The Role of CaMKK2 in Whole-Body Metabolism and Bone Remodeling

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Abstract

Obesity and metabolic dysfunction negatively impacts many tissues, including the skeleton. Calcium/calmodulin-dependent protein kinase kinase 2 (CaMKK2) is a serine/threonine protein kinase and key regulator of whole-body energy homeostasis. Previous studies have shown that male Camkk2$^{-/-}$ mice are protected from high-fat diet induced obesity, glucose intolerance and insulin resistance. CaMKK2 regulates hypothalamic control of appetite, hepatic gluconeogenesis, and insulin production by pancreatic β cells. Deletion of CaMKK2 has also been shown to enhance bone mass and strength in mice. The skeleton is recognized as a highly dynamic and metabolic tissue capable of regulating systemic glucose metabolism. The role of CaMKK2 in bone-mediate regulation of whole-body metabolism is unknown.

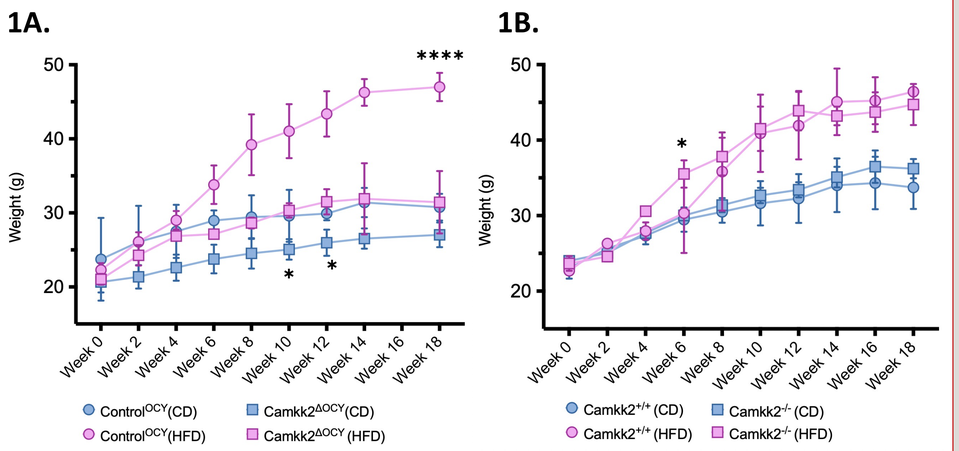
Hypothesis

We hypothesize that bone-targeted deletion of CaMKK2 from osteoblasts and osteocytes will concurrently improve glucose metabolism under normal and high fat diet and will simultaneously promote bone accrual.

Materials & Methods

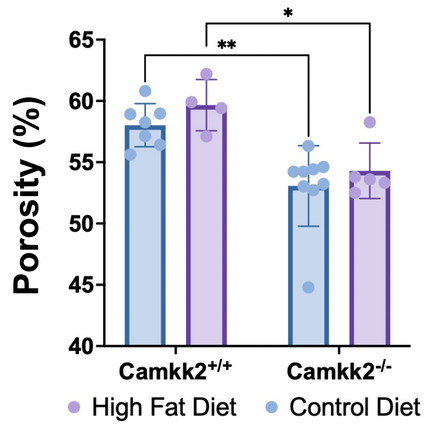
Animal studies were approved by the Indiana University School of Medicine (IUSM) Institutional Animal Care and Use Committee (IACUC). To generate *DMP1-Cre: Camkk2$^{fl/fl}$* (*Camkk2^{OCY}*) and *Dmp1-8kb-Cre^{+}: Camkk2$^{+/+}$ (Control)* mice, we used the Cre-LoxP recombination system and crossed *Camkk2$^{fl/fl}$* and dentin matrix protein 1 (*Dmp1-8kb*)-cre transgenic mice. Eight-week-old *Camkk2$^{+/+}$, , Camkk2$^{-/-}$, Control^{OCY},* and *Camkk2^{OCY}* mice (n=12/group) were randomly assigned to high-fat (60% kcal from fat), or nutrient matched control (10% kcal from fat) diets for a duration of 16 weeks. Diets contained equal amounts of protein, sucrose, vitamins, and minerals. Bbone mineral density (BMD) and body composition were measured using dual-energy X-ray absorptiometry (DEXA) beginning two weeks prior to the initiation of diets and monitored biweekly for an additional 16 weeks. Metabolic phenotyping including glucose tolerance test (GTT) and insulin tolerance test (ITT) was performed on male mice (n=12/group) prior to beginning diets and tested again after 16 weeks on diets. Following 16 weeks of control or high-fat diet (HFD) mice were euthanized and long bones were excised for analysis. Right femurs (n=6-7/group) were fixed in 4% paraformaldehyde (PFA) for 48 hours and transferred to 70% ethanol. The midshaft and distal femur microarchitecture were analyzed using micro-computed tomography (μCT). Statistical comparisons between groups were made using a two-way ANOVA. All values are presented as means ± standard deviation. *p*-value < 0.05 was considered significant.

Figure 1. Conditional deletion of CaMKK2 from osteoblasts and osteocytes protects from diet-induced obesity.



(1A.) Changes in body weight measured biweekly for *Control^{OCY}* and *Camkk2^{OCY}* on control and high fat diet. **(1B.)** Changes in body weight measured biweekly for *WT* and *Camkk2$^{-/-}$* on control and high fat diet**.** Values displayed represent mean ± standard deviation (n = 7/group; **** p<0.0001, and *p<0.01).

Figure 2. Global deletion of CaMKK2 reduces cortical bone porosity.



(2A.) Changes in Closed Porosity for *WT* and *Camkk2^{OCY}* following 16 weeks of either control or high-fat diet. Values displayed represent mean ± standard deviation (n = 7/group; ** p<0.01, and *p<0.01).

Results

Camkk2$^{\Delta OCY}$ showed significantly lower weight compared to Control $^{\Delta OCY}$ in the high-fat diet group starting at 8 weeks (**Fig 1A).** Camkk2$^{\Delta OCY}$ showed significantly lower weight compared to Control $^{\Delta OCY}$ in the control diet group at weeks 10 and 12 only **(Fig 1A).** The only timepoint where *Camkk2$^{-/-}$* had significant lower weight compared to WT was at week 6 in the high-fat diet group. There were no changes in the control diet groups. **(Fig 1B).** Closed porosity was significantly decreased in *Camkk2$^{-/-}$* compared to WT in control and high-fat diet groups (**2A)**.

Discussion

Conditional deletion of CaMKK2 from osteoblasts and osteocytes protected male mice from diet induced obesity, while global ablation of CaMKK2 did not. These results were somewhat unexpected, as previous studies have shown Camkk2$^{-/-}$ mice have are protected from diet-induced obesity; however, it is important to note fat content and duration of diets used in the study is different. Our results indicate that CaMKK2 and the skeleton play a key role whole-body metabolism. Closed porosity was significantly decreased in *Camkk2$^{-/-}$* mice suggesting that CaMKK2 also plays a protective role in bone in the setting of diet changes. This project is still ongoing, and more data and findings are forthcoming.