Evaluating the sex-dependent effects of manipulating osteocytic HIF-α expression on skeletal development

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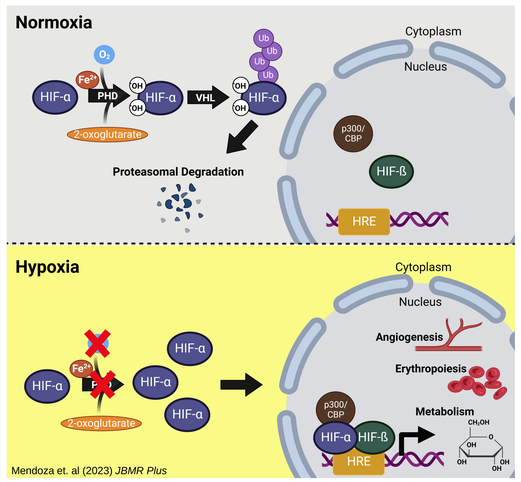
INTRODUCTION

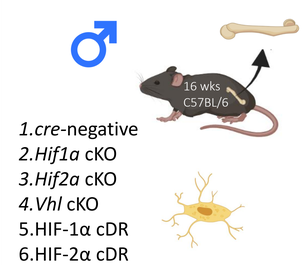


- Hypoxia pathway = osteoanabolic
- VHL is master regulator of HIF signaling
- Our previous studies show that:
  - Deletion of *Vhl* in osteocytes but not osteoblasts (*Dmp1-cre;Vhl* $^{f/f}$) generates a robust high bone mass phenotype in female mice[3].
  - We did not observe a reciprocal, low bone mass phenotype in osteocytes lacking HIF-1α in female mice[3].
- The status of the HIF2α isoform as necessary and/or sufficient for the *Dmp1-cre; Vhl* $^{f/f}$ HBM phenotype is currently unknown.

Objective: To evaluate how osteocytic HIF-1α isoform manipulation alters skeletal microarchitecture, bone mass and histomorphology in male mice, and determine sex-dependent effects

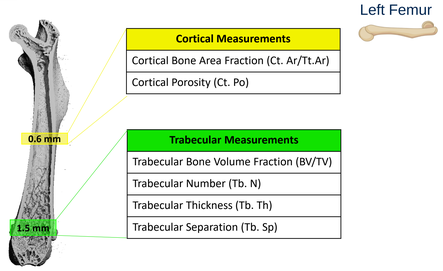
METHODS

Mice



1.*cre*-negative
2.*Hif1a* cKO
3.*Hif2a* cKO
4.*Vhl* cKO
5.HIF-1α cDR
6.HIF-2α cDR

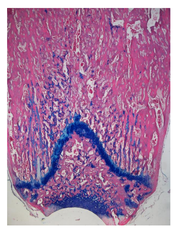
Micro-computed tomography (μCT 35; Scanco Medical AG)



Cortical Measurements
Cortical Bone Area Fraction (Ct. Ar/Tt.Ar)
Cortical Porosity (Ct. Po)

Trabecular Measurements
Trabecular Bone Volume Fraction (BV/TV)
Trabecular Number (Tb. N)
Trabecular Thickness (Tb. Th)
Trabecular Separation (Tb. Sp)

Histology



Hematoxylin, Eosin, and Alcian Blue staining

Protocol
Femora were: •Fixed, decalcified, processed, and paraffin-embedded •Cut into serial 5 μm sections via microtome Stained with: •Hematoxylin & Eosin / Alcian Blue •Safranin-O / Fast Green •Tartrate-Resistant Acid Phosphatase (TRAP)

RESULTS: Osteocytic degradation-resistant HIF-α isoforms

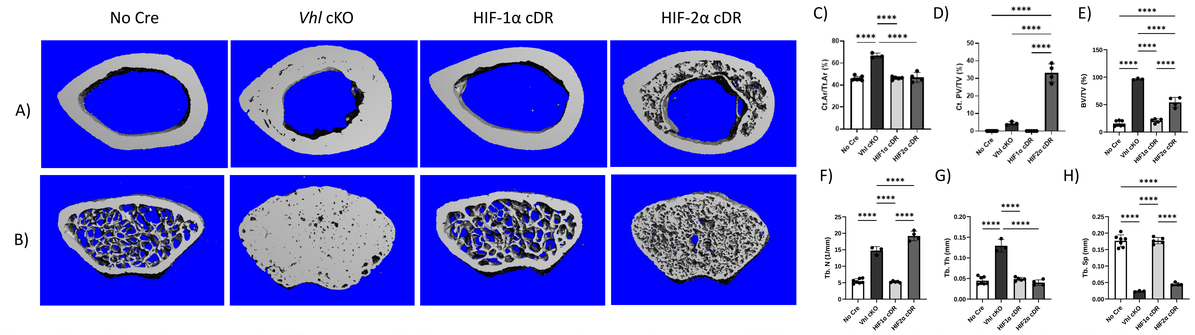


Figure 4. Degradation-resistant, osteocytic HIF2α generates a high bone mass phenotype. Representative transverse μCT 3D reconstructions from the femoral midshaft (A) and distal femoral metaphysis (B) from *cre*-negative, *Vhl* cKO, HIF-1α cDR, and HIF-2α cDR male mice. Quantitative cortical microarchitecture measurements of cortical bone area fraction (Ct.Ar/Tt.Ar, C) and cortical porosity (Ct.Po, D) from the femoral mid-diaphysis. Quantitative trabecular microarchitecture measurements of bone volume fraction (BV/TV, E), trabecular number (Tb.N, F), trabecular thickness (Tb.Th, G), and trabecular spacing (Tb.Sp, H) from the distal femoral metaphysis. Bars represent mean SD; n = 3–7 male mice per genotype; groups with *'s are statistically different from each other (****= p<0.0001).

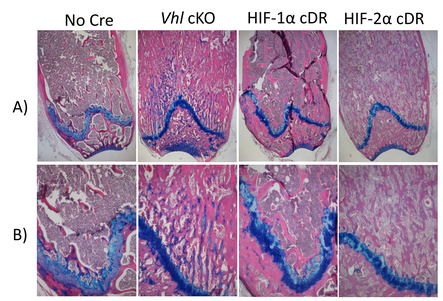


Figure 5. Degradation-resistant osteocytic HIF-2α generates increased bone turnover. Representative hematoxylin/eosin and Alcian blue staining of the distal femoral epiphysis at 4X magnification (A) and 10X magnification (B) from *cre*-negative, *Vhl* cKO, HIF-1α cDR, and HIF-2α cDR male mice .

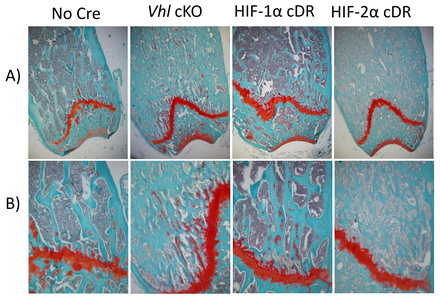


Figure 6. Degradation-resistant osteocytic HIF-2α does not generate residual calcified cartilage. Representative Safranin O/Fast Green staining of the distal femoral epiphysis at 4X magnification (A) and 10X magnification (B) from *cre*-negative, *Vhl* cKO, HIF-1α cDR, and HIF-2α cDR male mice .

RESULTS: Osteocytic *Hifa* isoform deletion

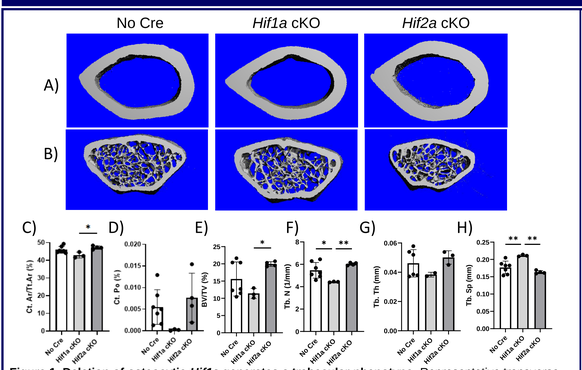


Figure 1. Deletion of osteocytic *Hif1a* generates a trabecular phenotype. Representative transverse μCT 3D reconstructions from the femoral midshaft (A) and distal femoral metaphysis (B) from *cre*-negative, *Hif1a* cKO, and *Hif2a* cKO mice. Quantitative measurements of cortical bone area fraction (Ct.Ar/Tt.Ar, C) and cortical porosity (Ct.Po, D) from the femoral mid-diaphysis. Quantitative measurements of bone volume fraction (BV/TV, E), trabecular number (Tb.N, F), trabecular thickness (Tb.Th, G), and trabecular spacing (Tb.Sp, H) from the distal femoral metaphysis. Bars represent mean SD; n = 3–7 male mice per genotype; groups annotated with *'s are statistically different from each other (* = p<0.05, ** = p<0.01).

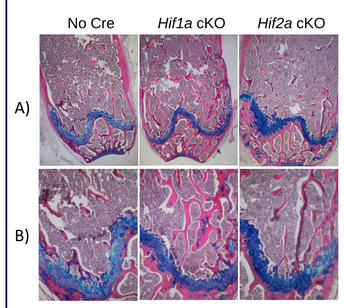


Figure 2. Osteocytic *Hifa* isoform deletion does not alter gross tissue morphology. Representative hematoxylin/eosin and Alcian blue staining of sagittal sections of the distal femoral epiphysis at 4X magnification (A) and 10X magnification (B) from *cre*-negative, *Hif1a* cKO, and *Hif2a* cKO male mice.

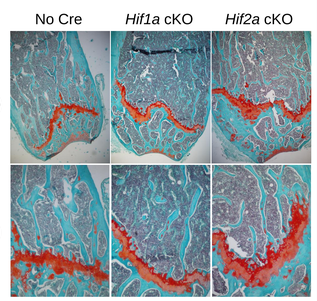


Figure 3. Osteocytic *Hifa* isoform deletion does generate residual calcified cartilage in the trabecular compartment. Representative Safranin O/Fast Green staining of sagittal sections of the distal femoral epiphysis at 4X magnification (A) and 10X magnification (B) from *cre*-negative, *Hif1a* cKO, and *Hif2a* cKO male mice.

SUMMARY & CONCLUSIONS

Our data suggests that there are sexually dimorphic differences in the *Hif1a* cKO mice:

1. Males showed decreased trabecular number and increased trabecular spacing compared to control mice, whereas the cortical compartment was unaffected
2. Females showed no significant difference in these parameters (Mendoza et. al (2023), *JBMR Plus*)

Male *Vhl* cKO, HIF-1α cDR, and HIF-2α cDR mice mirror results from the female mice

Future Directions:
- Compare male and female data to determine magnitude of skeletal changes
- Tartrate-Resistant Acid Phosphatase (TRAP) staining: osteoclast number/activity

REFERENCES

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