Effects of Combination Mechanical Loading and Raloxifene Treatment on Bone Matrix Composition and Mechanical Properties

JOHN G. DAMRATH IV[1], ALYCIA G. BERMAN[1], JOSEPH M. WALLACE[2]
[1] Weldon School of Biomedical Engineering, Purdue University, West Lafayette, Indiana, USA
[2] Department of Biomedical Engineering, Indiana University – Purdue University, Indianapolis, Indiana, USA

PURDUE UNIVERSITY
Weldon School of Biomedical Engineering
COLLEGE OF ENGINEERING

BBML

Introduction

- **Background:** Osteoporosis affects over 200 million adults[1], dramatically increasing the risk of fracture. Raloxifene (RAL) reduces fracture risk by up to 50% despite having minimal effects on bone mineral density, indicating improvements in bone quality[2]. Improving bone quality during periods of active bone formation via mechanical loading may be an effective way to increase overall bone strength in patients with osteoporosis.
- **Goal:** Determine the effects of combination mechanical loading and raloxifene treatment on bone tissue composition and material properties.

Methods

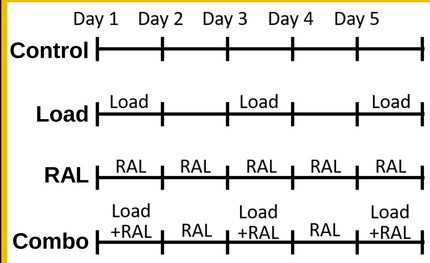


Figure 1: Ten-week old male C57BL/6 mice (n=14) underwent right tibial loading 3x/week for 6 weeks with the contralateral limb serving as a non-loaded control. Half of these mice were treated with 0.5 mg/kg raloxifene (RAL) 5x/week while half were untreated.

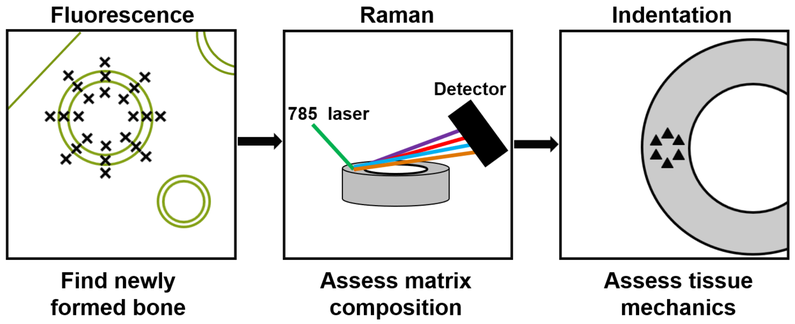
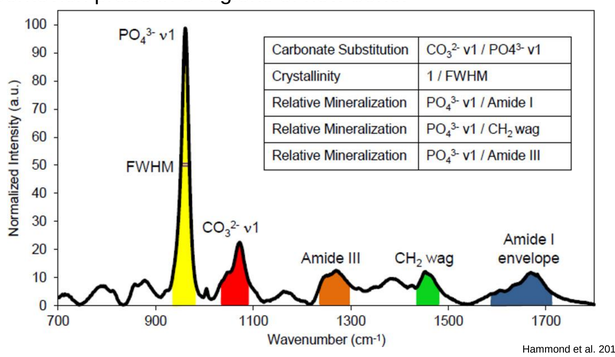


Figure 2: Tibiae were sectioned at 37.5% of the bone length from the proximal end, polished, and stored frozen in PBS-soaked gauze. Samples underwent fluorescent imaging to visualize calcein labels, indicating new bone formation. Raman spectroscopy was performed using a 785 nm laser with a 1 µm spot size in 5 regions of new bone. Nanoindentation was performed using a diamond Berkovich probe in 5 regions of new bone.



Hammond et al. 2014

Figure 3: Raman spectra were analyzed for matrix composition and averaged, yielding a single value for each matrix parameter[3].

Results

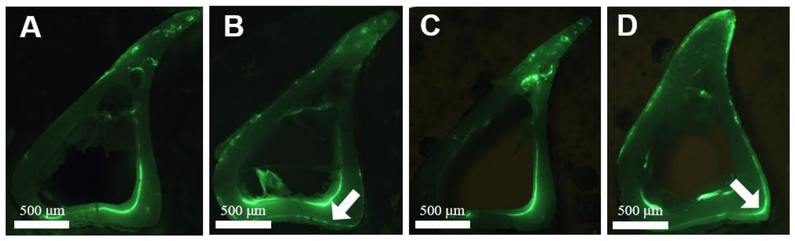


Figure 4: Fluorescent calcein labels were visualized on control (**A**), loaded (**B**), raloxifene-treated (**C**), and combination loaded and raloxifene-treated (**D**) tibiae. Loaded and combination treated tibiae demonstrated periosteal regions of new bone formation (arrows in **B** and **D**).

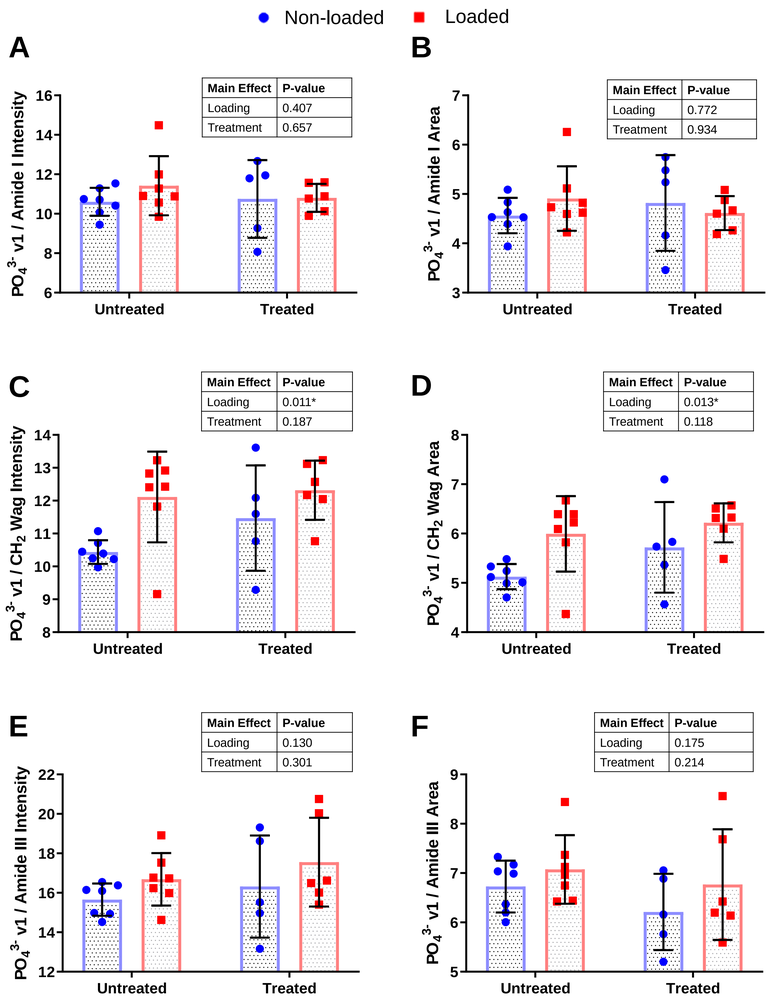


Figure 5: Relative mineralization was calculated as peak intensity and peak area ratios between the PO_4^{3-}v1 and Amide I (**A-B**), CH_2 wag (**C-D**), and Amide III (**E-F**). All P-values for interaction effects were > 0.05.

Results

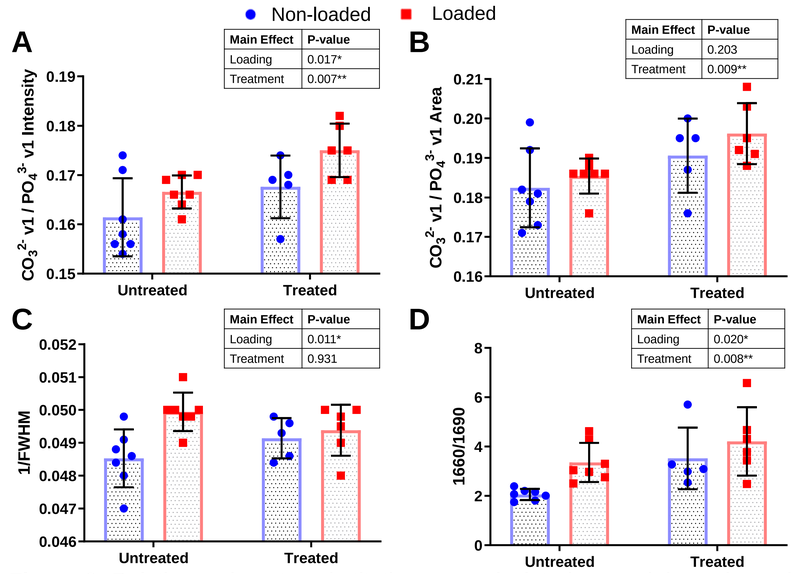


Figure 6: Relative carbonate substitution was calculated as peak intensity and peak area ratios between CO_3^{2-}v1 and PO_4^{3-}v1 (**A-B**). Crystallinity was calculated as the inverse of the full width at half maximum of PO_4^{3-}v1 (**C**). Relative collagen maturity was calculated as the area ratio of subpeaks within the amide I band at wavenumbers 1660 and 1690 cm^{-1} (**D**). All P-values for interaction effects were > 0.05.

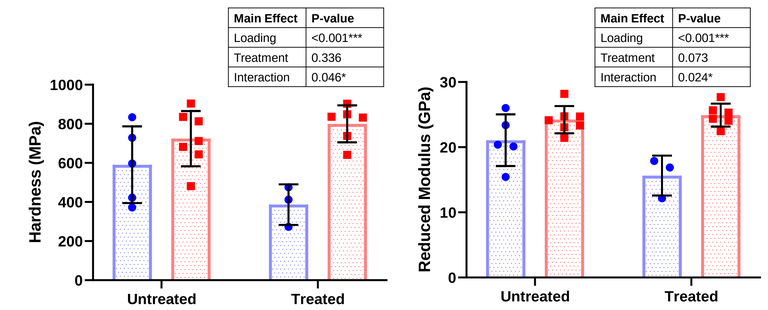


Figure 7: Hardness and reduced modulus were calculated as previously reported[3].

Conclusions and Future Work

Conclusion:

- Loading increased relative mineralization, carbonate substitution, crystallinity, and collagen maturity
- Raloxifene increased carbonate substitution and collagen maturity
- Loading increased tissue-level hardness and modulus

Future work:

- Combination treatment in models of osteogenesis imperfecta and diabetes

References

1. Sozen T, et al. Eur J Rheumatol. 2017;4(1):46-56
2. Ettinger B, et al. JAMA. 1999;282(7):637-45
3. Hammond M, et al. Bone. 2014;60:26-32