Effects of Osteocyte Perilacunar Remodeling on Bone Quality at the Nanoscale and Macroscale in a Murine Lactation Model

Amy Creecy[1], Hardi Patel[1], Joseph M. Wallace [1]
[1]Department of Biomedical Engineering, Indiana University Purdue University Indianapolis
Indianapolis, IN

Introduction

The osteocyte modifies its surroundings through perilacunar remodeling (PLR) during lactation. [1]

PLR may be a method by which the osteocyte regulates the mechanical properties of the whole bone [2]

We hypothesize that the nanomechanical properties of bone near the osteocyte will be altered compared to distant sites in cases of PLR where matrix has been demineralized

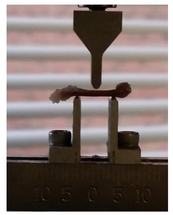
Study Design

Animals

- C57BL/6NHsd female mice (n=18) were bred and nursed pups for 3 weeks
- Mice were euthanized after weaning (**Lactation**) or given a 7-day recovery period (**Recovery**)
- Virgin controls were age-matched for comparison (**Control**) (n=10)

µCT/Mechanics

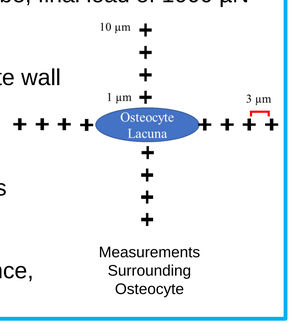
- Femurs scanned at 9.8 µm voxel to determine structure
- Broken in 3-point, displacement rate of 0.025 mm/sec
- Group comparisons performed with one-way ANOVAs and post-hoc Tukey's multiple comparisons tests were made when justified



Bending Test

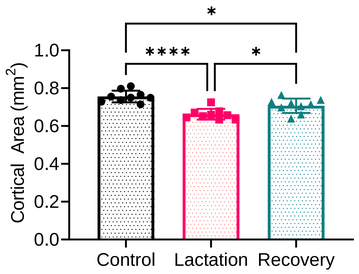
Raman/nanoindentation

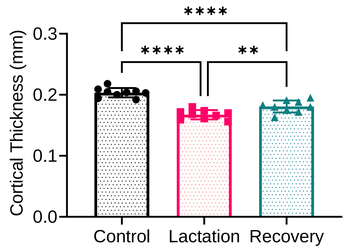
- Transverse cross-sections were cut from embedded tibiae
- Raman measurements were taken using a 785 nm laser
  - 8 accumulations taken per measurement
  - Mineral to matrix ratio (MMR) calculated by dividing phosphate peak area by Amide III peak area
- Nanoindentation was performed using a round probe, final load of 1000 µN
- Raman and nanoindentation measurements were performed starting at 1 µm away from the osteocyte wall and continuing every 3 µm until 10 µm.
- Measurements taken on 6 osteocytes/sample, averaged for each distance and across all samples in each group.
- Two way ANOVAs used to determine group, distance, and interaction effects

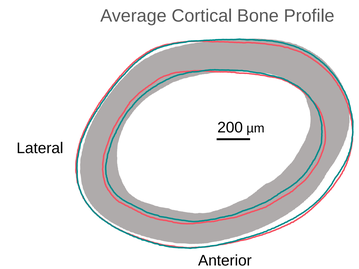


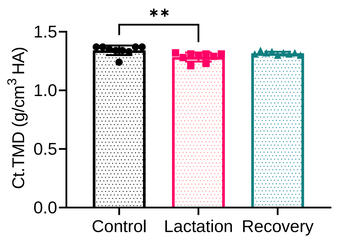
Measurements Surrounding Osteocyte

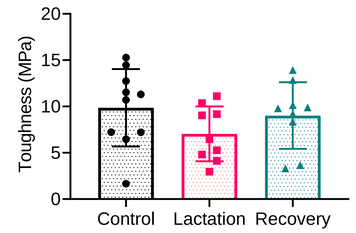
PLR in Lactation Causes Structural and Tissue Level Changes to Bone which are Partially Restored in the Recovery Group

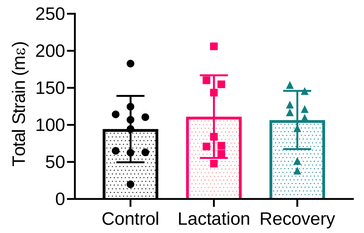


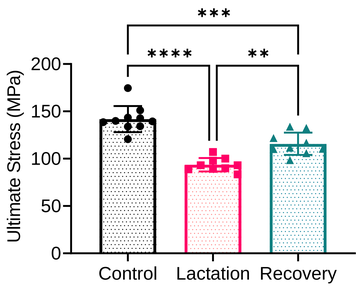


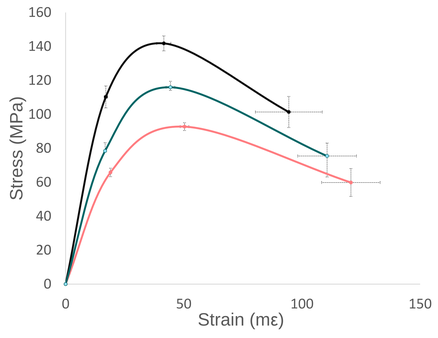






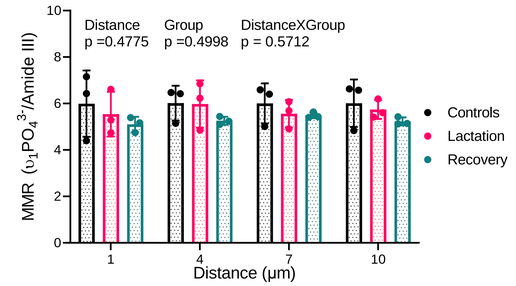


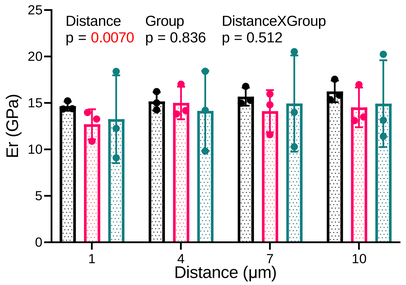


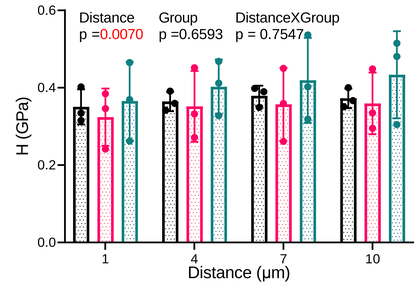


Lactation had lower cortical area, cortical thickness, and ultimate stress compared to controls, and these alterations were only partly reversed in the recovery group. Cortical tissue mineralization (Ct.TMD) was only lower in the lactation group. Toughness and strain did not alter between groups. Average cortical bone profiles are shown on the middle left and schematics of average stress-strain values are shown on the bottom right.

Nanoscale Mechanical Properties Changes with Distance from the Osteocyte Regardless of Group







Mineral to matrix ratio (MMR) was not different between groups. Modulus (Er) and hardness (H) were altered with distance from the osteocyte in all groups.

Discussion

Lactation caused TMD to decrease in cortical bone, which likely resulted in the decrease of ultimate stress.

Mineral to matrix ratio (MMR) was not changed on the nanoscale with distance from the osteocyte; however, despite this, modulus and hardness were affected by distance in all groups.

The effect of distance from the osteocyte on modulus can be another means by which the heterogeneity of bone tissue is increased, contributing to bone's overall fracture resistance.

Conclusion

While macroscale tissue level properties are altered in a model of PLR, distance from the osteocyte is the main factor influencing nanoscale mechanical properties

References

[1] Wysolmerski. *Bone*. 2013. [2] Kaya et al. *J Bone Miner Res.* 2017.

Acknowledgements

This work was funded by grants AR065971 and AR072609 from the NIH.