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Comparison Between Ovariectomy and Lactation Induced Bone Loss: A Dynamic Imaging Study

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Introduction

Lactation and postmenopausal osteoporosis are two hypoestrogenic physiological states that induce substantial changes in the bone mass and microarchitecture of women. During lactation, the body draws on calcium stores in the skeleton for milk production, resulting in substantial bone loss. In postmenopausal osteoporosis, ovarian hormone deficiency is responsible for bone loss.[1,2] What is remarkable about lactation-induced bone loss is that bone mineral density and structure are rapidly restored after weaning when estrogen levels return to normal.[1] In contrast, estrogen replacement post menopause can no longer reconstitute the deteriorated skeleton[3] in the manner that occurs in lactating women after weaning. Therefore, reversible changes in bone during lactation and weaning may provide important insight into pathogenesis and treatment of estrogen-deficiency induced bone diseases. A past study comparing lactating and ovariectomized (OVX) rats found that the resulting loss of bone in both groups followed similar patterns; however, lactation had a greater effect on trabecular bone loss than ovariectomy.[4] The purpose of this study was to explore the differences in structural mechanisms of bone loss and bone remodeling activities during lactation and postmenopausal osteoporosis in a rat model using high resolution *in vivo* micro computed tomography (μCT). We hypothesized that both types of bone loss would be caused by increased resorption, leading to rapid structural deterioration.

Methods

Eleven 4-month-old female Sprague Dawley rats were divided into two groups: OVX (n=5) and lactating rats (LAC, n=6). The LAC group underwent mating, pregnancy, delivery, and lactation, with litter size normalized to nine pups per dam. OVX rats were ovariectomized to simulate post-menopausal osteoporosis. A region 4.3 mm distal to the proximal tibia of each rat was scanned using an *in vivo* μCT system (VivaCT 40; SCANCO Medical AG) at an isotropic voxel size of 10.5 μm. LAC rats were scanned at days 0, 7, 14, and 21 after beginning lactation and OVX rats were scanned at days 0, 7, 14, and 28 after surgery. To track longitudinal changes, sequential *in vivo* μCT images were registered via landmark-initialized, mutual-information-based registration.[5] Trabecular bone volume fraction (BV/TV), number (Tb.N), thickness (Tb.Th), spacing (Tb.Sp), structure model index (SMI), connectivity density (Conn.D), and degree of anisotropy (DA) were calculated using Scanco software. A 1.58x1.58x1.05 mm^3 sub-volume corresponding to 150x150x100 voxels was selected from the primary spongiosa region of each μCT scan at days 0 and 7 for further analyses. Each pair of sub-volumes for days 0 and 7 further underwent a multi-step, precise registration procedure.[6] The aligned sub-volumes were then binarized and subjected to 3D *in vivo* dynamic bone histomorphometry to calculate bone formation rate (BFR/BS), bone resorption rate (BRR/BS), mineral apposition rate (MAR), and mineral erosion rate (MER).[6] Furthermore, the precisely registered sub-volumes were also subjected to an Individual Trabecular Dynamics (ITD) analysis to quantify incident rates of trabecular rod disconnection and plate perforation. Two-way repeated measures ANOVA tests were performed to compare the relative changes in 3D microstructure and ITD between LAC and OVX rats using baseline measures as covariates. Student's t-tests were used to compare bone histomorphometry and ITD measurements between LAC and OVX rats. For all tests, $p<0.05$ was considered a significant difference.

Results

3D microstructural analysis indicated significant trabecular deterioration in both OVX and LAC groups, in which OVX rats displayed a greater degree of bone loss (Figure 1). OVX rats experienced decreases in BV/TV, Tb.N, Tb.Th, Conn.D, and DA and increases in Tb.Sp and SMI over time (Figure 1 and Table 1). LAC rats experienced decreases in BV/TV, Tb.N, and Tb.Th, increases in Tb.Sp and SMI, with no significant changes in Conn.D and DA (Table 1). Comparisons of microstructural parameters between OVX and LAC groups indicated that OVX rats presented a 10% greater loss in BV/

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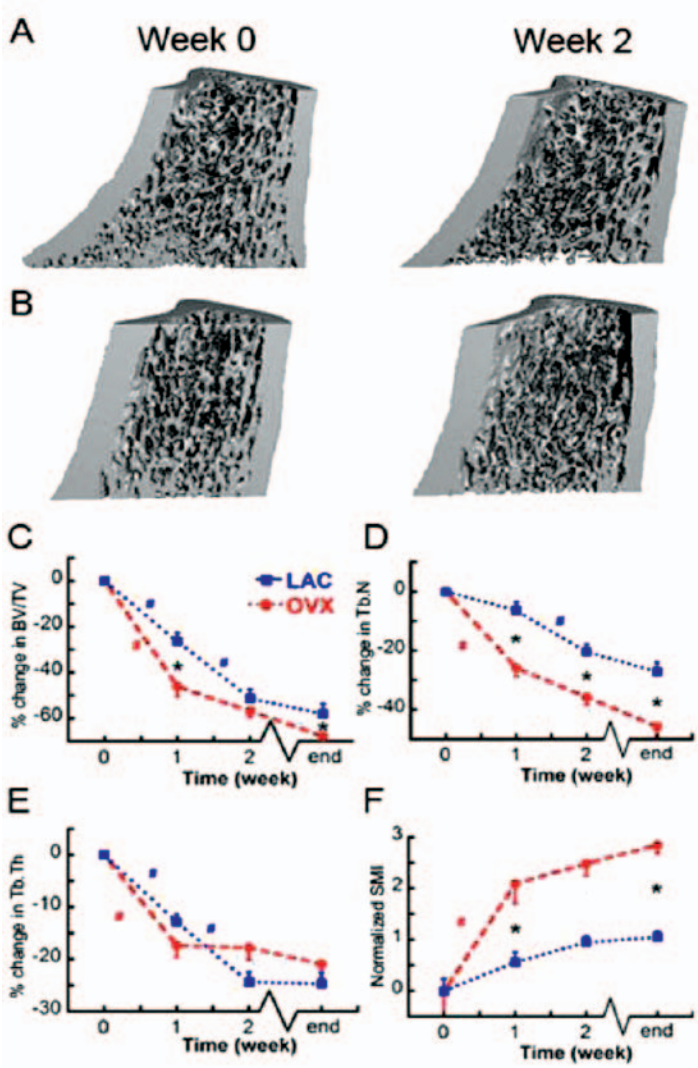

Figure 1. (A) Registered μCT images of the proximal tibia of an OVX rat on days 0 and 14. (B) Registered μCT images of the proximal tibia of a LAC rat on days 0 and 14. (C) % change of BV/TV (D) % change of Tb.N (E) % change of Tb.Th (F) Normalized SMI over time. # indicates differences between time point and * indicates differences between groups.

TV ($p=0.004$), 19% greater loss in Tb.N ($p<0.001$), 54% greater increase in Tb.Sp ($p<0.001$), and a greater increase in SMI by 1.8. There were no significant differences in Tb.Th, Conn.D, and DA between groups. ITD analysis suggests that rod disconnection was primarily responsible for changes in bone microarchitecture in both groups. From days 0 – 7, 9.0% and 10.5% of trabeculae underwent rod disconnection in the OVX and LAC groups, respectively, and 1.5% and 1.1% underwent plate perforation, respectively. There was no significant difference in any ITD parameters between the two groups. From 3D *in vivo* dynamic bone histomorphometry, we found that OVX rats experienced a greater degree of bone resorption from days 0 – 7 than did LAC rats (Figure 2). Specifically, the BRR/BS in OVX rats was 45% greater than in LAC rats ($p=0.02$) and the MER in OVX rats was 26% greater than in LAC rats ($p<0.001$). There were no differences in BFR/BS and MAR between the two groups.

Discussion

Both OVX and LAC rats displayed similar overall patterns of bone loss that were most pronounced in the OVX group. OVX surgery leads to significantly fewer, thinner, more spread out and rod-like trabecular bone network, with less connections and lower anisotropy. Changes in post-lactation bone share many similar structural mechanisms that lead to rapid bone volume loss, except for non-detectable changes in Conn.D and DA. At the end of the study, the loss of volume and number of trabeculae and the increase in trabecular spacing occurs to a greater extent in the OVX group. Furthermore, ITD analysis suggests that rod disconnections and plate perforations are critical mechanisms causing reduced integrity of the trabecular network, to a similar degree in both OVX and LAC rats. Interestingly, *in vivo* dynamic bone histomorphometry indicates a significantly greater bone resorption in OVX rats than in LAC rats. Although there was no statistical difference in bone formation rate between two groups, which may be due to highly variable data in the OVX group, the mean value of BFR/BS of OVX group is double that of the LAC group. These results suggest that there was a higher bone turnover rate

Table 1. Standard microstructural variables of interest represented as mean ± SEM. * indicates significant differences from baseline to end for % change, while a significant p-value indicates differences between groups ($p<0.05$).

	OVX			LAC			p-value
	baseline	end	%change	baseline	end	%change	
BV/TV	0.45±0.06	0.15±0.03	−68±4*	0.30±0.13	0.13±0.06	−58±−26*	0.004
Conn.D	202±24	44±8	−76±6*	114±50.8	49±22	−58±−26	0.97
SMI	−0.23±0.73	2.62±0.17	N/A	1.52±0.68	2.58±1.15	N/A	0.08
Tb.N*	6.56±0.22	3.56±0.26	−46±3*	5.18±2.32	3.77±1.69	−27±-12*	<0.001
Tb.Th*	0.092±0.009	0.072±0.005	−21±3*	0.082±0.037	0.061±0.027	−25±11*	0.11
Tp.Sp*	0.133±0.011	0.274±0.027	106±12*	0.167±0.074	0.253±0.113	52±23*	<0.001
DA	1.414±0.021	1.540±0.026	9±3*	1.602±0.716	1.695±0.758	6±3	0.03

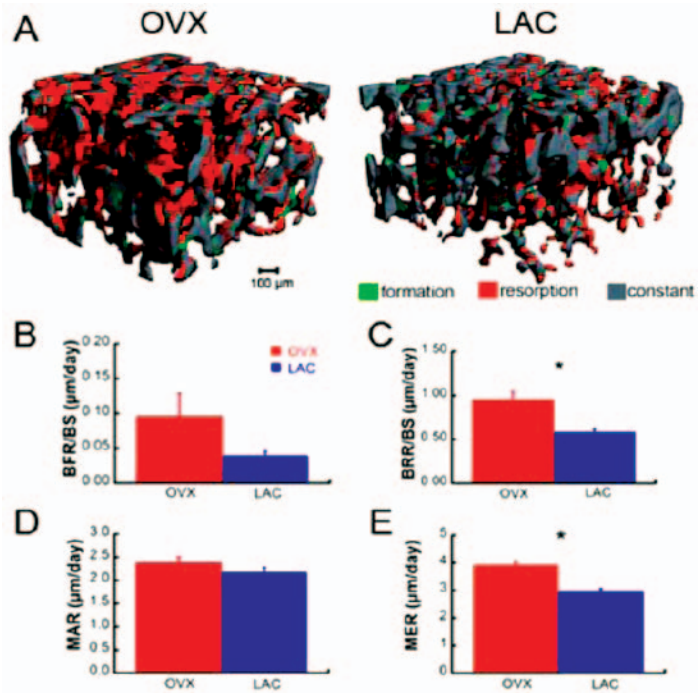

Figure 2. Results from 3D *in vivo* dynamic bone histomorphometry. (A) Representative 3D renderings of sub-volumes of trabecular region illustrating bone formation and resportion from days 0-7 in an OVC rat and LAC rat. (B) Comparison of BFR/BS. (C) Comparison of BRR/BS. (D) Comparison of MAR. (E) Comparison of MER. * Indicates significant difference between groups.

due to OVX than due to lactation. The inconsistency of this study's results with the previous literature may have resulted from excluding pregnancy from the study. Prior to pregnancy and ovariectomy, the rats shared similar microstructural parameters (data not shown). At the onset of lactation (d0) however, LAC rats had reduced trabecular bone volume and structural integrity (Table 1). The difference in baseline values between LAC and OVX rats seems to suggest that bone loss also occurred during pregnancy, which was not accounted for in this study. The results from this study suggest that both lactation-associated bone loss as well as estrogen deficiency-induced bone loss occur through similarly increased bone resorption and structural deterioration mechanisms.

Significance

Bone undergoes similar structural changes during these two physiological events, encouraging future study of post-lactation bone recovery which may be applicable to post-menoapausal osteoporosis treatment.

Acknowledgments

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