**A Sex Dimorphic Mechanism Links Protection from Bone Loss and Promotion of Gut Microbiome Diversity Induced by a Nutritional Intervention**

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We previously showed that a blueberry-enriched diet counteracts the effects of estrogen deficiency in female mice on bone, skeletal muscle, and peripheral fat, and alters the gut microbiome. Here, we compare between female and male mice the impact of the diet on the skeleton and the microbiome. Four-month old B6.129X1/J mice were gonadectomized or sham operated, each group separated in two groups, which then were fed either control AIN-93M diet (C) or AIN-93M diet containing 10% lyophilized/freeze dried Montgomery blueberry (MONT); N=9-20 females and N=10-26 males. In contrast to full protection from OVX-induced bone loss, MONT failed to prevent the loss in BMD induced by ORX. Further, while MONT completely prevented OVX-induced architectural deterioration of cancellous and cortical bone, the diet did not prevent the decrease in BV/TV, Tb.Th, Tb.N, Ct.Ar/Tt.Ar, and Ct.Th, quantified by micro-CT, induced by ORX. Likewise, MONT prevented OVX-induced increases in circulating resorption marker CTX, but it had no effect on CTX levels in ORX mice. We next quantified gut microbiome diversity, by analyzing fecal bacterial DNA using 16S rRNA gene sequences from high throughput paired end MiSeq technology. OVX or ORX did not alter the variety of bacterial communities (α-diversity) within either C or MONT-fed mice. However, MONT increased α diversity overall, and to a greater extent in females than in males, measured by the Shannon Index. MONT also shifted the relatedness of bacterial communities (β diversity) farther in females than in males, detected by two different metrics Jaccard distance (absence/presence of taxa), and weighted Unifrac (phylogenetic relatedness and relative taxa abundance). Moreover, MONT increased the prevalence of the taxon *Ruminococcus1* exclusively in females and not in males, detected by Analysis of Composition of Microbiomes. In summary, the MONT diet protects from estrogen, but not androgen deficiency-induced bone loss and architectural deterioration; and promotes a

healthier microbiome signature to a greater extent in females than males. These findings demonstrate that skeletal and microbiome responses to nutritional interventions are sex dimorphic and suggest a link between bone protection and gut microbiome diversity.

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