

Site-specific Characteristics of Bone in MYO9B Deficient Mice

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INTRODUCTION: Myosin IXB (MYO9B), an unconventional class IX myosin expressed in bone cells, plays a clear role in normal skeletal growth [1]. Global MYO9B deletion in mice leads to suppressed growth, particularly during early puberty, and bone-forming cells (osteoblasts) [1]. Initial analysis of femurs from MYO9B knockout mice highlights diminished trabecular (TB) and cortical bone (CB) at both 4 and 12 weeks of age, accompanied by a reduced bone formation rate and decreased mechanical stability [1, 2]. However, studies exploring the role of MYO9B in determining jawbone characteristics have been limited. Thus, the objectives of this study were to investigate the effects of MYO9B knockout on the characteristics of both the mandible and femur.

METHODS: C57BL/6 background MYO9B knockout mice were generated by a global deletion of the gene, as previously reported [3]. Following IACUC approval, 61 mice (12-weeks old) were assigned to mandible analysis using 16 wild-type (WT) mice (8 male and 8 female) and 15 KO mice (8 male and 7 female), and to femur analysis using 15 WT mice (7 male and 8 female) and 15 KO mice (7 male and 8 female). One hemi-mandible and one femur were randomly obtained from each mouse. These specimens were scanned using micro-computed tomography (micro-CT) at a voxel size of $10 \times 10 \times 10 \mu\text{m}^3$ for mandibles and $20 \times 20 \times 20 \mu\text{m}^3$ for femurs (Fig. 1a,b). Bone volume (BV) was calculated by counting whole bone (WB) voxels. For femurs, CB and TB voxels were digitally separated using a compartmentalizing method (Fig. 1a). In the mandible, WB voxels were isolated from the mental foramen to the distal end after removing the teeth (Fig. 1b). Alveolar bone (AB) voxels surrounding the teeth were also identified within 50 μm of the periodontal ligament.

Mean, standard deviation (SD), and low and high tissue mineral density (TMD) values were calculated at the mean and the lower and upper 5th percentiles of a TMD frequency plot for each region (Fig. 1c, d). Bone mineral density (BMD) was computed by dividing the total sum of TMD in each bone voxel by the total volume (TV), which includes bone, pores, and marrow cavity. Morphological parameters of femoral CB and TB were determined using analysis software provided by the micro-CT manufacturer.

A TB region located above the growth plate (GP) at the distal femoral condyle was digitally isolated to compute TB morphology, including trabecular bone fraction (BV/TV_{TB}), surface-to-volume ratio (BS/BV), number (Tb.N), thickness (Tb.Th), and separation (Tb.Sp). The GP at the distal metaphysis of the femur was identified using micro-CT images (Fig. 2). For the GP, total volume (TV_{GP}), bone volume (BV_{GP}), bone fraction (BV/TV_{GP}), surface area, mean thickness (Th.mean), and maximum thickness (Th.max) were recorded.

After non-destructive scanning, the femurs were mounted on a 3-point bending jig, and a 2 N preload was applied to confirm contact between the specimen and the loading apparatus (Fig. 3a). The hemi-mandibles were partially embedded for support and positioned within the jig for loading (Fig. 3b). Contact between the crown and the upper loading jig was confirmed with a 2 N preload. Throughout the mounting process, specimens were kept moist.

Dynamic mechanical analysis (DMA) was performed on the hemi-mandibles by applying a non-destructive compressive oscillatory force ($-3 \pm 2\text{N}$) at frequencies of 1, 2, and 3 Hz. For the femurs, DMA used a non-destructive oscillatory bending displacement with a mean level of 0.01 mm and an amplitude of 0.005 mm over a range of 0.5 to 3 Hz. Dynamic complex stiffness (K^*) was calculated from elastic (storage, K') and viscous (loss, K'') components using the equation: $*K = K' + iK''$. Tangent delta ($\tan \delta$), which indicates the ability to dissipate energy under loading, was calculated as K''/K' .

Following DMA testing, the same femur was fractured at a bending displacement rate of 0.5 mm/sec. Maximum force (F_{max}), displacement at failure (d_{max}), and toughness (U) were determined from the load-displacement curve (Fig. 2d). Analysis of variance (ANOVA) was used to compare parameters between WT and KO groups, with significance set at $p < 0.05$.

RESULTS SECTION: The femur in the KO group had significantly lower values of BV, TV, BV/TV , BV_{CB} , TMC, BMD, femur length, CB thickness (Ct.Th), minimum inertia (I_{min}), K^* , K , F_{max} , BV/TV_{TB} , Tb.N, Tb.SP, BV/TV_{GP} , GP surface area, Th.mean, and TMD_{GP} than that in the WT group ($p < 0.05$). However, $\tan \delta$ ($p = 0.03$) was higher in the KO group. For the mandible, no parameters showed significant differences between the WT and KO groups ($p > 0.09$). However, Low_{5WB} , Low_{5AB} , and $\tan \delta$ were marginally higher in the KO group compared to the WT group ($p < 0.07$).

DISCUSSION: Knockout of MYO9B diminished the bone quantity in the femur, while the TMD distribution was not significantly changed compared to the WT group. This bone loss in the KO group increased the fracture risk of the femur by reducing both dynamic and static mechanical properties. In contrast, mandibular bone was not significantly affected by MYO9B KO. This likely results from the higher osteogenic activity of BMSCs in the mandible. The shorter femur length in the KO group likely is a product of reduced growth plate thickness.

A limitation of the current study is that the mandibles and femurs were not collected from the same mice. Although the sample size was sufficient to draw conclusions for each site, we did not compare results between the two sites.

SIGNIFICANCE/CLINICAL RELEVANCE: MYO9B deficiency reduces femoral bone quantity, increasing fracture risk, but has no detectable effect on the mandible.

REFERENCES: [1] McMichael, B.K., et al., J Bone Miner Res, 2017. 32(10): p. 2103-2115. [2] Kim, D.G., et al., J Mech Behav Biomed Mater, 2018. 84: p. 99-107. [3] Hanley, P.J., et al., Proc Natl Acad Sci U S A, 2010. 107(27): p. 12145-50.

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