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Relationships Between Peak Bone Microstructure and Rate of Estrogen-Deficiency-Induced Bone Loss

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Introduction

Postmenopausal osteoporosis affects more than 200 million women worldwide[1]. Reduced estrogen levels post-menopause lead to accelerated bone remodeling, resulting in low bone mass and structural trabecular bone deterioration, which cause bone fragility and increased fracture risk[1-3]. Our previous study in rats suggested that variations in trabecular bone microstructure may impact the degree of bone loss following estrogen deficiency induced by ovariectomy (OVX) surgery[4]. We found that the rats that had undergone pregnancy and lactation had significantly thicker trabeculae and attenuated OVX bone loss than age-matched virgin rats. By pooling the data of reproductive and virgin rats together, we found that the percent reduction in bone volume fraction (BV/TV) was significantly correlated with baseline trabecular thickness (Tb.Th), connectivity density (Conn.D), and trabecular number (Tb.N). However, the reproductive history may impact OVX bone loss through other pathways; thus the ultimate relationship between trabecular microstructure and OVX bone loss remains requires further elucidation. Therefore, the objective of the current study is to longitudinally track the bone microstructural changes before and after OVX surgery in a homogeneous population of virgin rats in order to establish the relationship between peak bone microstructure and bone structural changes induced by estrogen deficiency. We hypothesize that the variations in peak bone microstructure can predict the extent of estrogen-deficiency-induced bone loss.

Methods

All animal experiments conducted in this study were approved by IACUC. 51 female Sprague Dawley rats underwent OVX surgery at age of 16-17 weeks and developed osteopenia for 4 weeks.

μCT imaging

μCT scans were performed on right proximal tibiae for all rats at week 0 (before OVX surgery) and week 4 (4 weeks post-OVX) using an in vivo μCT scanner (VivaCT 40, Scanco Medical AG, Brüttisellen Switzerland). A 4-mm region of the proximal tibia was scanned at 10.5 μm voxel size[5]. 3D image registration was conducted to detect a constant trabecular volume of interest (VOI) for each rat to evaluate trabecular microstructure at different time points (Figure 1). Standard trabecular bone structural parameters, such as bone volume/trabecular volume (BV/TV), trabecular thickness (Tb.Th), trabecular number, trabecular spacing (Tb.Sp), structure model index (SMI), and Conn.D were measured. Percent reductions between week 0 and week 4 were calculated for all parameters.

Statistics

Linear correlations and stepwise multiple linear regression analyses were performed to explore the relationship between the baseline (week 0) trabecular microstructural properties and corresponding percent decreases post-OVX. All data were divided into three tertiles, representing groups with Low, Medium, and High relative baseline Tb.Th (adjusted by baseline BV/TV), based on the residuals resulting from the linear correlation of baseline Tb.Th with BV/TV (Figure 2 D). One-way ANOVA with Bonferroni corrections was applied to compare % reductions in trabecular structural parameters among the 3 tertiles.

Results

All rats underwent significant bone loss over 4 weeks post-OVX (55.6±7.8% decrease in BV/TV, Figure 1). Correlation coefficients of linear regressions between baseline parameters and % reduction in trabecular bone microstructural properties are shown in Table 1. % decrease in BV/TV was not predicted by the baseline BV/TV, but it was significantly correlated to baseline Conn.D. Baseline Tb.Th was found to be the best predictor. All baseline structural parameters were significantly correlated to % decrease in Tb.Th. As shown in Table 2, stepwise multiple linear regressions showed that the combination of baseline Tb.Th and Conn.D was correlated with % reductions in BV/TV, Tb.Th, Tb.Sp and

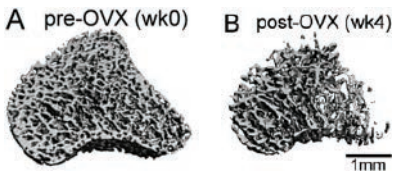

Figure 1. 3D rendering of a registered VOI of trabecular bone pre- and post-OVX.

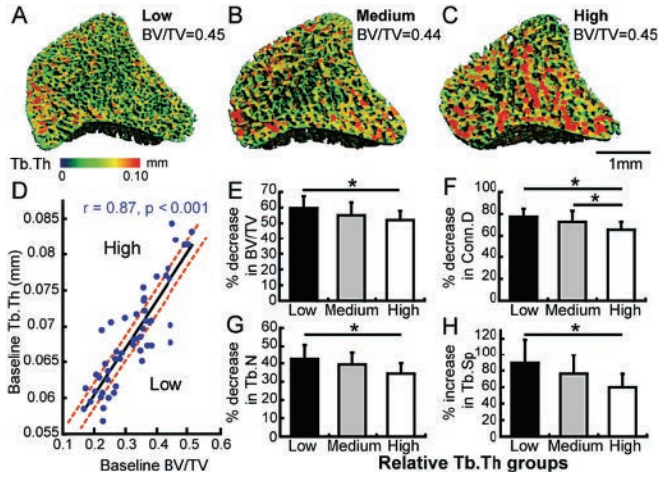

Figure 2. (A-C) Representative trabecular bone images in Low, Medium, and High relative Tb.Th groups. **(D)** Linear regression of baseline Tb.Th and BV/TV. **(E -H)** % changes in microstructural parameters in Low, Medium, and High relative Tb.Th groups. * indicates significant difference ($p < 0.05$).

Conn.D, indicating that baseline Tb.Th and Conn.D were the most important predictors. To further examine the influence of Tb.Th regardless of BV/TV on OVX bone loss, rats were stratified by the relative baseline Tb.Th (adjusted by BV/TV) into 3 groups (Figure 2 A-C). Baseline Tb.Th was significantly correlated to baseline BV/TV ($r=0.87$, $p<0.001$; Figure 2 D), and the corresponding residuals were applied to determine Low, Medium, and High relative Tb.Th groups. % decrease in BV/TV was 13% lower in the High group compared to the Low group (Figure 2 E), and % decrease in Conn.D in the High group was 15% and 10% lower than Low and Medium groups, respectively (Figure 2 F). Moreover, the % decrease in Tb.N and the % increase in Tb.Sp were 19% and 33% lower, respectively, in the High group than the Low group (Figure 2 GH).

Table 1. Correlation coefficients (r) between baseline trabecular parameters and % decrease in trabecular microstructure. Minus sign indicates negative correlation. *: $p < 0.05$, +: $p < 0.01$, #: $p < 0.001$.

% decrease	Baseline Parameters					
	BV/TV	Conn.D	SMI	Tb.N	Tb.Th	Tb.Sp
BV/TV	NS	0.28*	NS	NS	NS	NS
Tb.Th	0.74#	0.59#	−0.72#	0.67#	0.70#	−0.67#
Tb.Sp	NS	−0.31*	NS	NS	NS	NS
Tb.N	NS	NS	NS	NS	NS	NS
SMI	NS	NS	NS	NS	NS	NS
Conn.D	−0.41+	NS	0.44+	NS	−0.60#	NS

Table 2: Correlation coefficients (r) and independent predictors of stepwise multiple linear regression to predict the degree of bone loss by baseline trabecular structural parameters. Minus sign indicates negative correlat ion. *: $p < 0.05$, +: $p < 0.01$, #: $p <$

% decrease	r	Adjusted r	Independent predictors
BV/TV	0.46	0.41	Conn.D+, −Tb.Th+
Tb.Th	0.74	0.68	Conn.D+, Tb.Th#
Tb.Sp	0.51	0.48	−Conn.D#, Tb.Th+
Tb.N	NS		
SMI	NS		
Conn.D	0.68	0.66	−Tb.Th+, Conn.D+

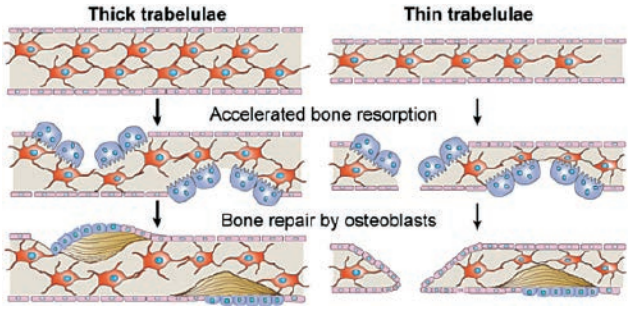

Figure 3. Schematics of accelerated bone resorption followed by osteoblast repair in (Left) a thick and (Right) a thin trabecula.

Discussion

This study investigated the relationship between the peak trabecular bone microstructure and the degree of estrogen-deficiency-induced bone loss. Over 4 weeks post-OVX, estrogen deficiency induced substantial trabecular bone loss and microstructural deterioration. Multilinear regression analysis revealed that the extent of bone loss was influenced by the baseline trabecular microarchitecture, most notably the trabecular thickness and connectivity. A tertile analysis of Tb.Th adjusted for BV/TV suggested that, given the same bone mass (BV/TV), rats with thicker trabeculae had attenuated loss in the volume, number, and connectivity of the trabecular bone network and reduced expansion in the spacing between trabeculae. Our working hypothesis is that the increased bone remodeling in response to estrogen deficiency has variable effects depending on the trabecular thickness. For thick trabeculae, the resorbed bone can be repaired by subsequent osteoblast activities (Figure 3 Left). However, thin trabeculae may be disconnected during remodeling and cannot be repaired (Fig.3 Right). Therefore, trabecular network of low connection but thick trabeculae may be protective against OVX-induced structural deterioration. Future studies with a longer post-OVX duration are necessary to elucidate the effects of peak trabecular bone microstructure on OVX bone loss.

Acknowledgements

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