ASBMR 2021 ANNUAL MEETING

ASBMR The American Society for Bone and Mineral Research

October 1 – 4, 2021

# Positive Compositional and Material Properties Effects Following Raloxifene Treatment in a Mouse Model of Osteogenesis Imperfecta


**R.K. Surowiec** [1,2] , **R. Rai** [3] , **J.G. Damrath** [2], **M.R. Allen** [1,2] **and J.M. Wallace** [2]

[1]Anatomy, Cell Biology and Physiology, Indiana University School of Medicine (IUSM), Indianapolis, IN, USA

[2]Biomedical Engineering, Indiana University Purdue University – Indianapolis (IUPUI), IN, USA

[3]Biochemistry and Molecular Biology, IUSM, Indianapolis, IN, USA

IUPUI DEPARTMENT OF BIOMEDICAL ENGINEERING

INDIANA UNIVERSITY SCHOOL OF MEDICINE


## INTRODUCTION

- Raloxifene (RAL), an FDA-approved selective estrogen receptor modulator, can improve bone matrix quality and mechanical properties in a bone cell-independent manner through modulation of bone hydration [**1-3**].
- Solid state nuclear magnetic resonance (ssNMR) spectroscopy is a compelling tool that can detect matrix tissue properties, including bound and free water, and structural changes in the collagen-hydroxyapatite interface [**4-5**].
- Osteogenesis imperfecta (OI) is a rare genetic skeletal disorder of the collogen resulting in severely diminished bone quality and increased fracture risk [**6**].

## AIM

We sought to determine the therapeutic effects of RAL on **compositional** and **material properties** in a model of OI.

## METHODS

**Animals and Treatment**:

- Eight wk old homozygous (OIM-/-) male osteogenesis imperfecta mice, a model for severe OI type III, and wildtype (WT) littermates
- Treated with RAL (0.5 mg/kg 5x a wk) for 8 wks or untreated (UN) controls. Mice sac'd at 16 wks.

**Solid State Nuclear Magnetic Resonance (ssNMR)**

- proximal/distal ends removed, marrow flushed, and 2 tibiae per group combined for acquisition.
- Sequences: $^{1}$H MAS ssNMR (total water), $^{1}$H-$^{31}$P heteronuclear correlation (HeTCor) (bound water), $^{31}$P relaxation parameter $T_1$ (matrix integrity), and $^{13}$C (structural matrix collagen).

**Co-localized Raman Spectroscopy + Nanoindentation**

- 1 tibia per group was embedded (methyl methacrylate), sectioned within the diaphysis region, and polished.
- Raman: 785 nm laser, 1.3 µm spot size (6 s exposure, 50% laser power, 10 accumulations) at 5 points within 4 intracortical regions for 20 points per sample.
- Nanoindentation: fully immersed in PBS, spherical diamond probe, colocalized with Raman sites (30 s loading period, 45 s hold at 1,000 µN, 30s unloading period).

**Statistics**

- Effects of RAL vs. UN within each genotype were assessed by Student's t-tests.

### Total Water

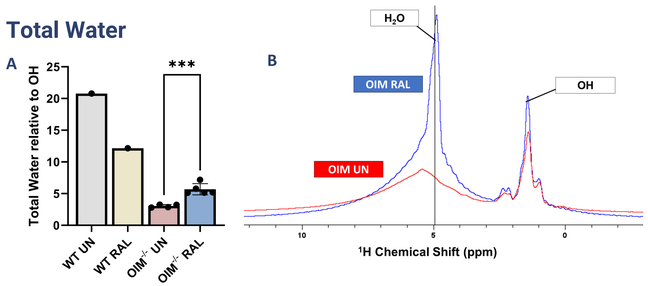


*Figure1. **A)** OIM RAL mice demonstrated a sig. increase in total water compared to OIM UN measured via $^{1}$H ssNMR. This was not observed in WT RAL where total water was instead lower with treatment. ***p=0.0007. One WT RAL and 1 WT UN NMR experiment has been conducted at this time. **B)** Representative $^{1}$H chemical shift spectra (ppm) from the OIM RAL (blue) treated and OIM UN (red) mice demonstrate the higher total water content observed in the cortex. Peaks associated with water ($H_2O$) and inorganic OH denoted.*

### Bound Water Ratio

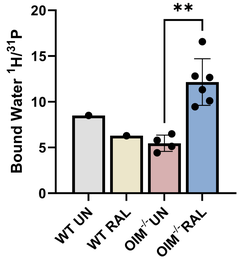


*Figure 2. The $^{1}$H-$^{31}$P heteronuclear correlation (HeTCor) experiment resolves peaks at 0.4 ppm (OH) and 4.8 ppm (bound water) where bound water peak intensity depends on coupling with various $^{1}$H protons in bone. HeTCor illuminates the dipolar coupling between $^{1}$H and $^{31}$P nuclei, thus the ratio shows bound water content near inorganic surfaces. We observed OIM RAL sig. increased the ratio of bound water vs. OIM UN (**p=0.0011). This increase in bound water was not observed in WT RAL vs. WT UN mice.*

### $T_1$ Relaxation

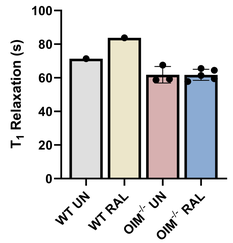


*Figure 3. There was no sig. change in $^{31}$P $T_1$ relaxation with RAL treatment in the OIM mice (p=0.9788). $T_1$ relaxation increased, indicative of less disorganized matrix, in WT but additional mice are needed to determine if this increase is sig.*

### $^{13}$C (collagen)

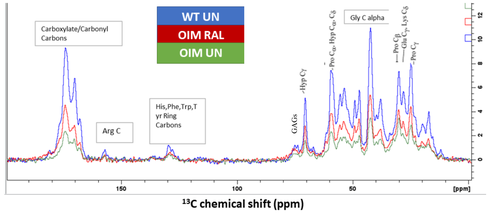


*Figure 4. $^{13}$C spectra principally represents resonance from type 1 collagen in bone. The OIM UN (green) exhibited much broader spectral lines compared to OIM RAL (red) and WT UN (blue). Specifically, GAG peaks at 76 ppm have nearly disappeared in OIM UN and the carbonyl peaks (~176 ppm) are less pronounced and have begun to merge. These peaks become more clearly resolved with RAL treatment.*

### Co-localized Raman Spectroscopy + Nanoindentation

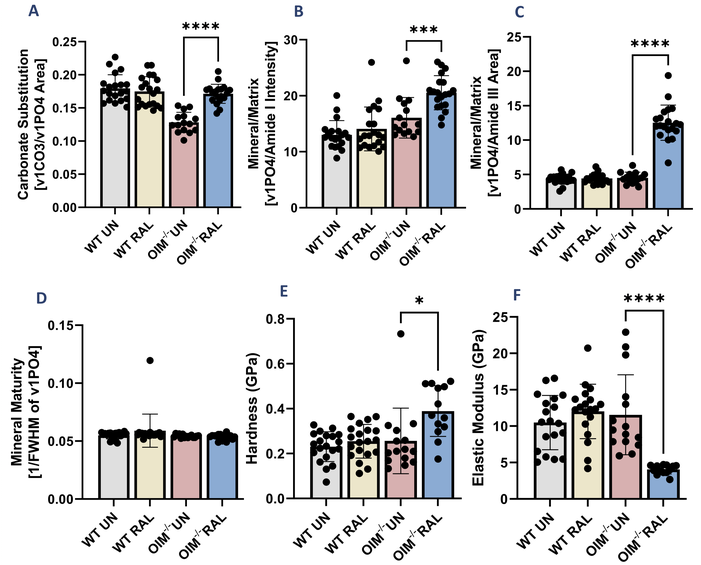


*Figure 5. **A)** Type B carbonate substitution, relative mineralization (Phosphate/Amide I, **(B)**; Phosphate/Amide III, **(C)**), but not mineral maturity **(D)** were all elevated due to RAL treatment within the OIM genotype but not in WT. OIM bone treated with RAL had a significantly increased hardness **(E)** and significantly reduced modulus **(F)**. *p=0.01, ***p= 0.0004, ****p<0.0001.*

## CONCLUSIONS

- This study demonstrates **RAL induced bone quality changes in a model of OI,** but not healthy conditions.
- **In OIM -/-, RAL increased the mineral/matrix ratio**, a metric that correlates positively with bone strength.
- **OIM-/- increased total water** in the system increased and, using 2D HetCor NMR, this was influenced by **increased bound water,** a metric that has historically positively correlated with increased bone toughness.
- Initial nanoindentation findings indicate RAL treatment in the OIM bone **increased hardness** while decreasing elastic modulus suggesting a less stiff but stronger matrix.

## REFERENCES


1. Gallant MA, et al. *Bone cell-independent benefits of raloxifene on the skeleton: a novel mechanism for improving bone material properties*. Bone. 2014.
2. Allen MR, et al. *In Vivo UTE-MRI Reveals Positive Effects of Raloxifene on Skeletal-Bound Water in Skeletally Mature Beagle Dogs*. J Bone Miner Res. 2015.
3. Bivi N, et al. *Structural features underlying raloxifene's biophysical interaction with bone matrix*. Bioorg Med Chem. 2016.
4. Rai RK. Total water, phosphorus relaxation and inter-atomic organic to inorganic interface are new determinants of trabecular bone integrity. PLoS One. 2013.
5. Rai RK, et al. *Predominant role of water in native collagen assembly inside the bone matrix*. J Phys Chem B. 2015.
6. Martin E, Shapiro JR. *Osteogenesis imperfecta: epidemiology and pathophysiology*. Curr Osteoporos Rep. 2007.


## ACKNOWLEDGEMENTS


This work supported, in part, by the NIH (AR067221, AR072609 and T32DK120524-02) and the NSF (1952993).


## CONTACT INFORMATION


**Rachel Surowiec:** rksurowi@iu.edu

**Lab Website:** https://bbml.sitehost.iu.edu/