesis was formulated in view of studies showing that numerous factors can affect mdx accumulation and repair [9-11] in the development of a stress fracture, including age, gender, load strain-mode (compression, tension, shear), and the amount of previous strain-mode-specific adaptation. In this perspective, we hypothesized that resorption in EST deficiency would show a strong strain-mode association (medial 'compression' vs. lateral 'tension').

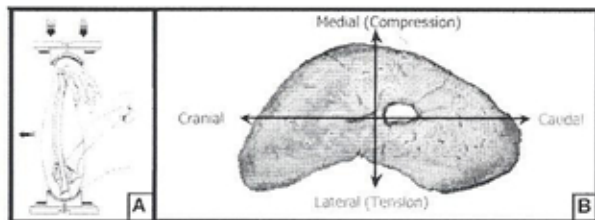


Fig.1 A) Diagram of the rat ulna-loading model B) Orientation of rat ulna in cross-section

METHODS:

Following IACUC approval, 36 five m.o. female Fischer 344 rats were obtained from the NIH. 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 μ strain (20-25 N). This produced ~85% of fracture displacement, which has been shown to cause a "high" level of mdx [12]. 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 CO₂ inhalation. The ulnae were fixed in EtOH, and bulk stained en bloc in 1% basic fuchsin (Mallinckrodt Baker, Inc.). Ten transverse sections from the mid-third diaphysis were ground to 50 μ m. Mdx entities were quantified in medial (compression) and lateral (tension) regions (Fig. 1B) using a PCM-2000 confocal microscope (Nikon). Digitized cross-sectional images were imported into Adobe Photoshop CS2 and resorption area was quantified using Scion Image Beta 4.02.

RESULTS:

Fig. 2 (* = statistically significant) shows results of resorption space data (as % cortical area, CA). Intra-group medial ('compression') vs. lateral ('tension') comparisons showed a significant difference in the OVX group with increased resorption in the lateral (lat) cortex. In the inter-group comparisons (right half of figure), there was a significant difference in the lat. cortex with increased resorption in the OVX group. There was also a significant difference in the medial (med.) cortex with increased resorption in the OVX-EST group.

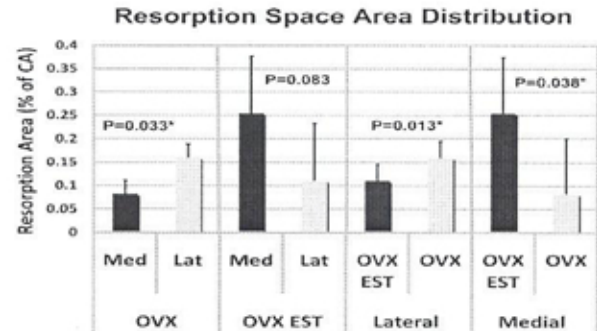


Fig. 2 Resorption Space Area (% cortical area) versus Estrogen Status and Strain-Mode [Medial ('compression') vs. Lateral ('tension')]

DISCUSSION:

These results most strongly support our second hypothesis — in EST deficient animals a strain-mode association is revealed by greater resorption in the lateral 'tension' cortex than the medial 'compression' cortex. This finding correlates with the observation that stress fractures in human limb bones are associated with strain mode, especially when a shifting neutral axis causes tension in a region that was microstructurally adapted for compression. Results of our previous study [4], which examined the same tissues as the present study, also showed that there was significantly increased diffuse matrix injury (DMI) and decreased coarse and fine linear mdx in the EST deficient group. This effect was most prominent in the lateral 'tension' cortex. The paradoxically increased DMI in the setting of increased resorption could be fundamentally important because DMI also becomes more prevalent in the femoral shaft of postmenopausal/aging human females and could contribute to their skeletal fragility [11]. It appears that EST deficiency results in repair-directed resorption of larger mdx (e.g., coarse and fine linear) while DMI mdx is somehow not detected. One possible explanation for this is that the more typical mdx entities (coarse and fine linear) sufficiently disrupt osteocyte homeostasis and thereby activate the process of osteoclastic migration — DMI does not appear to do this. Furthermore, the reduced DMI in the EST repleted animals (OVX-EST) suggests that EST may provide a protective effect for subtle damage; thereby requiring a higher threshold of damage for the accumulation of DMI. Finally, our intra-group analysis revealed that the OVX-EST group had increased resorption in the medial cortex along with our previous findings of increased coarse linear mdx [4]. It is conceivable that early EST exposure leads to acute resorption in the medial cortex but may require longer EST deficiency or increased ambulation time to repair linear mdx entities. Therefore, EST status combined with osteocyte damage may play a vital role in the type of mdx and its temporal repair, and in this way influence the etiology of both stress and fragility fractures.

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