

## Ketogenic diet induces bone loss in adult mice and may reduce the anabolic effect of exercise.

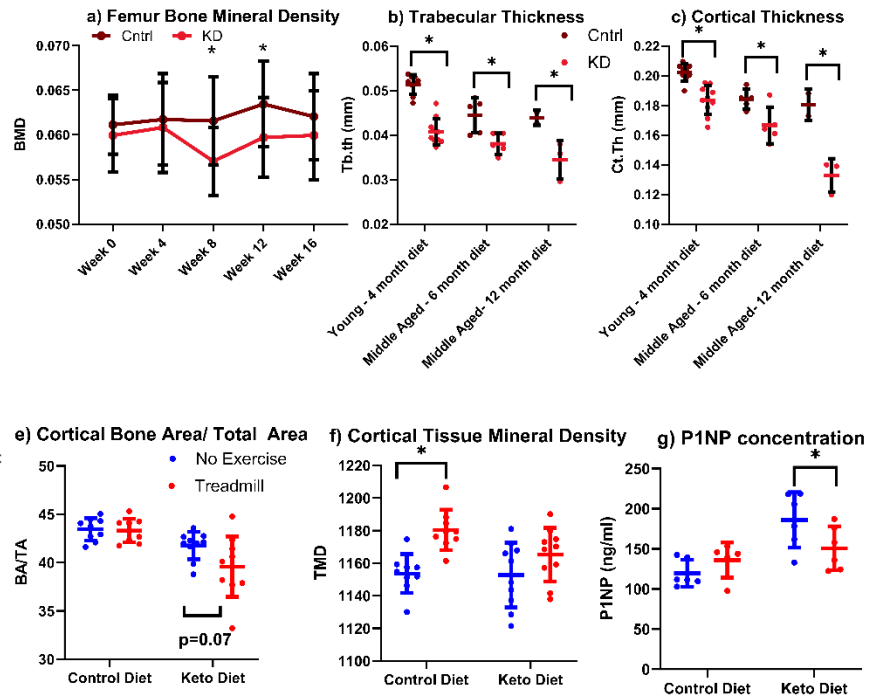
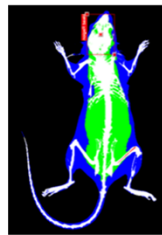
Benjamin Osipov<sup>1</sup>, Sophie V. Orr<sup>1</sup>, Yu-Yang Lin<sup>1</sup>, Ritvik S. Punati<sup>1</sup>, Maryam Rahmati<sup>1</sup>, Suraj J. Pathak<sup>2</sup>, Kei Takahata<sup>3</sup>, Keith Baar<sup>2</sup>, Blaine A. Christiansen<sup>1</sup>  
<sup>1</sup>University of California Davis Health, Department of Orthopaedic Surgery; <sup>2</sup>University of California Davis, Department of Neurobiology, Physiology and Behavior; <sup>3</sup>Graduate School of Saitama Prefectural University, Saitama, Japan

**Disclosures:** None

**INTRODUCTION:** Ketogenic Diet (KD), a diet high in fat and low in carbohydrate, has been used to treat refractory epilepsy in children and weight management in adults. KD is associated with bone loss and increased fracture risk in children prescribed KD for drug refractory epilepsy, but the effect of KD on bone in older individuals has not been well studied. Furthermore, prior studies in athletes found that KD decreased markers of bone formation and increased markers of bone resorption relative to athletes on a diet with normal carbohydrate content, suggesting KD may impair bone formation in response to exercise. In this study, we assessed the effect of KD and exercise on bone quantity and strength in mice. We hypothesized that compared to animals eating a control diet (CD), KD mice would exhibit lower bone quantity and altered mechanical properties, and the magnitude of the effect would increase with diet duration. We further hypothesized that KD would reduce the anabolic effect of exercise on bone quantity and mechanical properties.

**METHODS:** This study used a total of 60 male 3-month-old (Young) C57BL/6J mice that were placed on a KD or control diet (CD) for 3 months. Mice

were then randomly assigned to normal cage activity or 15 days of treadmill running at 12 m/s for 30 minutes a day. To assess longer term effects of KD, we collected a convenience sample of 15 12-month-old male (Middle-aged) C57BL/6J mice randomly assigned to receive 11.6 kCal/day in the form of KD or CD. 10 mice were euthanized 6 months after the start of the dietary intervention and 5 were euthanized at 12 months. 3-month-old mice received DEXA scans at 4-week intervals. For all age groups, cortical and trabecular bone microarchitecture of the femur midshaft and distal metaphysis were quantified via micro-computed tomography, and mechanical properties of the femur midshaft were quantified via 3-point bending. Serum was collected and bone remodeling markers P1NP (for bone formation) and CTX-1 (for bone resorption) were assessed in the 3-month-old cohort via enzyme-linked immunosorbent assay (ELISA). Data were analyzed using multifactorial ANOVA with Tukey's HSD post-hoc comparisons.



**RESULTS:** 3-month-old KD mice exhibited a lower femur BMD than CD mice 8 weeks after diet intervention began (Fig a). 4 months of KD was associated with a significant decline in cortical thickness and trabecular bone volume fraction and thickness (Fig b,c). In 12-month-old mice, 6 months of KD also significantly decreased bone quantity and femoral midshaft stiffness and max force, and the difference between CD and KD groups was larger in the 12 month than the 6 month diet intervention group (Fig b,c,d). In 3-month-old mice, exercise was associated with a significant increase in cortical tissue mineral density only in CD mice (Fig f). Exercised KD mice showed a trend towards decreased bone area/total area fraction compared to unexercised KD mice, which was not evident in CD mice (Fig e). For P1NP, there was a significant interaction between diet and exercise. KD was associated with elevated P1NP compared to mice on CD (Fig g). Unlike CD animals, P1NP significantly decreased in exercised KD animals compared to unexercised controls. CTX-1 did not differ due to diet but was lower in exercised animals.

**DISCUSSION:** Consistent with our initial hypotheses, KD was associated with considerable cortical and trabecular bone loss and altered bone remodeling. Deficits in bone quantity and material properties increased with the duration of the diet. Defects in bone mass were not the result of decreased collagen synthesis since P1NP levels were elevated on KD. Exercise did not increase cortical tissue mineral density to the same extent in KD mice as CD mice and may have even decreased cortical thickness in KD mice. This is supported by bone formation markers decreasing in KD mice after exercise.

**SIGNIFICANCE/CLINICAL RELEVANCE:** These findings suggest that KD-induced bone loss occurs in skeletally mature individuals and may significantly increase fracture risk. Currently, KD is only considered a fracture risk for juveniles, but these findings indicate that KD, especially in the long term may increase fracture risk in adults. Attempts to treat epilepsy, lose weight, or attenuate the effects of aging via KD may need to consider the potential impact of this diet on bone health and fracture risk. KD may reduce the anabolic effect of exercise. This is important given the growing interest in using KD for enhancing athletic performance, and it suggests that exercise may not prevent bone loss on KD.

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