Age-Related Bone Loss in Mouse Lumbar Vertebrae is Affected by Region, Sex, and Level: Implications for Spinal Loading and Analysis Methods

Charu Jain[1*], Neharika Bhadhouria[1*], Justin Tiao[1], Jonathan Huang[1], Yunsoo Lee[1], Saad Chaudhary[1], Andrew Hecht[1], Nilsson Holguin[1], James Iatridis[1]
[1]Dept. of Orthopedics, Mount Sinai Health System, New York, NY
*Authors contributed equally.
Email: charu.jain@icahn.mssm.edu **Disclosures**: All authors (NA).

INTRODUCTION: Vertebral bone loss with aging is a key contributor to spinal fragility, instability, and increased risk of vertebral fractures, particularly at the upper lumbar levels. The loss of vertebral bone density and structure with age is well-documented in humans, animal studies especially in mice typically examine a single vertebral level (e.g., L4 or L5)[1,2] or limited vertebral regions[3], often overlooking the biomechanical and anatomical heterogeneity that may influence susceptibility to bone loss. Thus, there remains a need for a comprehensive preclinical study in mice evaluating whether aging and sex influence spatial patterns of vertebral bone loss across spinal levels and within specific vertebral regions. Furthermore, mouse vertebral studies have methodological variability in defining regions of interest (ROIs), highlighting a need for analyses to clarify ROI selection. This study evaluated age- and sex-related changes in trabecular and cortical bone properties across the lumbar spine using micro-CT analysis in male and female C57BL/6J mice. We also compared multiple ROIs to determine their influence on detecting region and level-specific changes. We hypothesized that age-related bone loss would be most pronounced at L1 and in the cranial vertebral region, particularly in females, like humans.

METHODS: The IACUC-approved study used lumbar vertebrae (L1–L6) from male and female C57BL/6J mice at three age groups: young-adult (4 months), middle-aged (12 months), and old (24 months) (n=4/age/sex). Vertebrae were scanned using micro-computed tomography (VivaCT 40, Scanco) at a voxel size of 9.9 µm. Trabecular bone was assessed for bone volume fraction (Tb.BV/TV), volumetric bone mineral density (vBMD), trabecular thickness (Tb.Th), number (Tb.N), and spacing (Tb.Sp). Cortical bone analysis was assessed for cortical thickness (Ct.Th), porosity (Ct.Po), and tissue mineral density (TMD). Three regional analysis approaches were applied: full-vertebra, standardized 30-slice and 1/3-vertebral reconstructions from cranial, middle, and caudal vertebral regions[4]. Two-way ANOVA compared the bone properties across age, sex, vertebral level, and region with Tukey post-hoc tests, and α=0.05. Pearson correlation was used to compare 30-slice and 1/3 reconstructions to full-vertebra.

RESULTS: Age reduced trabecular BV/TV and vBMD across all lumbar levels in both sexes ($p<0.0001$), with the most pronounced decline observed at L1 **(Fig. 1A, B).** In female mice, BV/TV declined by 47.3% at L1 from 4 to 24 months; in males, the loss was 36.0%. L6 also showed significant loss in females but remained relatively preserved in males. Cranial regions showed the most consistent and severe age-related trabecular BV/TV loss (37% in females, 23% in males), while middle regions were more stable **(Fig. 1C, D).** Cortical bone showed less sensitivity to aging; Ct.Th remained largely unchanged across levels and sexes, although Ct.Po increased at L1 in aged females. TMD varied significantly by spinal level but not age. Correlation analyses revealed that the 1/3-vertebra method had stronger agreement with full-vertebra measurements than the 30-slice method. However, the 30-slice approach captured greater regional variability in Ct.Th and BV/TV, especially in cranial regions. These findings suggest that cranial regions of L1 are particularly vulnerable to aging-related bone loss and that the 30-slice method is sufficiently sensitive to detect these spatial trends despite its smaller sampling volume.

DISCUSSION: This study showed vertebral bone loss in aging mice is spatially heterogeneous and sex dependent. The greatest loss of trabecular bone occurred at L1 and L6 in females, with males showing a similar but less severe pattern. This aligns with findings from human studies, which consistently report that the upper lumbar spine, particularly L1, is more prone to osteoporotic fractures due to its location at the thoracolumbar junction and its exposure to high shear forces[5]. Conversely, the lower lumbar spine experiences greater axial loads and is more susceptible to disc degeneration, often promoting adjacent osteoporosis[6]. We also found that cranial regions of vertebral bodies were most sensitive to trabecular bone loss, likely due to higher mechanical loading and stress concentration at these endplate-adjacent sites. Cortical bone, though less affected overall, exhibited increased porosity at L1, particularly in females, which may further compromise structural integrity. Sex differences in bone loss patterns are also well-characterized in humans, with females experience greater trabecular and cortical bone loss compared to age-matched males[7]. Our findings are consistent with human imaging studies showing regional variation in age-related vertebral deterioration[8]. In the context of the literature, our results show important similarities in age-related bone loss between mice and humans despite mice being quadrupeds. Additionally, we validated the 30-slice method as a practical and effective alternative to full-vertebra analysis, capable of detecting level- and region-specific changes. Our findings emphasize the importance of considering both vertebral level and regional anatomy in preclinical models of aged bone and support the utility of simplified analysis methods for detecting regional age-related bone loss.

SIGNIFICANCE/CLINICAL RELEVANCE: This study reveals that mouse vertebral bone aging follows level- and region-specific patterns, most prominently in cranial regions of L1, mirroring human fracture risk. Simplified analysis methods such as 30-slice reconstructions can capture these trends, offering practical approaches for preclinical osteoporosis research and spine fragility modeling.

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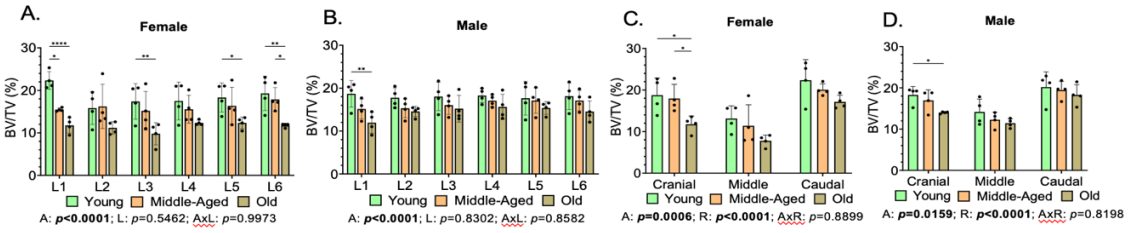

Figure 1. ***Age-Related Vertebral Trabecular Bone Loss Occurs at All Levels and is Pronounced at L1 and L6 in Females and L1 in Males*** *(A, B) Quantification of trabecular BV/ across full vertebral bodies in 4-, 12-, and 24-month female and male mice. (C, D) Quantification of trabecular BV/ across cranial, middle, and caudal regions of the lumbar spine (L1–L6) in 4-, 12-, and 24-month female and male mice.*