Cortical Bone Tissue Mineral Density is Affected by Ovariectomy in the Mouse

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Introduction: Bone tissue quality affects fracture risk. Tissue-level bone mineral density (tBMD), a correlate of ash fraction, has been reported to be altered in osteoporotic humans and animal models [1,2]. We and others have postulated that tBMD is tightly regulated to prevent damage initiation and accumulation in both collagen fibers and mineral crystals.

In this study we examined the structural and tissue-level cortical responses to both ovariectomy (OVX) and aging in mice. Depletion of estrogen by OVX is associated with decreased apparent-level bone mineral density (aBMD). However, tBMD effects have not been clearly demonstrated. Adult mouse cortical bone presents a system for the study of the short-term effects of OVX on tBMD since this bone normally exhibits few osteonal units or other large pores, and presents minor structural changes due to aging or OVX during adulthood [3,4]; two factors that may obscure the measurement of tBMD.

Methods: Adult mice (C57Bl/6J, 16-week-old, n=30) were bilaterally ovariectomized (OVX) or sham operated (SHAM control) under avertin anesthesia. Mice (n=3-8 per group) were euthanized at baseline (t=0) or 1, 4 and 8 weeks post surgery. Tibias were immediately removed and fixed in 10% neutral-buffered formalin. All procedures were approved by IACUC.

Cortical structure and tissue mineral density (tBMD) were assessed with quantitative microCT. Structural measures included tribial length, cortical width, perimeters (periosteal and endosteal), areas (marrow, cortical, and total) and moments of inertia. Each microCT scan (GE Healthcare eXplore Locus SP) was completed in 8 hours at 12-µm isotropic voxel resolution and included a density calibration phantom containing air, water, and a hydroxyapatite standard (SB3; Gammex RMI) to allow determination of tBMD by converting grayscale values to mineral density values using a density calibration curve from the scanned phantom and then averaging mineral content values over all bone voxels. Mid-diaphyseal traits were quantified for a region comprising 20% of tribial length. Values for each mouse were the average of both tibias. Differences between groups were evaluated by 2-way ANOVA with Fisher PLSD post-hoc testing. Results are expressed as means ± SD.

Results: Body mass was not different between OVX and SHAM control at each time point (p>0.05). However, body mass for both was greater than baseline control at 4 weeks post surgery (5%) and remained so at 8 weeks (p<0.05). Uterine mass was lower in OVX versus SHAM at each time point (>50%, p<0.05).

No structural measures, including moments of inertia (Fig. 1), were significantly affected by age or OVX. However, tissue-level bone mineral density (tBMD) was greater in both OVX and SHAM control at 8 weeks post surgery versus baseline (p<0.05). Furthermore, at 8 weeks but not earlier time points, tBMD was lower in OVX versus SHAM control (p<0.05).

Discussion: Age and ovarian status are important determinants of bone structure and tissue density. Our cortical structure results largely agree with previous studies of age and OVX related effects in the femur of similarly aged C57Bl/6 (B6) mice. The majority of transverse diaphyseal cortical bone accrual and increase in mechanical structural properties are completed by 16-20 weeks of age in B6 mice [3,4]. Additionally, this cortical bone undergoes very little short-term structural response to OVX, as confirmed by the current study, and cortex thinning (due to narrow expansion and low periosteal bone formation) is only observed beginning at 16 weeks post OVX [4]. Overall, post-OVX structural changes in the mouse agree with the 2-phase bone loss of post-menopausal osteoporosis: a short-term loss of cancellous structure and a long-term gradual loss of cortical structure.

Compared to the vast literature representing the knowledge-base of structural effects of aging and estrogen depletion, very little is understood about their influence on bone tissue quality. Our data indicating a short-term increase in cortical tissue density with age are opposed to the only previous study in mouse bone in which Archimedes principle was used [3]. However, in our data with microCT was an order of magnitude lower than attained with the Archimedes technique (2% versus 25%) [3]. Also, our data are in agreement with a bulk of literature indicating that during senescent periods bone tissue age tends to correlate with mineralization [5]. Importantly, high turnover-based increases in bone formation are not present after OVX in this model [4]. Thus, newly formed and less mineralized bone should not contribute to the changes in tBMD observed. Accordingly, the changes seen in the current study must result from the effects of estrogen loss on the existing bone matrix.

Additional data for the effects of estrogen depletion on tBMD are unavailable for comparison or insight into mechanism. However, the in vivo effects on 17 week-old mice of another hormone; glucocorticoid, suggest that short-term (4 weeks) alterations in osteocyte viability, independent of bone mass as measured by aBMD, contributes to tissue-level properties and bone strength [6]. In addition to considering this indirect effect of cell viability or function on matrix mineralization we must also consider if the short-term effect of estrogen depletion is a direct biochemical one, influencing the manner in which existing tissue matrix mineralizes. These are important issues given that even small differences in tBMD may have profound effects on bone quality.

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References: