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The Effects of a Caloric Restrictive Diet on Bone Mineral Density and Bone Strength in Male and Female Rats

Haley Folta
Chapman University, folta101@mail.chapman.edu

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Folta, H. N., Gerston, A., Lee, K., Wong, Q., Sumida, K.

Abstract

The purpose of this study was to determine the existence of sex differences following a 40% caloric restrictive diet and its impact on tibial bone mineral density (BMD) and bone strength between male and female rats. Thirty-two, six-week old Sprague Dawley rats (16 males and 16 females) were randomly divided into an *ad libitum* fed control group (MC, n=8 and FC, n=8) and a pair fed diet group placed on the 40% caloric restriction (MD, n=8 and FD, n=8) for a 6 week period. The caloric restrictive diet was equivalent to the normal fed diet in vitamin and mineral content where the only difference was 40% less calories. After 6 weeks, there were no significant interaction effects, therefore main effects (i.e., sex and diet) were examined. While the tibial BMD was equivalent between males (0.206 ± 0.003 g/cm^2) and females (0.207 ± 0.004 g/cm^2), bone strength (amount of force required to break the tibia expressed in Newtons, N) was significantly greater for males (112.0 ± 2.4 N) compared to females (74.8 ± 3.1 N). The BMD was significantly lower for caloric restrictive fed groups (0.200 ± 0.003 g/cm^2) compared to normal fed animals (0.213 ± 0.003 g/cm^2). In like manner, bone strength significantly lower for diet fed animals (86.5 ± 5.6 N) compared to control fed animals (100.3 ± 5.1N). The results indicate that caloric restriction lowers BMD and bone strength irrespective of sex. However, bone strength was greater for males compared to females.

Introduction

Caloric restriction is a common strategy to help prevent obesity. However, caloric restrictive diets may not be beneficial for all components of the body; calories, an energy source, is necessary for bone growth. In support, diet programs have been associated with bone loss (Compston et al., 1992) and Peneau (2013) noted that dieting tends to be more common in women than in men. In addition, osteoporosis tends to impact more women than men (Shapses & Riedt 2006). Maintaining peak bone mass during the growth period can minimize the incidence or lower the severity of osteoporosis (Whiting et al. 2004). However, Kreipe & Forbes (1990) noted that female adolescents who employ diets elevated their risk of osteoporosis later in life. To date, studies examining the impact of caloric restrictive diets in male adolescents are lacking. While human studies are advantageous, identifying sex differences are prone to many confounding variables such as differences in: activity levels, diets, and genetics. In contrast, the use of an animal model can minimize many of the confounding variables associated with human studies. Therefore, the purpose of this study was to determine the existence of sex differences from a caloric restrictive diet and its impact on bone mineral density and bone strength during the growth period. We hypothesized that caloric restriction would have a more deleterious impact on female animals resulting in lower BMD and bone strength compared to males.

Materials and Methods

This study was approved by Chapman University's Institutional Review Board. 48 young male Sprague Dawley Rats were housed individually and placed on a reverse 12 hour light/dark cycle with access to water *ad lib*. Animals from each sex were randomly separated into three groups of 8. 8 female and 8 male rats were sacrificed to determine baseline values. The remaining 32 rats were split into 4 groups: females with no diet restriction (FC, n=8), females with a 40% diet restriction (FD, n=8), males with no diet restriction (MC, n=8), and males with a 40% diet restriction (MD, n=8). Those with no diet restrictions were able to eat *ad lib*. Each diet fed animal was paired with a control fed animal and received 40% less food each day for a 6 week period. The foot pellets were special ordered from Diet, Inc. and the "diet restriction" pellets contained more vitamins and minerals so that the only variable was the caloric intake.

Sample Collection

After the 6 weeks, animals were anesthetized and the tibia of the right hind limb as well as the whole left hind limb were dissected. The entire left hind limb was also frozen in liquid nitrogen and stored at -80°C immediately after removal. The tibia was stored in a 50/50 ethanol/saline solution.

Bone Mineral Density and Bone Strength Testing

The bone mineral density (BMD) of the tibia, was determined via a Dual Energy X-ray Absorptiometry (DXA) from the left hindlimb. The markers used to ensure consistency and proper positioning for every bone were the condyle and malleolus curvatures of the tibia. Three measurements were taken of each bone, and then averaged to get the reported BMD. The coefficient of variation for the 3 measurements was 2.13 ± 0.23%. Bone strength was determined via a three-point bending rig from the right tibia. A medial-to-lateral force was applied to the mid-shaft of each bone, with a deformation rate set to 0.9 mm/sec for each bone. The maximal force until breakage, F_{max} (units=N), was the peak of the deformation curve.

Statistics

A 2 factor (sex x diet) ANOVA was used to determine interaction effects. If a significant F ratio was found, Tukey's Post-Hoc was used. Main effects were examined using a Student's t-test with a p value set at 0.05. All values are expressed as the Mean ± SE.

Results

	Initial Body Weight (g)		Final Body Weight (g)	
	male	female	male	female
Baseline	263.60±5.06	181.00±1.74		
Control	265.84±2.17	182.39±4.69	485.98 ± 15.32	275.48±8.93
Diet	265.09±2.67	188.44±3.61	352.26±6.59	203.31±7.30

Table 1. Initial body Weight and Final Body weight of both sexes for the Baseline (BF, n=8, BM, n=8), Control (FC, n=8 and MC, n=8), and Diet Groups (FD, n=8 and MD, n=8).

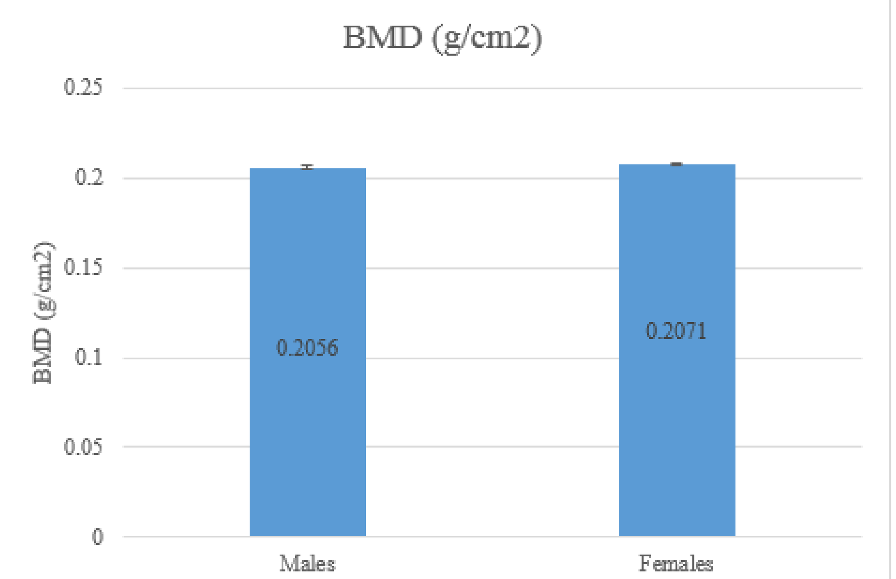

Figure 2. Bone Mineral Density (g/cm^2) of the tibia comparing males (M, n=16) and females (F, n=16).

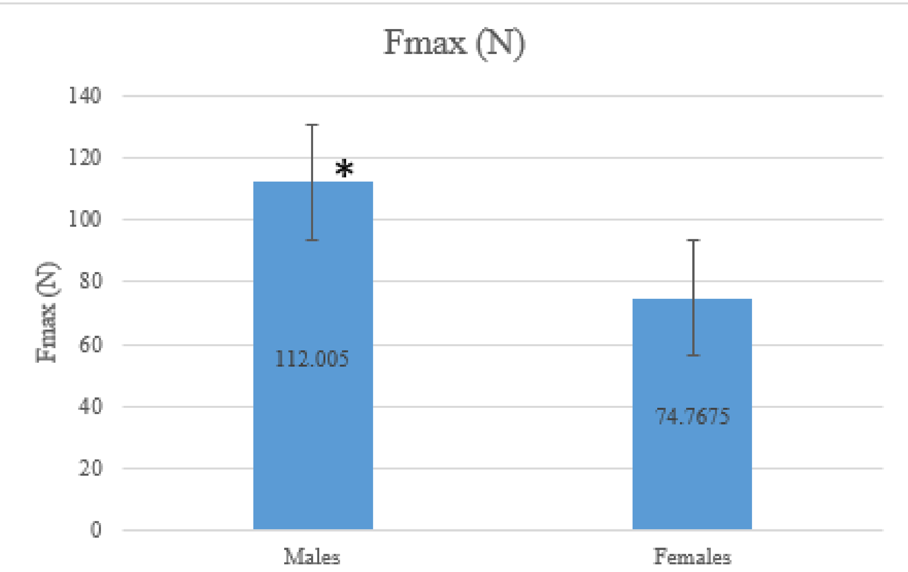

Figure 4. F_{max} (N) of the tibia comparing males (M, n=16) and females (F, n=16). *Significant difference between Males vs. Females.

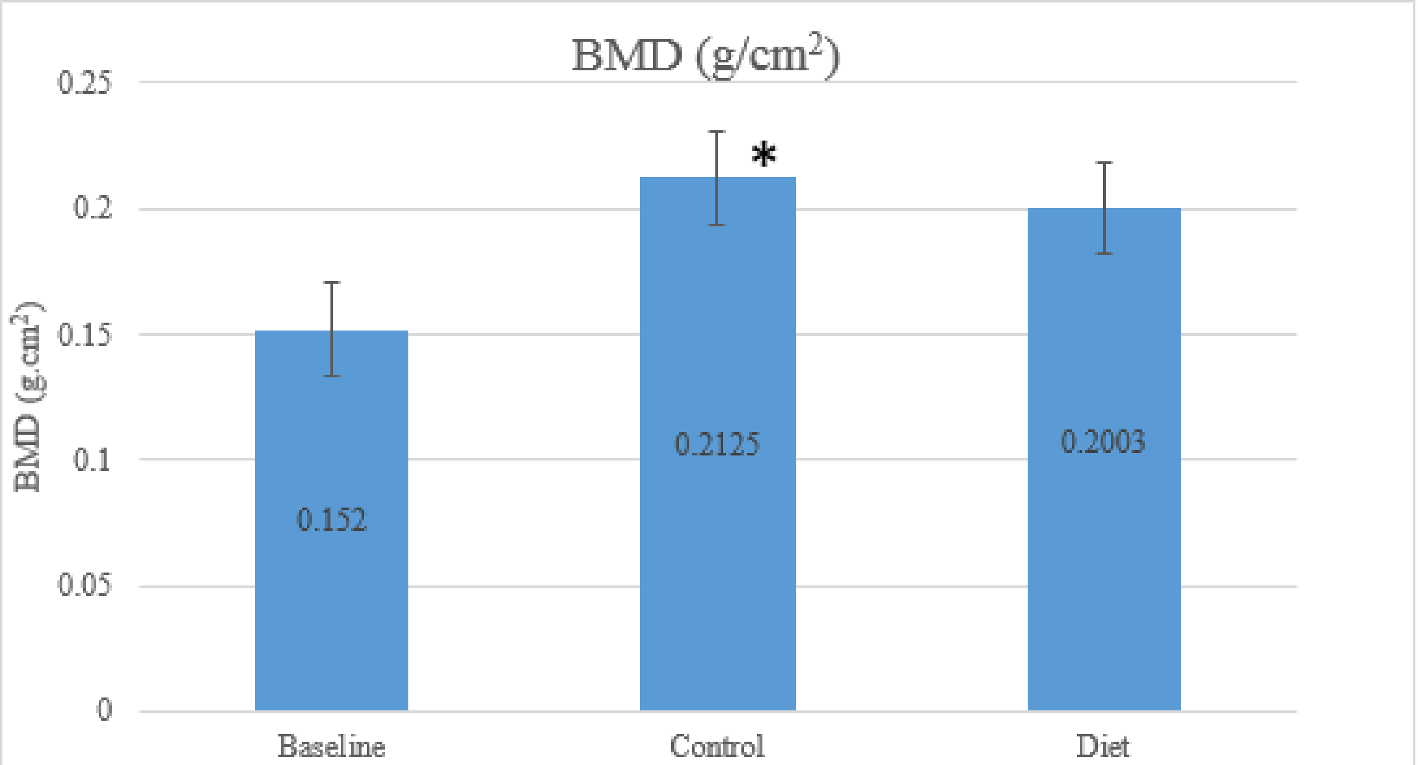

Figure 1. Bone Mineral Density (g/cm^2) of the tibia for the baseline group sacrificed immediately (B, n=16), regularly fed group allowed to eat *ad lib.* (C, n=16), and the 40% caloric restricted group (D, n=16). *Significant difference between Control vs. Diet.

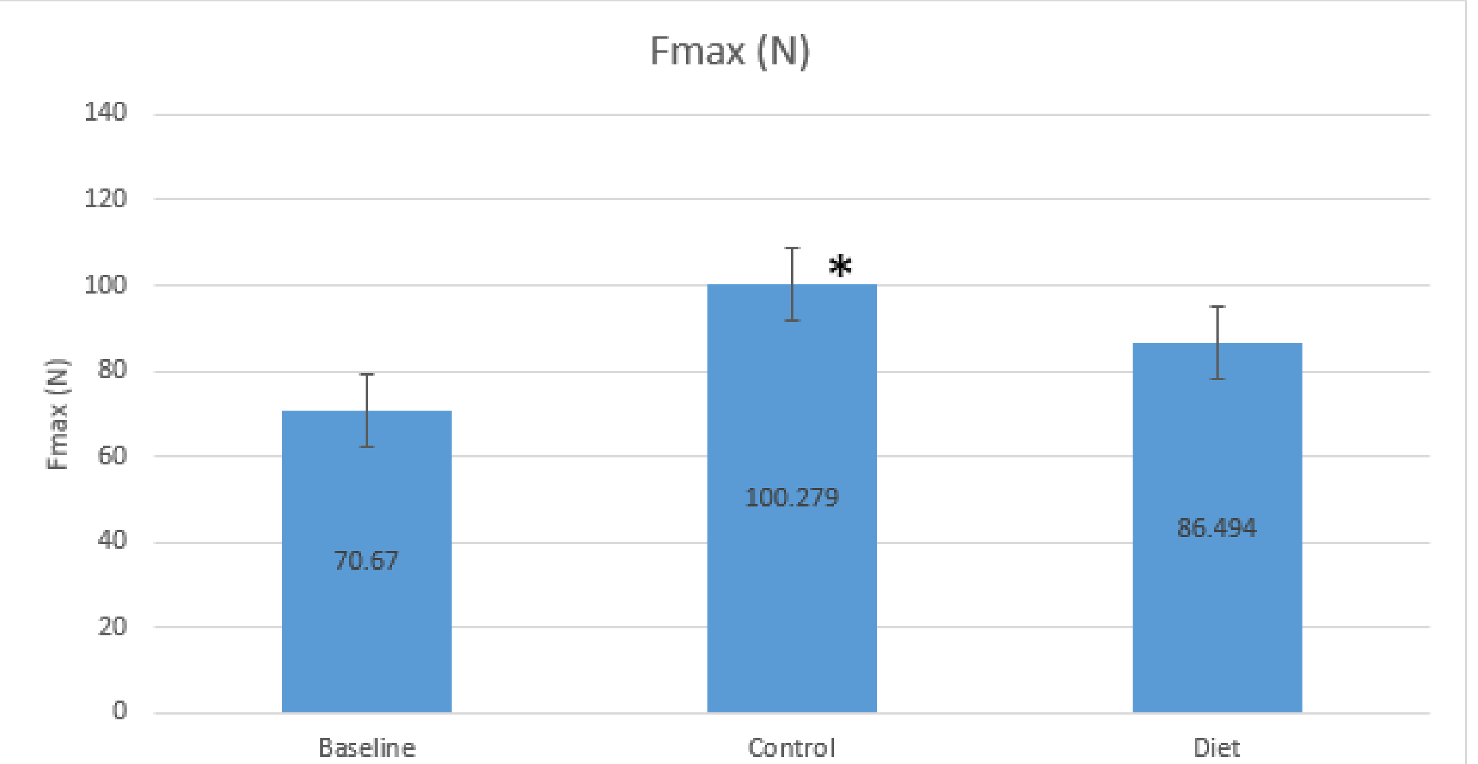

Figure 3. F_{max} (N) of the tibia for the baseline group sacrificed immediately (B, n=16), regularly fed group allowed to eat *ad lib.* (C, n=16), and the 40% caloric restricted group (D, n=16). *Significant difference between Control vs. Diet.

Summary and Conclusions

- The caloric restrictive diets resulted in significantly lower body weights for both males and females.
- No interaction effects were found so main effects (i.e. diet and sex) were determined where caloric restricted animals had lower BMD (0.200 ± 0.003 g/cm^2) compared to normal fed animals (0.213 ± 0.003 g/cm^2), similarly, bone strength was found to be significantly lower for diet fed animals (86.5 ± 5.6 N) when compared to control fed animals (100.3 ± 5.1N).
- In contrast, the BMD was equivalent between males (0.206 ± 0.003 g/cm^2) and females (0.207 ± 0.004 g/cm^2), however, males were found to have significantly higher bone strength (112.0 ± 2.4 N) compared to females (74.8 ± 3.1 N).
- There were no interaction effects between females and males on the caloric restriction, therefore, our hypothesis was not supported.
- Since bone strength was greater in males compared to females, this suggests possible sex differences in bone architecture.

Works Cited

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