Dual Deletion of Hypoxia Inducible Factor-1a and -2a Protects Against Age-Related Cortical Bone Loss

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INTRODUCTION: Osteocytes are key regulators of skeletal homeostasis *via* integration of local and systemic signals that govern osteoblast-mediated bone formation and osteoclast-mediated bone resorption[1]. Osteocytes sense and respond to oxygen deprivation *via* hypoxia inducible factors (HIFs). Under normoxia, HIFs (HIF-1α and HIF-2α) are hydroxylated then degraded *via* Von Hippel-Lindau (VHL)-mediated ubiquitination. Hypoxia prevents oxygen-dependent hydroxylation, leading to HIF-α stabilization, its nuclear translocation, and activation of target genes[2]. HIFs are key mediators in bone development and skeletal maintenance. Our previous studies have shown that osteocyte-specific deletion of either *Hif1a* or *Hif2a* in female adult mice had no impact on skeletal phenotype[4]. Preliminary data indicate that a similar trend is observed in male mice. Given the established role of HIFs in bone development and remodeling, this study utilized dual osteocytic *Hif1a* and *Hif2a* deletion mice to determine whether both *Hifa* paralogs are required for healthy skeletal development and skeletal maintenance during aging. We hypothesized that HIFs work in a compensatory manner and dual deletion of *Hif1a* and *Hif2a* will negatively impact skeletal architecture with age.

METHODS: *Hif1a*$^{f/f}$ (#007561) and *Hif2a*$^{f/f}$ (#008407) mice were purchased from Jackson Laboratory and bred with mice expressing Cre-recombinase driven by 10-kb-*Dmp1* promoter (*Dmp1-Cre*). Matings produced male and female progeny with osteocyte-enriched dual deletion of *Hif1* and *Hif2a* cKO (dcKO). Both male and female mice were euthanized at 4 months and 18 months of age and left femora were scanned (μCT 35; Scanco Medical AG) at 55 kVP, 145mA, 6μm voxel size[5]. Trabecular bone volume fraction (BV/TV) and trabecular number (Tb. N) were quantified within a 1.5-mm span of the distal femoral metaphysis. Cortical parameters, including cortical area fraction (Ct. Ar/Tt. Ar) and cortical thickness (Ct. Th), were assessed within a 0.6-mm segment of the mid-diaphysis. Sample sizes ranged from n=3-11 for female mice and n=3-12 for male mice. Data were analyzed using multiple comparison ordinary two-way ANOVA tests, where $p<0.05$ was considered statistically significant. All animal work was conducted in accordance with institutional guidelines.

RESULTS: Dual deletion of *Hifa* paralogs revealed sex-dependent effects on skeletal aging that varied between skeletal compartments. MicroCT analyses revealed that cortical bone mass was preserved in aged male dcKO mice and dramatically enhanced in aged female dcKO mice (**Fig. 1A-B**). Both male and female dcKO mice demonstrated an increase in cortical thickness (**Fig.1C**) where significant decreases in cortical porosity were also observed in female mice (*data not shown*). Deletion of *Hifa* paralogs did not prevent age-related trabecular bone loss in either sex (**Fig.1D-E**).

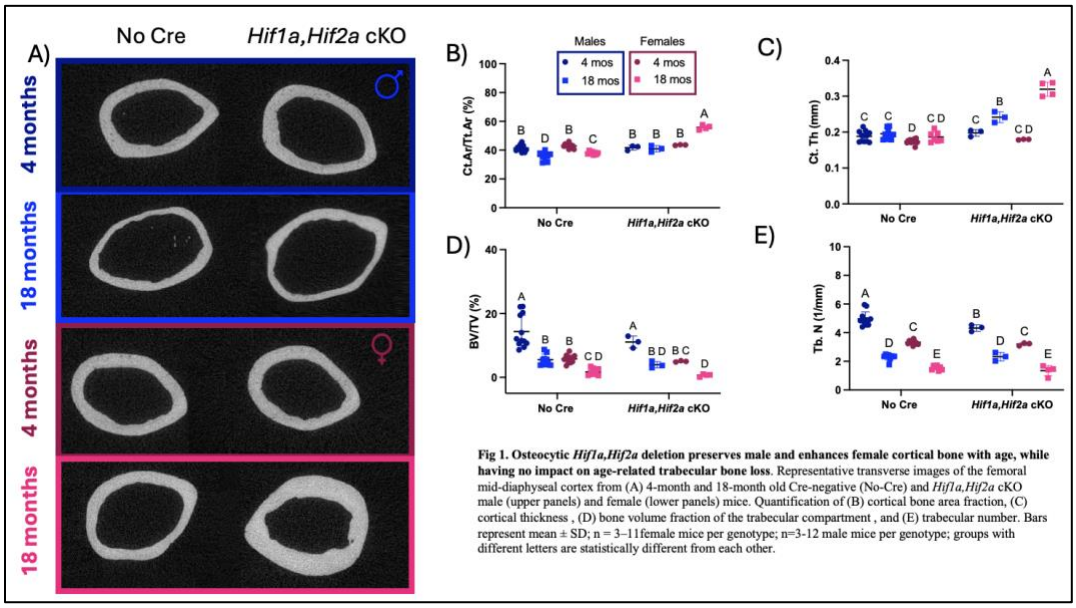

Fig 1. Osteocytic *Hif1a,Hif2a* deletion preserves male and enhances female cortical bone with age, while having no impact on age-related trabecular bone loss. Representative transverse images of the femoral mid-diaphyseal cortex from (A) 4-month and 18-month old Cre-negative (No-Cre) and *Hif1a,Hif2a* cKO male (upper panels) and female (lower panels) mice. Quantification of (B) cortical bone area fraction, (C) cortical thickness , (D) bone volume fraction of the trabecular compartment , and (E) trabecular number. Bars represent mean ± SD; n = 3–11female mice per genotype; n=3-12 male mice per genotype; groups with different letters are statistically different from each other.

DISCUSSION: The role of HIF signaling in the aging skeleton remains poorly understood. Our study demonstrates that dual deletion of *Hifa* and *Hif2a* in osteocytes had no effect on age-induced bone loss in the trabecular compartment but preserved bone mass in the cortex. These data suggest that the activity of HIFs during aging contribute to the molecular mechanism underlying bone loss in the cortical but not trabecular compartment. Further, the accumulation of bone in the cortex of aged female dcKO mice suggests that HIF activity in female mice may be more strongly catabolic than in males with aging. This correlates with unpublished data from our lab that demonstrate that dual stabilization of HIF-1α and HIF-2α in osteocytes leads to cortical bone loss in 4-month-old female, but not male mice. Loss of HIF signaling enhances cortical bone mass with aging, suggesting a novel osteoanabolic approach to combat senile osteoporosis which demands greater sustained mechanistic interrogation.

SIGNIFICANCE/CLINICAL RELEVANCE: Age related bone loss is a major contributor to fractures and morbidity in the aging population, yet the molecular mechanisms that protect bone remain unclear. Our results reveal that dual HIF deletion enhances cortical bone in aged females and preserved it in aged males, highlighting a novel pathway that could inform sex-specific strategies to prevent age associated bone deterioration.

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