Temporal Bone Quality and the Bone Inflammatory State During Progressive Estrogen Deficiency Following Ovariectomy in Rats: New Insights into the Sequence of Bone Fragility

[1]Peter Olayooye, [3]Landon Y. Tak, [1]Oli Culpepper, [2,3]Alec N. Laplant, [2]Pearl-Marie Andoh, [1]Peter Jalaie, [3]Corinne E. Metzger, [1,3]Rachel K. Surowiec
[1]Purdue University, [2]Indiana University Purdue University Indianapolis, [3]Indiana University School of Medicine rsurowie@purdue.edu
Disclosures: PO (N), LT (N), AL (N), OC (N), PA (N), PJ (N), CM (N), RS (N)

INTRODUCTION: Post-menopausal osteoporosis, characterized by estrogen deficiency, is a leading cause of osteoporotic fractures, yet early fracture prediction remains challenging in this population with limited advancements in recent decades. Estrogen deficiency induces well-established effects that modify bone, including a systemic pro-inflammatory state leading to altered bone turnover, favoring bone loss. These changes are linked to diminished mechanical properties and heightened fracture susceptibility. A less recognized alteration associated with fractures is the reduction in bone hydration, a change not easily detected using standard clinical methods that typically focus on measuring variations in bone mass and mineral density. We propose that estrogen deficiency-induced inflammation contributes to changes in bone turnover and reductions in bone matrix water. Further, we hypothesize that changes in water content occur before bulk mineral changes and can be effectively captured using a clinically relevant magnetic resonance imaging (MRI) technique providing an earlier time point for detecting bone fragility.

METHODS: Two-month-old female Sprague Dawley rats (n=48) underwent ovariectomy (OVX, n=24) to induce estrogen-deficiency bone loss or were sham-operated (n=24) as controls. A subgroup of n=8 rats per group was sacrificed at 2, 5, and 10 weeks post-surgery (**Fig. 1A**) to assess the temporal relationships of inflammation, bone matrix water, bone turnover, and standard clinical imaging outcomes. All animal procedures received Institutional Animal Care and Use Committee approval. The right tibiae underwent dual-energy X-ray absorptiometry (DXA) for BMD, micro computed tomography (microCT) for geometry and microarchitecture (9µm resolution), and ultrashort echo time (UTE) MRI (9.4T) for calculating bound water. The left tibiae were formalin-fixed and underwent microCT (12um) to analyze a 1mm section below the proximal growth plate for trabecular microarchitecture. The proximal tibial midshaft was decalcified, embedded in paraffin, and underwent immunohistochemistry staining for TNF-alpha and RANKL. Data were analyzed by t-test between sham and OVX within each time point with effect sizes.

RESULTS: By 2 wks post-surgery, OVX rats demonstrated significantly lower body mass compared to Sham (**Fig.1B**), which continued through week 10. DXA detected a significantly lower tibial BMD in the OVX group by 10 wks only (**Fig.1C**). Trabecular bone volume of the tibia was lower in OVX at all time points assessed, but midshaft cortical bone area was not altered between OVX and sham until 5- and 10-wks post-surgery (**Fig.1D**). **Figure 1E** depicts a representative UTE MRI images from each group and initial bound water index calculation in the cortical bone indicate lower bound water in the OVX rats. Complete MRI analysis is ongoing. At the 2-wk timepoint, the percentage of osteocytes positive for TNF-alpha were not different between groups, but by 5- and 10-wks post-surgery osteocyte TNF-alpha was 2-4 fold higher in OVX vs. age-matched shams (**Fig.1F**). Similarly, there were no differences in the percentage of osteocytes positive for RANKL at 2-wks, but RANKL was 2.5-3-fold higher in OVX rats by 5- and 10-wks (**Fig.1F**).

DISCUSSION: Overall, these data demonstrate the progression of estrogen deficiency bone loss with cortical bone alterations detectable in the 5- and 10-week timepoints post-surgery prior to detectable changes in BMD measured via DXA. Bone inflammatory markers and RANKL, a key osteoclastogenesis regulator, were altered by 5-weeks within the cortical bone, but not at 2-weeks indicating the progressive nature of the bone inflammatory response following estrogen deficiency. Ongoing work includes finalizing UTE MRI assessment to track the dynamic changes of bone water and its temporal relationship to inflammation markers during progressive estrogen deficiency-induced bone loss. Further, histomorphometric measures of bone turnover and mechanical testing to extract whole bone mechanical and estimated material properties assessing the composition/function relationship are underway.

SIGNIFICANCE/CLINICAL RELEVANCE: Unraveling the relationship between inflammation and bone hydration and understanding the temporal evolution of these changes could shed light on novel aspects of bone physiology and pave the way for innovative therapies aimed at reducing fracture risk. Moreover, if alterations in bone matrix water, detected through UTE MRI, precede standard clinical in vivo measures, early intervention becomes possible when bone treatment is most effective.

ACKNOWLEDGEMENTS: We thank the Purdue/IUSM Engineering in Medicine Pilot Grant Program.

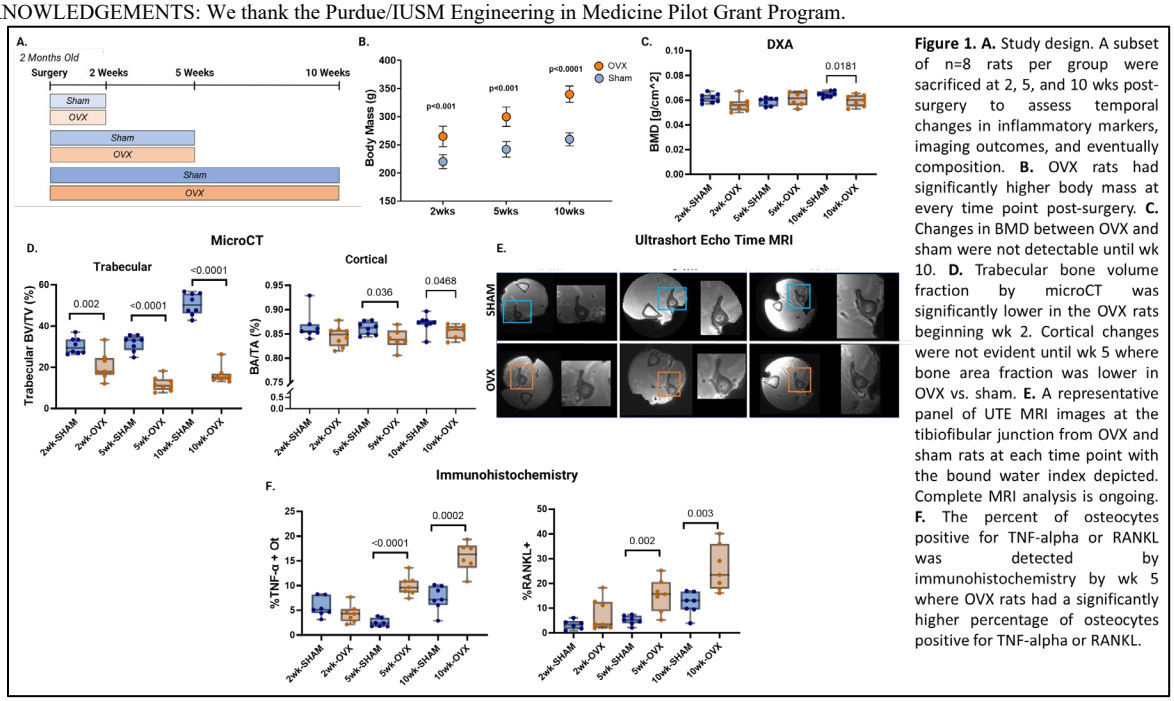

Figure 1. A. Study design. A subset of n=8 rats per group were sacrificed at 2, 5, and 10 wks post-surgery to assess temporal changes in inflammatory markers, imaging outcomes, and eventually composition. **B.** OVX rats had significantly higher body mass at every time point post-surgery. **C.** Changes in BMD between OVX and sham were not detectable until wk 10. **D.** Trabecular bone volume fraction by microCT was significantly lower in the OVX rats beginning wk 2. Cortical changes were not evident until wk 5 where bone area fraction was lower in OVX vs. sham. **E.** A representative panel of UTE MRI images at the tibiofibular junction from OVX and sham rats at each time point with the bound water index depicted. Complete MRI analysis is ongoing. **F.** The percent of osteocytes positive for TNF-alpha or RANKL was detected by immunohistochemistry by wk 5 where OVX rats had a significantly higher percentage of osteocytes positive for TNF-alpha or RANKL.