Ex vivo Raloxifene Treatment of the Human Radius Improves Whole Bone Biomechanical Behavior

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INTRODUCTION: Improvements to bone quality are key to reduce fracture risk. Raloxifene (RAL) is an FDA approved selective estrogen receptor modulator used as a pharmaceutical treatment for osteoporosis. Clinically, RAL decreases fracture risk without significant change in bone quantity (i.e. BMD or t-score) [1]. Pre-clinical work employs RAL as a model for changes to bone quality; in vivo and ex vivo animal studies [2] and ex vivo human cortical bone [3,4] exhibit improved mechanical properties with RAL exposure. Investigations of RAL treatment on whole human bone mechanics have not been performed. We hypothesize that RAL treatment of whole human bones will increase damage tolerance. We further hypothesize that an increase in damage tolerance can be attributed to changes in hydration from RAL treatment as measured with UTE-MRI as bound water index [5].

METHODS: Twelve fresh-frozen human right radii (6 female, 6 male; mean age 83.5 y; no known bone disease; Indiana University School of Medicine Anatomical Donation Program). The proximal and distal ends were removed, and soft tissue and marrow were cleared. Samples were allocated to Raloxifene (RAL) or Vehicle (VEH) by a sex-balanced age "snake-draft". Bones were soaked ex vivo for 14 days at 37 °C in either 2 µM raloxifene in PBS with DMSO or as VEH (PBS + DMSO), with solution changes every other day [2]. HR-pQCT images were obtained prior to soaking (voxel size = 60.7 µm, SCANCO Medical). UTE-MRI (Siemens 3T) images were obtained prior to and immediately after soaking using two echoes ($TR/TE1/TE2$ = 12/0.04/2.80 ms, voxel size = 0.7 mm). The Bound Water Index is calculated as $BWI = (I_{TE1}\text{-}I_{TE2})/I_{TE1}$ where image intensity of TE1 captures fast- and slow-relaxing water, while image intensity of TE2 isolates slow-relaxing (pore) water. UTE images were imported into 3D Slicer, along with STL segmented HR-pQCT. STL segmentations were rigidly aligned to the UTE-MRI space to ensure anatomical correspondence between modalities. Binary masks derived from the aligned segmentations were then applied to compute BWI using in-house algorithms developed in MATLAB, isolating cortical bone while excluding background signal. After overnight PBS thaw at 4 °C, three-point bending experiments were performed at the mid-diaphysis (span-to-depth = 8, strain rate = 0.001 /s, Instron). Radii were oriented with their anterior border along the span and loaded on the posterior surface. Critical damage was determined using $D_c = 1 - F_{fx}/F_{lin}$, where F_{fx} is force at fracture and F_{lin} is the extrapolated linear elastic force at fracture. Mechanical analysis also determined the 0.2% offset stress ($S_{0.2}$), ultimate stress, strain to failure, and toughness based on standard procedures. Micro damage events were recorded by an acoustic emission (AE) sensor (Physical Acoustics Corporation) attached to the proximal, lateral surface at a sampling rate of 5 MSPS (AEWin). AE signals were pre-amplified by 40 dB and processed by a 40 dB activation threshold and frequency range of 20 kHz to 500 kHz. The number of AE events were classified into pre- and post-0.2% offset domains, Figure 1. Statistical analysis was performed using un-paired non-parametric t-test (Mann-Whitney) between sex-specific treatment groups and a two-way ANOVA to evaluate the effects of sex and treatment on all samples (Prism v10).

RESULTS SECTION: A stress-strain graph with accumulated AE data for a representative sample is shown in Figure 1. For the present sample group, RAL treatment did increase critical damage in males at a statistically significant level relative to VEH ($D_{c,RAL}$ = 0.51, $D_{c,VEH}$ = 0.35, p = 0.05). RAL treatment had no significant effect on damage tolerance in female specimens ($D_{c,RAL}$ = 0.56, $D_{c,VEH}$ = 0.52, p = 0.50); On average, female bones exhibited higher critical damage than male ($D_{c,M}$ = 0.43, $D_{c,F}$ = 0.54, p = 0.12), Figure 2(a). Treatment and biological sex did not significantly impact the 0.2% offset stress of the sample group, but their interaction was significant (p = 0.04). The 0.2% offset stress was lower in females compared to males, and the 0.2% offset stress was increased by RAL treatment in females compared to VEH (p = 0.05), Figure 2(b). The number of AE hits after the 0.2% offset normalized by area was found to be linearly correlated with the value of critical damage (r = 0.652, p = 0.022), Figure 3. Preliminary analysis of the UTE-MRI shows average BWI increased due to RAL treatment (+9 %) while for VEH BWI remained rather constant (-2%). However, variability between specimens in RAL and VEH was significant, as was the intra-specimen variability of BWI.

DISCUSSION: Raloxifene treatment has been previously shown to enhance bone material properties without significant changes in bone quantity. The present ex vivo whole-bone biomechanical experiments combined with UTE MRI–derived hydration metrics seek new insights into how raloxifene may alter cortical bone failure characteristics. Change in bone biomechanical behavior were observed for male and female specimens, but the variable effected were different; male specimens had increased damage tolerance and female specimens had increased strength. Female radii possess significant smaller cross section area, Figure 2(c). In [3,4] it was argued that bone size would affect the success of the apparent treatment outcomes, including higher strength in smaller bones and increased damage tolerance in larger bones. AE hits were found to relate to damage. This indicates that RAL acts by providing crack arrest features to microcracks such that a larger number of microcracks can accumulate without fracture instability. Changes in BWI were assessed on the whole diaphysis which is likely to contribute to the large scatter in the BWI data; further investigations on BWI changes in smaller ROIs is needed. The present study is limited by a small sample size (n=12) of aged donor specimens. Thus, further local investigation on individual specimens will be conducted. This includes micro-indentation, scanning electron microscopy of fracture surfaces, local BWI measures and stress analysis by finite element methods.

SIGNIFICANCE/CLINICAL RELEVANCE: This is the first study to investigate RAL effects on whole bone mechanics and elucidates possible mechanisms contributing to fracture and fracture risk in patients with osteoporosis.

REFERENCES: [1] Cranney et al. (2002) [2] Gallant et al. (2014) [3] Arnhart et al. (2024) [4] Gallaway el al. (2025) [5] Jacobson et al (2024)

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IMAGES AND TABLES:

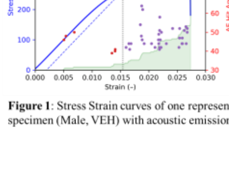

Figure 1: Stress Strain curves of one representative specimen (Male, VEH) with acoustic emission hits.

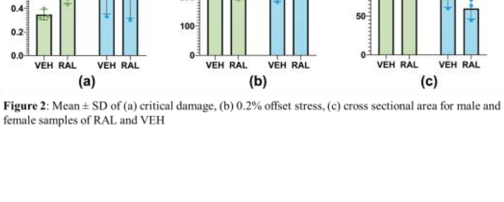

Figure 2: Mean ± SD of (a) critical damage, (b) 0.2% offset stress, (c) cross sectional area for male and female samples of RAL and VEH

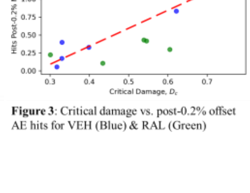

Figure 3: Critical damage vs. post-0.2% offset AE hits for VEH (Blue) & RAL (Green)