Delineating the Effects of Pregnancy and Lactation on Rat Maternal Bone Responses to Future Estrogen Deficiency

Rebecca Chung[1], Yihan Li[1], Chantal de Bakker[1], Carlos Osuna[1], Justin Leggin[1], Zachary Davis[1], Liyun Wang[2], X. Sherry Liu[1]

[1]McKay Orthopaedic Research Laboratory, University of Pennsylvania, Philadelphia, PA

[2]Department of Mechanical Engineering, University of Delaware, Newark, DE

Introduction

- Reproduction-induced bone changes
  - Dramatic changes in bone mass and bone microarchitecture during pregnancy, lactation, and post-weaning recovery [1,2]
  - History of reproduction followed by lactation exerts a protective effect against bone loss due to estrogen deficiency [3]

Clinical Barrier: Nearly 60% of mothers are unable to or do not breastfeed for as long as they intend [4]

Objective: To investigate changes in bone structure, mechano-responsiveness, and osteocyte activities in response to estrogen deficiency in virgin and reproductive rats with a reproductive history with and without lactation

Hypothesis: Absence of lactation will differentially alter the skeletal response to estrogen deficiency

Materials and Methods

Animal Protocol

- 3 groups of female Sprague Dawley rats
  - Virgin, reproductive without lactation (Preg-NL), and reproductive with lactation (Lactation)
    - Reproductive rats: 2-3 consecutive cycles of 3-wk pregnancy, 3-wk lactation and 6-wk post-weaning recovery (Lactation), or 9-wk recovery (Preg-NL)
  - Age 10-12 months: bilateral ovariectomy (OVX) surgery to induce estrogen deficiency

Bone microstructural analysis (n=17-20/group)

- *In vivo* µCT imaging of the tibia using VivaCT40 (Scanco Medical) at 10.5 µm resolution for all groups
  - 1st reproductive cycle: time points corresponding to baseline, parturition, end of 3-wk lactation, and 3- & 6-wk post-weaning recovery in Lactation group
  - pre-OVX, 4-,8-, and 12-wks post-OVX

In vivo dynamic loading (n=6-8/group)

- Left tibia: 2-wk dynamic, compressive uniaxial loading at 6-wks post-OVX [5]
  - Peak load: 45 N (~1500 µε) applied at tibial shaft
  - Frequency: 2 Hz (0.15s ramp up, 0.15s ramp down, and 0.2s dwell time)
  - Time: 5 min/day for 2-wks (5 days/wk)
- Right tibia: Non-loaded control
- *In vivo* µCT scans performed on both tibiae at day 0 (before loading) and day 14 (after loading)

Histomorphometry (n=5-6/group)

- 6µm sections of paraffin embedded non-loaded tibial cortices
  - Immunostained MMP13 and CtsK to assess peri-lacunar/canalicular remodeling (PLR) enzyme expression
  - Ploton silver staining to examine lacunar structure

Statistics:

- One-way ANOVA with Bonferroni corrections

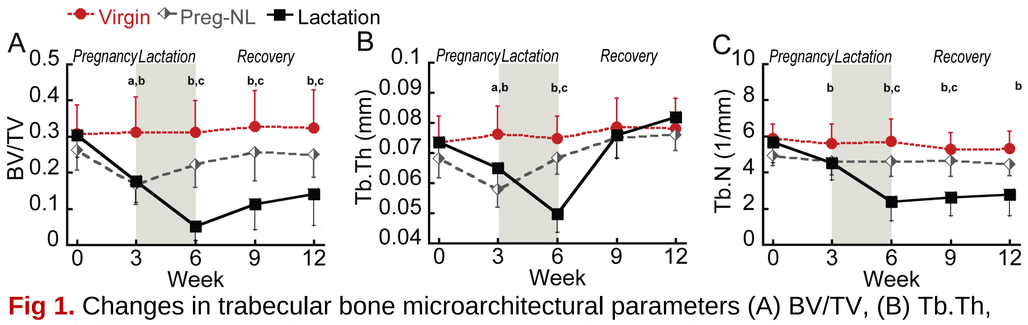

Fig 1. Changes in trabecular bone microarchitectural parameters (A) BV/TV, (B) Tb.Th, and (C) Tb.N during the first reproductive cycle. p<0.05: a: Virgin ≠ Preg-NL; b: Virgin ≠ Lactation; c: Preg-NL ≠ Lactation.

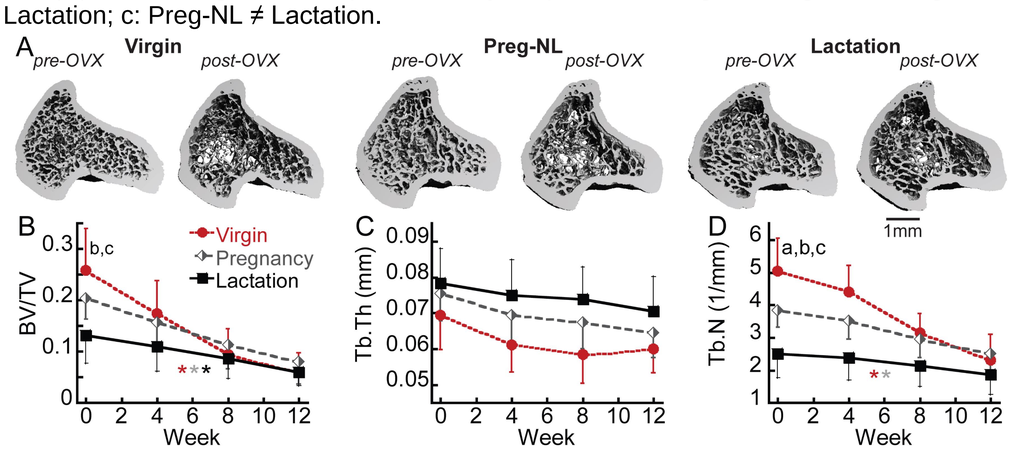

Fig 2. (A) 3D renderings of Virgin, Preg-NL, and Lactation rats pre- and 12 weeks post-OVX. (B-D) Post-OVX changes in trabecular bone microstructure. p<0.05: * week 0 ≠ week 12; a: Virgin ≠ Preg-NL; b: Virgin ≠ Lactation; c: Preg-NL ≠ Lactation.

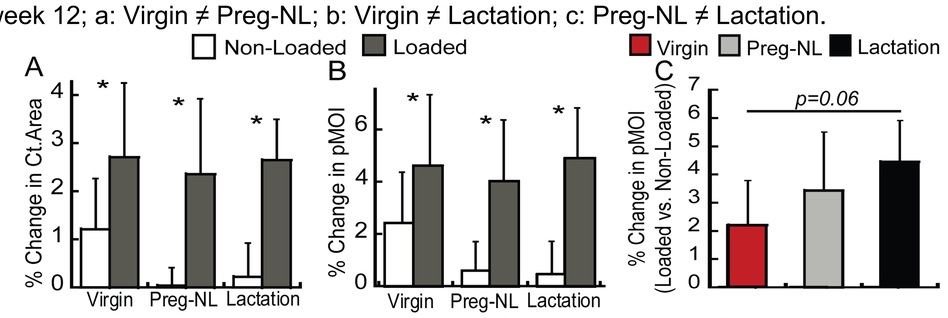

Fig 3. Comparisons between % change in (A) Ct.Area and (B) pMOI between loaded and non-loaded tibia. (C) % change in pMOI in loaded relative to non-loaded tibia. *p<0.05.

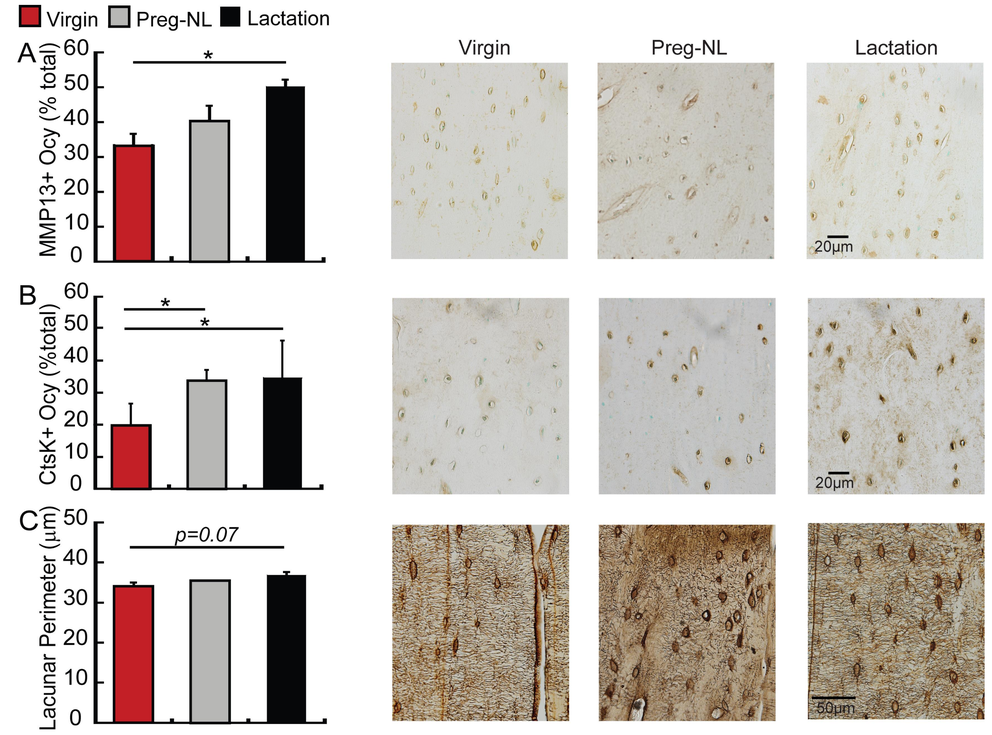

Fig 4. Comparisons between Virgin, Preg-NL, and Lactation rats under OVX conditions in (A) percent MMP13-positive osteocytes, (B) percent CtsK-positive osteocytes, and (C) lacunar perimeter. *: p<0.05.

References

[1] Kovacs CS, Physiol Rev., 2015; [2] de Bakker CM *et al.,* JBMR., 2017; [3] de Bakker CM *et al.*, JBMR, 2018; [4] Odom EC *et al.*, Pediatrics., 2013; [5] Fritton *et al.*, Bone., 2005; [6] Qing H *et al.*, JBMR, 2012; [7] Li Y *et al.*, SB3C, 2019.

Acknowledgements

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Results

Comparison of microstructural changes

- First reproductive cycle
  - Pregnancy: 35% reduction in BV/TV for Preg-NL and 42% for Lactation rats but minimal changes for Virgins (Fig 1A)
  - Lactation: Continued deterioration of Tb.Th (-30%) and Tb.N (-51%) for Lactation rats while Preg-NL rats began to recover (Fig 1BC)
  - 6-wk post-weaning recovery: Full recovery of Tb.Th for both reproductive groups and no difference compared to Virgins (Fig 1B)
- End of 3 reproductive cycles
  - 35% greater BV/TV and 35% greater Tb.N in Preg-NL compared to Lactation rats, but 27% less BV/TV and 31% less Tb.N compared with Virgins (Fig 2)
- 12-wk post-OVX period
  - 62% and 53% decrease in BV/TV at a relatively lower rate for Preg-NL and Lactation rats compared to Virgins (77%, Fig 2AB)
  - Similar changes in Tb.N while no change in Tb.Th found in any group (Fig 2CD)
  - Differences among all groups prior to OVX no longer existed at 12-wks post-OVX (Fig 2D)

Changes in midshaft cortex induced by dynamic loading

- Significant loading responses in tibial Ct.Area and pMOI in all 3 post-OVX groups (Fig 3AB)
- Trend toward greater response in pMOI detected between Lactation and Virgin rats (Fig 3C)

Investigation of PLR enzymatic expression

- 17% greater MMP13+ osteocytes in Lactation rats than Virgins (Fig 4A)
- 13% and 14% greater CtsK+ osteocytes in Preg-NL and Lactation rats compared to Virgins (Fig 4B)

Comparison of lacunar size

- Trend toward 8% greater lacunar size in Lactation rats compared to Virgins (Fig 4C)

Discussion

- Different extent of trabecular bone changes after pregnancy with and without lactation
  - Preg-NL rats: anabolic response after parturition
  - Lactations rats: continued microarchitecture deterioration during lactation
- Attenuated bone loss by 12-wks following OVX in both reproductive groups
- Lactation *vs.* Preg-NL rats:
  - Greater mechano-responsiveness of tibial midshaft at 6-wks post-OVX
  - Mechanical stimuli on osteocytes enhanced through PLR [6,7]
- Conclusion: Reproduction, especially if followed by lactation, may exert a protective effect throughout the maternal lifespan

Significance

- New insights for the development of strategies to engage mothers to breastfeed longer