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The Impact of a 20% vs. a 40% Caloric Restrictive Diet on Bone Mineral Density During the Growth Period in Male Rats

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Abstract

Purpose: The purpose of this study was to determine the impact of a 20% and a 40% caloric restrictive diet on bone mineral density (BMD) and bone strength in male rats during the growth period. **Methods:** 32 male rats were randomly divided into: control groups (C20, n=8 and C40, n=8), a group fed the 20% caloric restrictive diet (D20, n=8), and a group fed the 40% caloric restrictive diet (D40, n=8). An animal in the caloric restrictive diet group was matched and pair fed with an animal in the control group for 6 weeks. Each caloric restrictive diet contained additional vitamins and minerals so that the only variable was a restriction on the amount of calories consumed (i.e. 20% or 40% reduction in caloric intake). **Results:** There was a 17.5% reduction in body weight for D20 compared to C20. In like manner, there was a 27.5% reduction in body weight for D40 compared to C40. The left tibia BMD for D40 (0.2001± 0.0015 g/cm^2) was significantly lower compared to C40 (0.2111 ± 0.0048 g/cm^2). Further, the Fmax (the amount of force required to break the tibia expressed in Newtons, N) for D40 (105.73 ± 2.39 N) was significantly lower than C40 (118.73 ± 2.67 N). In contrast, there was no significant difference in tibial BMD or Fmax between D20 and C20. **Conclusion:** The results suggest that although both caloric restricted groups had a reduction in body weight, only the 40% diet group had a significant decrease in BMD and bone strength.

Introduction

A reduced caloric intake can be used to lower the effects of certain diseases such as obesity as well as natural human aging (McNeill, 2014). However, a caloric restricted weight loss program has the potential to lower bone mineral density (BMD). Villareal (2006) reported that in a random study of women and men, a decrease in body weight following caloric restriction resulted in lower levels in regional BMD, especially in the lumbar spine, total hip, and intertrochanter. When non-obese, older women underwent a caloric restricted diet, the results showed that moderately low caloric intake of about 80% of the recommended minimum daily intake (RMDI) did not exhibit negative effects on bone growth. However, with an extremely low caloric intake of about 55% of the RMDI (RMDI consisted of total calories, vitamin D, calcium, phosphorus, and protein), there were decreases in femur BMD (Caporaso, 2011). A caloric restricted diet is one of the contributing factors that leads to more bone loss (Caporaso, 2011). Also, a decreased caloric intake during growth has been associated with lower bone mass ultimately leading to osteoporosis in adulthood (Devlin, 2010). However, the level of caloric restriction that is detrimental to bone loss is still unknown. The purpose of the current study was to determine the impact a 20% vs. a 40% caloric restricted diet on BMD. We also determined bone strength using a three point bending instrument. Given that bone formation is an energy requiring process, we hypothesized that the 40% caloric restricted diet will show more detrimental differences in BMD and bone strength compared to the 20% caloric restricted diet.

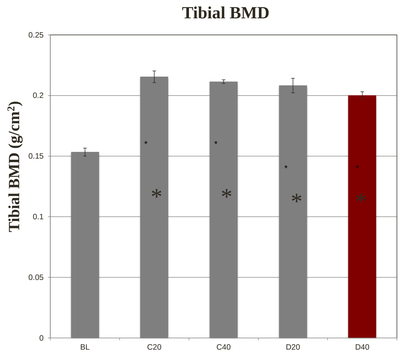
Results

Table 1: Body Weight

Group	Initial BW (g)	Final BW (g)
BL	263.60 ± 5.06	-
C20	231.50 ± 3.35	479.25 ± 12.90
C40	265.84 ± 2.17	485.98 ± 15.32
D20	231.75 ± 2.77	395.50 ± 11.05
D40	265.09 ± 2.67	352.26 ± 6.59

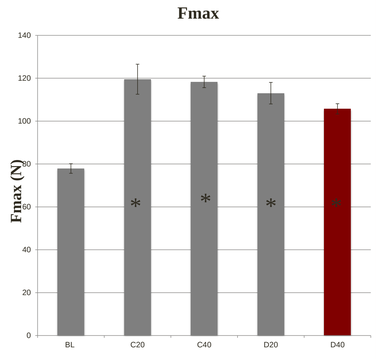
Data are expressed as mean ± SD
BW, body weight; BL, baseline (n=8)**;** C20, control group for 20% caloric restriction (n=8); C40, control group for 40% caloric restriction (n=8); D20, 20% caloric restriction group (n=8); D40, 40% caloric restriction group (n=8)

Figure 1: BMD



Data are expressed as mean ± SD
* indicates significant difference vs. BL (baseline)
Red indicates significant difference compared to the 40% control group
BMD, bone mineral density; BL, baseline (n=8)**;** C20, control group for 20% caloric restriction (n=8); C40, control group for 40% caloric restriction (n=8); D20, 20% caloric restriction group (n=8); D40, 40% caloric restriction group (n=8)

Figure 2: Fmax



Data are expressed as mean ± SD
* indicates significant difference vs. BL (baseline)
Red indicates significant difference compared to the control group
Fmax, maximal load to failure; BL, baseline (n=8)**;** C20, control group for 20% caloric restriction (n=8); C40, control group for 40% caloric restriction (n=8); D20, 20% caloric restriction group (n=8); D40, 40% caloric restriction group (n=8)

Methods

Animals

Chapman University Institutional Review Board approved the experimental protocol for this research. Forty male Sprague-Dawley rats were obtained and housed individually. They were exposed to a 12-hour light/12 hour dark schedule, which was carried out with a timer. During the first week, the rats were given free access to the food and water. Following this first week, the forty rats were separated into five groups: a baseline group (BL, n=8) that was sacrificed immediately, a control group (C20, n=8) that was fed *ad libitum*, a 20% caloric restricted group (D20, n=8), a control group that was fed ad libitum (D40, n=8), and a 40% caloric restricted group (D40, n=8).

Diet

The control rats were matched and pair fed each day with their corresponding diet rats. The caloric restricted rats were given 80% or 60% of what the control rats had eaten the previous day, thus restricting their caloric intake by 20% or 40% for a total of 6 weeks. Each caloric restrictive diet contained additional vitamins and minerals so that the only variable was a restriction on the amount of calories consumed.

Sample Collection and Chemical Analyses

After 6 weeks, animals were sacrificed. The right tibia was dissected from the rat and then cleaned of any remaining soft tissues. The bones obtained were then placed into a scintillation vial that contained a 50/50 ethanol/saline solution. The left hind limb was amputated and kept frozen at -80 ºC until its subsequent analysis for BMD.

Bone Mineral Density Measurements

A dual energy x-ray absorptiometry (DXA) was used to measure the BMD of the tibia of the left hind limb. The left hind limb was thawed and positioned on the DXA to scan its entirety. The condyle and malleolus curvatures of the tibia were used as anatomical markers to ensure that the positioning of the left hind limb was correct. This was used to ensure that the bone mineral density (BMD) results were accurate. Three consecutive BMD measurements of the left hind limb were conducted with the hind limb being repositioned for every measurement and the coefficient variance (mean ± standard error) was 2.065 ± 0.601.

Bone Strength Measurements

A three-point bending test was conducted at room temperature to measure bone strength. The test was carried out using a three-point bending rig that was placed on a stage of a texture analyzer instrument. Before conducting this test the right tibia were rinsed and submerged in saline for 24 hours at room temperature. The texture analyzer was calibrated with a standard weight and then the right tibia were dried before being placed onto the rig. The deformation rate of the test was set to 0.9 mm/sec before a medial to lateral force was applied to the midshaft of each bone. The parameter of bone strength that was measured was the maximal load to failure (F_{max}, N).

Calculations and statistics

An ANOVA was used for all the comparisons with a significant value set to $P < .05$. When there was a significant F-value, Fisher's PSLD post hoc test was used.

Summary

1) Both calorically restricted groups showed reductions in body weight.

2) Only the 40% calorically restricted group had significant decreases in BMD and bone strength.

3) The 20% calorically restricted group did not have any significant differences in BMD or bone strength.

4) Collectively, the results support our hypothesis pertaining to the greater loss of bone mass from a 40% caloric restrictive diet compared to a 20% caloric restrictive diet.

Conclusion

1) Further investigation is required to determine how much caloric restriction between 20% and 40% will be detrimental to bone mineral density.

2) While this study examined the impact of caloric restriction in growing males, the impact of caloric restriction in growing females is unknown.

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