

Dietary Prune Rapidly Increases Trabecular Bone Volume in Healthy Female Mice and Bone Benefits are Lost Following Discontinuation

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Disclosures: None.

INTRODUCTION: Osteoporosis and low bone mass is a silent affliction that will impact 50% of women and 25% of men in the US at some point in their lifetime. Current treatment strategies are effective at preventing further loss, as well as some options to mildly increase bone density. Unfortunately, these strategies can be costly, are inconvenient, and are not without side-effects. Thus, there is a critical need to develop novel therapies and approaches that are cost-effective, convenient, and side-effect free. Our lab and others have established the gut as a critical and unique target to prevent several etiologies of bone loss in both preclinical models and human studies. These studies have primarily focused on preventing bone loss through supplementation with pre- and probiotics as well as gut barrier enhancers. Recently, prunes, a natural fruit and prebiotic, have emerged as an effective option to both prevent and reverse primary osteoporosis in animal models as well as prevent further loss of total hip bone mineral density in post-menopausal women after one year of treatment. We have recently demonstrated that dietary prune supplementation is a novel approach to prevent glucocorticoid-induced bone loss in female mice. In addition, we have also shown a time-dependent increase in trabecular bone volume in healthy female mice, culminating in a 3-fold increase following 16-weeks of dietary prune, something we have not observed with any of our previous gut targeted treatments. While our lab and others have now shown bone benefits of dietary prune supplementation in healthy mice, it is unknown if the prunes need to be supplemented continuously to maintain bone benefits, or if the benefits are lost following discontinuation. In preliminary studies we found that bone benefits of 10-day dietary prune treatment were completely lost following 20 days of prune discontinuation. The timeline of how quickly the benefits were lost however remain unclear. Thus, the goal of these studies was to better understand the response to prune diet discontinuation and re-administration in healthy female mice. Based on the magnitude of responses observed in previous studies, we hypothesized that the bone benefits would not be completely lost following diet discontinuation and any loss that occurred would be rapidly regained following re-administration of the prune diet.

METHODS: All animal experiments were approved by Michigan State University IACUC. **Animal Experiments:** 16-week-old female C57BL/6J mice were treated with iso-caloric, isoflavone free AIN-93M diets. The control diet did not contain prune powder while the prune diet contained prune powder at 25% w/w. Mice were fed diets for periods of 4 weeks, 5 weeks, 6 weeks, or 7 weeks (n=10/group at each time point). The pattern of discontinuation and re-administration of diets can be found in **Figure 1. Micro-Computed Tomography (μCT):** Fixed femurs were scanned using a GE Locus Explore μCT, were phantom calibrated, and analyzed blinded to treatment group. Each scan contained bones from each group at a certain time point. Trabecular bone was analyzed in a region of the distal femur extending 10% of the total bone length towards the diaphysis proximal to the distal metaphysis and excluded cortical bone. Cortical bone was measured at the midshaft of the femur within a 2x2x2 mm cube. **Statistical Analysis:** All analyses were completed within GraphPad Prism via One-Way ANOVA and statistical significance set to $p < 0.05$.

RESULTS: As expected, μCT analyses of distal femur trabecular bone following 4, 5, 6, and 7 weeks of 25% prune diet resulted in significant increases ($p < 0.0001$) in trabecular bone volume/total volume (BV/TV%, **Figure 1**). To assess the bone response after discontinuation of the prune diet, we replaced prune diet with control diet for 1, 2, or 3 weeks following 4 weeks of prune supplementation (E, H, L respectively). Surprisingly, after 1 week of discontinuation, there was a significant reduction in BV/TV% compared to age matched prune diet treated mice ($p = 0.0032$), however, BV/TV% was still increased compared to controls ($p = 0.0093$) indicating that there was some reversal of bone benefit, but the benefits were not completely lost. After 2 or 3 weeks of discontinuation the benefits were completely lost and, in both cases, returned to age matched control values for BV/TV%. To assess how rapidly the bone benefits could be regained after discontinuation, mice were treated with prune diet for 4 weeks, switched to a control diet for 1 week, and then switched back to the prune diet for 1 or 2 weeks (I and M respectively). Even 1 week of prune diet after the discontinuation period was sufficient for the bone benefits to be restored when compared to age matched prune diet treated mice and the results were similar after 2 weeks of prune diet following the discontinuation period. Trabecular micro-architecture was also assessed and the results for trabecular number, spacing, and thickness followed appropriately with the changes in BV/TV%.

DISCUSSION: For the first time, we demonstrate that the bone benefits of 4-week treatment with dietary prune are partially lost following 1 week of prune diet discontinuation and completely lost following 2 weeks of discontinuation of the prunes in healthy female mice. We also show that the bone benefits can be completely regained with re-administration of the prune diet for as little as 1 week following discontinuation, suggesting that prunes may be eaten intermittently and still maintain their positive effect on bone health. Further studies are necessary to better understand the mechanism by which these rapid changes in bone volume occur and the functional effects on bone health.

SIGNIFICANCE/CLINICAL RELEVANCE: Since low bone mass remains an important health problem in the US and around the world despite effective treatment and prevention strategies, there is a critical need to discover novel strategies to prevent this. These results demonstrate that dietary prune supplementation is an effective option to improve bone volume prior to age or disease related declines and that it may be used intermittently and still achieve similar bone benefits.

ACKNOWLEDGEMENTS: Funding for this study was provided by California Prunes and the California Prune Board and fellowship support was provided by NIH National Center for Complementary and Integrative Health (NCCIH, F30 AT012416).

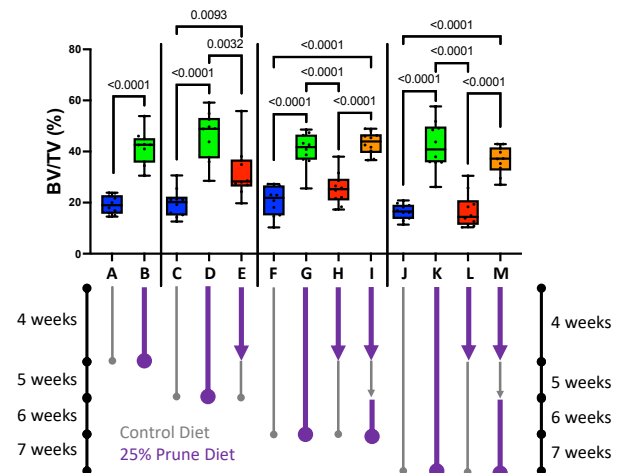


Figure 1. Experimental timeline and distal femur bone volume/total volume % (BV/TV%). Statistical analyses completed via One-Way ANOVA at each time point. n=10/group.