The Effect of Reduced Osteopontin Expression on Bone Morphology and Mechanical Properties in a Novel Heterozygous Mouse Model

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INTRODUCTION: Osteopontin (OPN) is a non-collagenous protein in the extracellular matrix in bone that binds the collagen fibril layer together and inhibits hydroxyapatite crystal growth, ultimately regulating a bone's fragility. Its role is well-studied in the wildtype and knockout phenotypes, and the knockout of OPN leads to increased fragility and impaired matrix quality, independent of changes to bone mass.[1,2] Phosphorylation further regulates OPN activity, and dephosphorylated bone demonstrates susceptibility to fracture, similar to OPN knockout models.[3] However, it has been assumed that OPN is haplosufficient, where having only one functional copy of a gene is sufficient to maintain normal biological function. Hence, little to no work has been done with an OPN heterozygous model, and there is currently a lack of evidence characterizing the changes in bone that occur when there is a physiological deficiency, but not a complete absence, of OPN. This study hypothesized that heterozygous osteopontin mouse bone may exhibit altered morphology, mechanical properties, and phosphorylation compared to wild-type counterparts.

METHODS: We utilized the OPN+/- mouse male model with sample sizes consistent with literature (N=12 WT, 21 OPN +/- radii, ages 6-8 months). Bone morphology was assessed via micro-CT imaging, and mechanical properties were evaluated through three-point bending tests. Validation was performed on extracted bone proteins using ELISA to confirm reduced OPN protein content in bone. Phosphorylation levels in bone proteins were measured using biochemical assays. Only male mice were used at this time to eliminate confounding interactions with sex hormones. T-tests were used to determine statistical significance between groups at a p-value <0.05. Appropriate institutional review was obtained for all experiments.

RESULTS SECTION: ELISA confirmed significantly decreased OPN protein content in OPN+/- mice (-53.57%, p=0.04). Micro-CT analysis revealed that bones with reduced OPN were smaller and less slender, with decreased total cross-sectional area (-6.14%, p=0.012), cortical area (-7.61%, p=0.004), and slenderness (total cross-sectional area/length) (-9.42%, p=0.001). Mechanical testing showed that reduced OPN decreased maximum load (-11.14%, p=0.037) but increased elastic modulus (+29.82%, p=0.01), yield force (+28.74%, p=0.02), and yield stress (+47.66%, p=0.0005). Ultimate stress, work to fracture, post-yield displacement, and post-yield work to fracture remained unchanged (p>0.05). Phosphorylation levels showed a trend toward an increase in OPN+/- mice, but the difference was not statistically significant (+63.79%, p = 0.30).

DISCUSSION: While previous studies have found that osteopontin does not affect bone mass or morphology even when knocked out[1, 4], we report altered bone morphology in the heterozygous model. The mechanical testing trends towards a stiffer profile and being more prone to fracture, which may mechanistically be attributed to increased crystal growth in the absence of OPN. Although it is possible that phosphorylation of the bone without sufficient OPN increases interfilament distancing and is partly responsible for the observed mechanical effects[3], we do not find significant differences in phosphorylation, and further study is needed to elucidate this interaction.

SIGNIFICANCE/CLINICAL RELEVANCE: This study characterizes altered bone morphology and mechanical susceptibility at whole bone and tissue-level properties for the first time in an osteopontin heterozygous mouse model. We challenge the assumption that osteopontin is haploinsufficient and call for future work in this more physiologically relevant phenotype.

REFERENCES: **1**. Thurner PJ, et al. Bone. 2010 Jun;46(6):1564-1573. **2.** Depalle B, et al. Acta biomaterialia. 2021 Jan;120:194-202. **3.** Bailey S, et al. Elife. 2020 Dec;9:e58184. **4.** Bailey S, et al. Ann N Y Acad Sci. 2017;1409(1):79-84.

IMAGES AND TABLES:

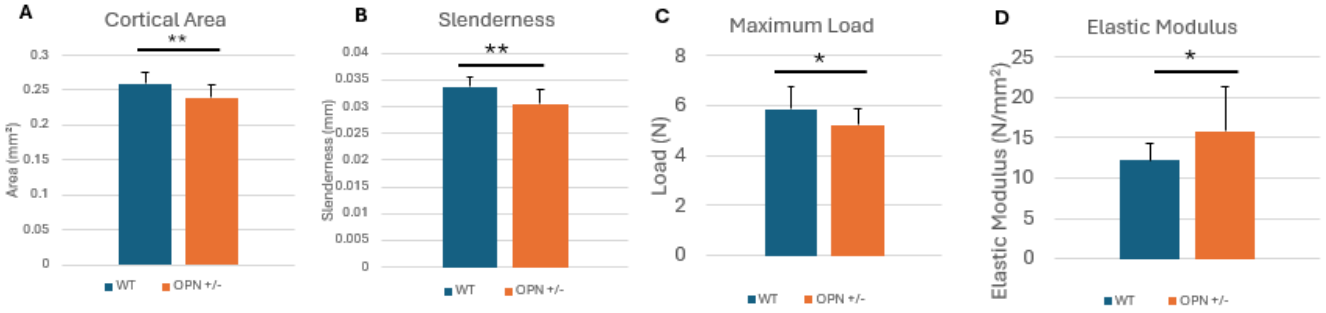

Figure 1. A and B. Micro-CT determined cortical area and slenderness (cross-sectional area/length). **C**. Crystallinity determined from inverse of full width at half maximum of phosphate signal. **D.** Summary of significant mechanical properties. Blue bar= WT; orange bar= OPN +/-. Error bars represent standard deviation. * indicates significance at $P < 0.05$, ** indicates $P < 0.01$.