Microarchitectural Adaptations in Rat Maternal Bone Induced by Pregnancy and Lactation Exert Protective Effects against Future Estrogen Deficiency

Chantal de Bakker
Laurel Leavitt
Hongbo Zhao
Yihan Li, MSE
Casey Krickus
Mengting Huang
Wei-Ju Tseng, MSE
X. Sherry Liu, PhD

McKay Orthopaedic Research Laboratory
University of Pennsylvania

Introduction

Pregnancy and lactation induce dramatic maternal bone loss, which recovers partially post-weaning[1]. Although reproductive history does not increase the risk of developing postmenopausal osteoporosis[1], recent studies utilizing high-resolution computed tomography (CT) found that the effects of reproduction on maternal bone microstructure persist long after weaning[2-4], forming a paradox. We hypothesized that reproduction may induce changes in the bone structural and/or cellular response to future estrogen deficiency, resulting in an altered pattern of postmenopausal bone loss. To test this hypothesis, we investigated the skeletal effects of ovariectomy (OVX) surgery in rats with and without a history of pregnancy and lactation.

Methods

Animal Experiments

All experiments were IACUC approved. Female, SD rats were divided into two groups: Reproductive and Virgin. Starting at age 3 months, reproductive rats underwent 3 cycles of pregnancy and lactation, with a 6-week post-weaning recovery period between each cycle. At age 12 months, all rats underwent OVX surgery to induce estrogen deficiency.

Microstructural Analysis

17 rats (9 reproductive, 8 virgin) underwent in vivo μCT imaging of the proximal tibia prior to OVX, as well as 4, 8, and 12 weeks post-OVX (10.5μm, vivaCT 40, Scanco Medical) for the evaluation of trabecular and cortical bone microstructure. Whole-bone stiffness was estimated through finite element analysis (FEA).

Cell Activities

17 rats (9 reproductive, 8 virgin) were euthanized at 4 weeks post-OVX. Tibiae were harvested and processed for MMA embedding. Longitudinal sections were stained with Goldner's Trichrome, and the numbers and surfaces of osteoblasts and osteoclasts (N.Ob/BS, N.Oc/BS, Ob.S/BS, Oc.S/BS) were quantified within the secondary spongiosa.

Effects of Baseline Microstructure on Bone Loss

Stepwise multiple linear regression was performed to identify the baseline trabecular parameters that were most predictive of the degree of post-OVX bone loss. To further evaluate the role of trabecular thickness, individual trabecular dynamics (ITD) analysis[5] was performed. A trabecular volume of interest (VOI) was identified within the registered μCT scans made prior to and 4-weeks post-OVX, and was subjected to individual trabecular segmentation (ITS), to isolate individual trabecular elements. The extent of bone loss and changes in connectivity were tracked for each trabecula, and the baseline characteristics associated with connectivity deterioration were identified.

Results

Over 12 weeks post-OVX, virgin rats underwent 76%, 87%, 52%, and 22% decreases in bone volume fraction (BV/TV), connectivity density (Conn.D), trabecular number (Tb.N), and whole-bone stiffness, respectively ($p<0.05$), with no change in trabecular thickness (Tb.Th, Fig 1). In contrast, reproductive rats showed

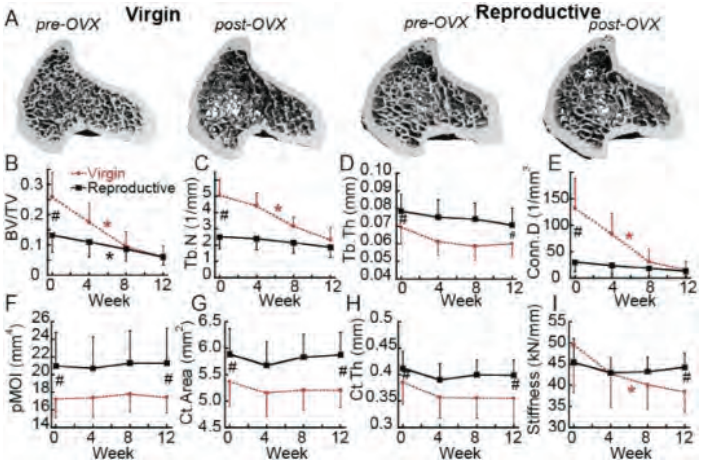

Figure 1. (A) 3D renderings of virgin and reproductive tibiae pre- and 12 weeks post-OVX. **(B-E)** Post-OVX changes in trabecular microstructure **(F-H)** Post-OVX changes in cortical bone structure **(I)** changes in whole bone stiffness after OVX.

a 53% decrease in BV/TV, with no changes in Conn.D, Tb.N, Tb.Th, or whole-bone stiffness. Prior to surgery, reproductive rats had 49%, 77%, and 50% lower BV/TV, Conn.D, and Tb.N, respectively, than virgins ($p<0.05$), but by 12 weeks post-OVX, these parameters were not different between the two groups. Reproductive rats had 13-17% greater Tb.Th, as well as 22%, 10-13%, and 7-12% greater polar moment of inertia(pMOI), cortical area (Ct.Area), and cortical thickness (Ct.Th) than virgins throughout the study ($p<0.05$). Because of the differential post-OVX reductions in whole-bone stiffness between the two groups, virgin rats had 13% lower whole-bone stiffness than the reproductive group by 12 weeks post-OVX. Histomorphometry indicated that virgin and reproductive rats had highly similar osteoblast and osteoclast numbers and surfaces at 4 weeks post-OVX (Fig 2). Multiple linear regression showed that the combination of baseline Tb.N and Tb.Th was most strongly associated with the percent decrease in BV/TV, with an adjusted r2 = 0.69. ITD analysis (Fig 3) showed that virgin rats underwent a 125-179% greater rate of rod disconnection and plate perforation than the reproductive group. Furthermore, the trabeculae that underwent connectivity deterioration were significantly less thick than those that remained intact after OVX ($p<0.05$). Analysis of the overall distribution of trabecular thicknesses demonstrated a greater mean and variance of trabecular thicknesses in the reproductive group, as compared to virgins. However, isolation of the subset of trabeculae that underwent connectivity deterioration indicated that, for both groups of rats, a highly similar population of trabeculae, with a reduced thickness, underwent microstructural decay.

Figure 2. Cell activities 4 weeks post-OVX in virgin and reproductive rats.

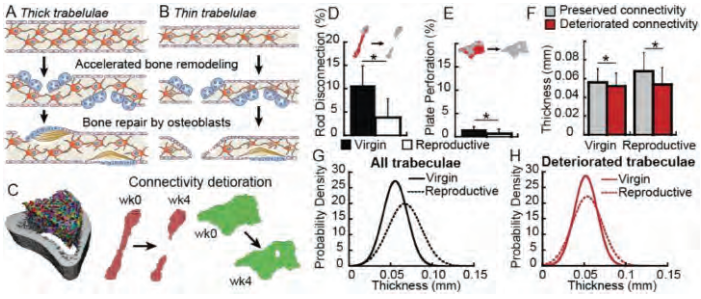

Figure 3. (A-B) Schematics of estrogen-deficiency-induced bone resorption, followed by osteoblast repair in **(A)** thick and **(B)** thin trabeculae. **(C)** Schematics of ITD analysis. Rate of **(D)** rod disconnection and **(E)** plate perforation. **(F)** Mean thickness of deteriorated and intact trabeculae post-OVX. **(G-H)** Probability density distribution of thickness of **(G)** all trabeculae and **(H)** deteriorated trabeculae. *$p<0.05$.

Discussion

Results from this study confirm the long-lasting effects of reproduction on maternal bone, as prior to OVX, reproductive rats showed inferior trabecular microarchitecture compared to virgins. After OVX, reproductive history resulted in a reduced bone loss rate, such that, by 12 weeks post-OVX, baseline differences in trabecular microstructure between reproductive and virgin rats were eliminated. In addition, reproductive rats showed elevated robustness of cortical bone throughout the experiment, and by 12-weeks post-OVX, reproductive rats had greater whole-bone stiffness than virgins, suggesting that reproductive history may have a protective effect on postmenopausal bone strength. Taken together, histology and ITD results indicate that reproductive-history-induced differences in OVX response did not result from alterations in bone cell activities, but instead were likely due to differences in baseline trabecular microstructure, and, in particular, trabecular thickness. The thicknesses of trabeculae undergoing structural decay were highly similar between reproductive and virgin rats, demonstrating that, regardless of reproductive history, the same population of thinner trabeculae, was responsible for the post-OVX connectivity deterioration. This is likely due to the increased susceptibility of thin trabeculae to undergo perforation or separation as a result of elevated bone remodeling[6]. The larger proportion of thick trabeculae in the reproductive group may explain the protective effect on post-OVX bone loss.

Significance

The effects of reproduction on bone health are unclear: pregnancy and lactation have long-lasting effects, but do not increase long-term fracture risk. This study shows that the unique microstructure of post-reproductive bone confers protective effects against postmenopausal bone loss.

References

1. **Kovacs CS**, *Physiol Rev* 2016
2. **Brembeck P *et al.***, *J Clin Endocrinol Metab* 2015
3. **Bjornerem A *et al.***, *J Bone Miner Res* 2016
4. **de Bakker CM *et al.***, *J Bone Miner Res* 2017
5. **Altman AR *et al.***, *J Biomech Eng* 2015
6. **Liu XS *et al.***, *Bone* 2008.