Role of CaMKK2 in Mechanical Stimulation of Bone

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Introduction

There is a need for anabolic therapies to treat degenerative bone diseases. Mechanical loading stimulates anabolic and inhibits catabolic pathways of bone leading to increased bone formation. Osteocytes are the bone cell type responsible for coordinating the mechano-stimulatory pathways in bone. Calcium/ calmodulin-dependent protein kinase kinase 2 (CaMKK2) is a serine/threonine protein kinase with roles in bone remodeling. CaMKK2 global deletion results in lower osteoclast differentiation and higher osteoblast formation and activity.

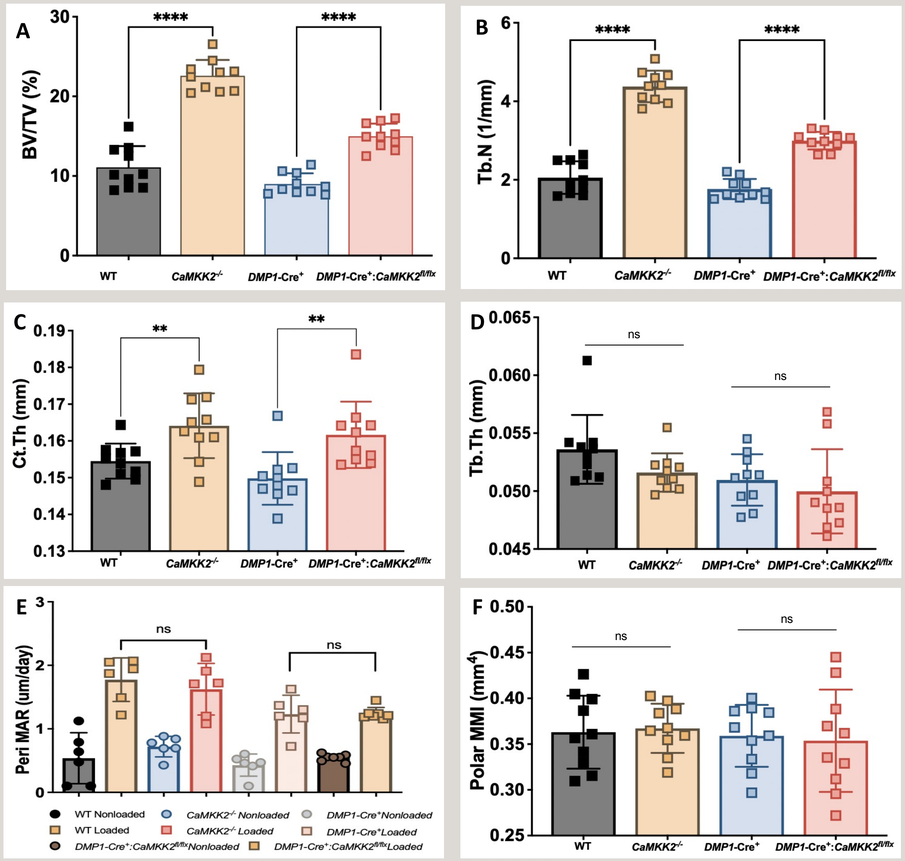
Hypothesis

We hypothesize that global and osteocyte specific loss of CaMKK2 in female mice will result in higher bone formation under mechanical loading compared to non-loaded limbs.

Materials & Methods

Animal studies were approved by the Indiana University School of Medicine (IUSM) Institutional Animal Care and Use committee (IACUC). Conditional deletion of *Camkk2* in osteocytes was achieved by crossing *Camkk2*$^{flox/flox}$ mice with dentin matrix protein1 (*Dmp1* -8kb, osteocyte-specific) -Cre transgenic mice, to generate *Dmp1*-Cre:*Camkk2*$^{-/-}$ (osteocyte-specific) and *Dmp1-Cre*$^{+}$ (Cre^{+} only) control mice. The right tibiae of 18-week-old female WT, *Camkk2*$^{-/-}$, *Dmp1*-Cre^{+}, and *Dmp1*-Cre: *Camkk2*$^{-/-}$ mice were loaded at 2 Hz for 220 cycles while anesthetized and peak forces were predetermined for each genotype. The left tibiae served as non-loaded internal controls. Mice were loaded on days 1, 3, 5, 8 and 10 of the experiment using an electro actuator (Bose ElectroForce 3200; EnduraTEC, Minnetonka, MN, USA). Calcein and alizarin red were administered intraperitoneally on days 9 and 16 respectively to measure bone formation using dynamic histology. Mice were sacrificed on day 19. Left and right tibiae were collected and fixed in 4% paraformaldehyde for 48 h and stored at 4ºC in 70% ethanol. Micro-computed tomography (µCT) was performed on both loaded and non-loaded tibiae to analyze bone microstructure in the proximal tibia and cortical midshaft. (N=10/genotype). Undecalcified tibiae were embedded in polymethyl methacrylate and cross-sections were taken of the tibial midshaft for dynamic histology (N=5/genotype). Statistical comparisons between groups were made using Welch's t-test . All values are presented as means ± standard deviation. *p*-value <0.05 was considered significant.

Figure 1. Impact of global or osteocyte-specific loss of CaMMK2 in mechanically loaded female mice

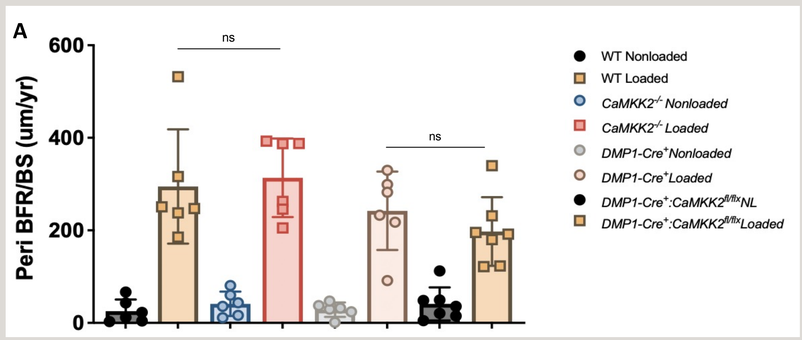


(1A) *Camkk*$^{-/-}$ and *Dmp1*-Cre: *Camkk2*$^{-/-}$ mice showed significantly higher levels of (BV/TV %), (1B) trabecular number , and (1C) cortical midshaft thickness when compared to WT and Cre^{+}-only control mice.

(1D) Trabecular thickness and (1E) mineral apposition rate did not show any significant changes between all genotypes.

(1F) There was no significant change in mineral apposition among all genotypes. Quantification for loaded tibia.
N=10; mean ± SD; p-values: **** p<0.0001, and **p<0.01.

Figure 2. CaMKK2 effect on bone formation rate in mechanically loaded and non-loaded female mice



Bone formation rate/ bone surface (BFR/BS) between loaded *Dmp1*-Cre: *Camkk2*$^{-/-}$ and Cre^{+}-only mice and WT loaded and CaMKK2 loaded mice did not show any significant changes. Quantification for loaded tibia.
N=6; mean ± SD; p-values: **** p<0.0001, and ***p<0.001.

Discussion

Global and osteocyte-specific deletion of CaMKK2 in female mice have higher %BV/TV, higher Tb. N, higher Ct. Th, compared to WT and Cre^{+}-only control mice. Importantly, BFR/BS did not show any changes between all genotypes. These findings suggests while CaMMK2 does impact bone microstructure in loaded mice, it plays no role in bone mass accrual in mechanically stimulated mice. Further understanding of the role that CaMKK2 plays in the process of bone remodeling may allow the development of novel therapies to target various degenerative disease states. *This project is still on-going and more data has yet to be analyzed. In addition, the role of CaMMK2 in mechanically stimulated males is concurrently being investigated by our lab.*

Acknowledgements

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