Bone mineral density and content during weight cycling in female rats: effects of dietary amylase-resistant starch

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Recommended Citation
Brown, Ian L.; Ambia-Sobhan, Hasina; Huang, Abigail E.; Shapses, Sue A.; Jagpal, Sugeet; Bogden, John D.; Kemp, Francis W.; and Birkett, Anne M.: Bone mineral density and content during weight cycling in female rats: effects of dietary amylase-resistant starch 2008, 1-12.
https://ro.uow.edu.au/hbspapers/1690

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Bone mineral density and content during weight cycling in female rats: effects of dietary amylase-resistant starch

Abstract
ABSTRACT: BACKGROUND: Although there is considerable evidence for a loss of bone mass with weight loss, the few human studies on the relationship between weight cycling and bone mass or density have differing results. Further, very few studies assessed the role of dietary composition on bone mass during weight cycling. The primary objective of this study was to determine if a diet high in amylase-resistant starch (RS2), which has been shown to increase absorption and balance of dietary minerals, can prevent or reduce loss of bone mass during weight cycling. METHODS: Female Sprague-Dawley (SD) rats (n=84, age = 20 weeks) were randomly assigned to one of 6 treatment groups with 14 rats per group using a 2x3 experimental design with 2 diets and 3 weight cycling protocols. Rats were fed calcium-deficient diets without RS2 (controls) or diets high in RS2 (18% by weight) throughout the 21-week study. The weight cycling protocols were weight maintenance/gain with no weight cycling, 1 round of weight cycling, or 2 rounds of weight cycling. After the rats were euthanized bone mineral density (BMD) and bone mineral content (BMC) of femur were measured by dual energy X-ray absorptiometry, and concentrations of calcium, copper, iron, magnesium, manganese, and zinc in femur and lumbar vertebrae were determined by atomic absorption spectrophotometry. RESULTS: Rats undergoing weight cycling had lower femur BMC (p<0.05) and marginally lower BMD (p=0.09) than rats not undergoing weight cycling. In comparison to controls, rats fed RS2 had higher femur BMD (p<0.01) and BMC (p<0.05), as well as higher values for BMD and BMC measured at the distal end (p<0.001 and p<0.01) and femoral neck (p<0.01 and p<0.05). Consistent with these findings, RS2-fed rats also had higher femur calcium (p<0.05) and magnesium (p<0.0001) concentrations. They also had higher lumbar vertebrae calcium (p<0.05) and magnesium (p<0.05) concentrations. CONCLUSION: Weight cycling reduces bone mass. A diet high in RS2 can minimize loss of bone mass during weight cycling and may increase bone mass in the absence of weight cycling.

Keywords
Bone, mineral, density, content, during, weight, cycling, female, rats, effects, dietary, amylase, resistant, starch

Disciplines
Arts and Humanities | Life Sciences | Medicine and Health Sciences | Social and Behavioral Sciences

Publication Details
Bogden, J. D., Kemp, F. W., Huang, A. E., Shapses, S. A., Ambia-Sobhan, H., Jagpal, S., Brown, I. L. & Birkett, A. M. (2008). Bone mineral density and content during weight cycling in female rats: effects of dietary amylase-resistant starch. Nutrition and Metabolism, 5 (34), 1-12.

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This journal article is available at Research Online: https://ro.uow.edu.au/hbspapers/1690
Nutrition & Metabolism

Research

Bone mineral density and content during weight cycling in female rats: effects of dietary amylose-resistant starch

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Abstract

Background: Although there is considerable evidence for a loss of bone mass with weight loss, the few human studies on the relationship between weight cycling and bone mass or density have differing results. Further, very few studies assessed the role of dietary composition on bone mass during weight cycling. The primary objective of this study was to determine the effect of amylose-resistant starch (RS), which has been shown to increase in weight and balance of dietary minerals, can prevent or reduce loss of bone mass during weight cycling.

Methods: Female Sprague-Dawley (SD) rats (n = 80, age = 20 weeks) were randomly assigned to 1 of 4 treatment groups with 10 rats per group using a 2 x 3 experimental design with 2 diets and 3 weight cycling protocols. Rats were fed calcium-deficient diets without RS controls or diets high in RS (18% by weight) throughout the 21-week study. The weight cycling protocols were weight maintenance/eight, 1 round of weight cycling, or 2 rounds of weight cycling. After the rats were euthanized, bone mineral density (BMD) and bone mineral content (BMC) of femur were measured by dual energy X-ray absorptiometry, and concentrations of calcium, copper, iron, magnesium, manganese, and zinc in femur and lumbar vertebrae were determined by atomic absorption spectroscopy.

Results: Rats undergoing weight cycling had lower femur BCP (p < 0.05) and marginally lower BMD (p = 0.09) than rats not undergoing weight cycling. In comparison to controls, rats fed RS2 had higher femur BMD (p < 0.05) and BMC (p < 0.05), as well as higher values for BCP and BMC in the proximal end (p = 0.001 and p < 0.05) and femoral neck (p = 0.01 and p < 0.05). Consistent with these findings, RS-fed rats also had higher femur calcium (p < 0.05) and magnesium (p = 0.001) concentrations. They also had higher lumbar vertebrae calcium (p < 0.05) and magnesium (p < 0.05) concentrations.

Conclusion: Weight cycling reduces bone mass. A diet rich in RS2 can minimize loss of bone mass during weight cycling and may increase bone mass in the absence of weight cycling.

Background

Although obesity has a number of well-documented adverse effects on health, obese men and women have higher bone mineral density (BMD) and bone mineral content (BMC) than their age and gender-matched counterparts with lower body weights [1]. Weight cycling is the repeated loss and regain of body weight, and occurs frequently in obese and overweight men and women in their attempts to lose weight and maintain a lower body weight. Weight cycling may also occur in men and women of normal body weight or in those with eating disorders, such as anorexia nervosa. The goal of intentional weight loss is to reduce fat mass, but there is typically an accompanying sustained loss of lean body mass, including bone mass [1]. Although there is convincing evidence for a loss of bone mass with weight loss [1], there are only a few human studies on the relationship between weight cycling and bone mass or density. A small number of epidemiologic studies demonstrate that a history of weight cycling is associated with a reduction in bone mineral density or an increased risk of hip and other fractures in men and women [2-5]. In one of these studies [5], greater weight variability in a sample of about 20,000 women and 19,000 men was associated with relative risks of hip fracture of 2.07 for women and 2.70 for men. In another study of 169 premenopausal women [3], weight cycling was associated with significant (p < 0.01) decreases in BMC at the lumbar spine and distal radius. In the above studies, the significant relationships between weight cycling and hip fracture or BMC persisted even after correction for body weight. In more recent studies, Sgroedl et al. [6] found that weight cycling, in elderly Hawaiian men was a risk factor for femur fracture, and Bacon et al. [7] reported that chronic dieting in 30-45 year-old obese women was associated with lower bone mass. In contrast, other investigators found that weight cycling did not reduce bone mass in competitive athletes [4] and overweight, sedentary, premenopausal women [7]. The varying results of the above and other human studies on the relationship between weight cycling and bone mass may be due to the many factors that cannot be adequately controlled in human studies, including subject characteristics, compliances with the study protocols, and patterns of weight loss. Furthermore, a history of weight cycling may define a subgroup of people at risk for bone loss due to other factors.

It is not known if dietary composition during weight cycling can influence bone mass, quality, and strength; specific diet components that may influence bone during weight cycling include vitamins D and K, calcium, magnesium, several essential trace minerals, and the resistant starches. Resistant starches (RS1, RS2, RS3, RS4, and RS5) are a subgroup of fibers that occur naturally in foods or can be produced during food processing [8-11]. Total resistant starch is estimated to be about 10% (range = 2-20%) of ingested starches in Western diets [8]. The resistant starches are not digested by mammalian enzymes in the mouth, stomach, or small intestine, and are fermented in the colon [8-11]. Categorization of the five types of resistant starch is based on the factors that explain their resistance to degradation in the upper gastrointestinal tract. These factors include physical entanglement of the starch by the major cellular structures (RS4), the physical structure of the starch granules (RS5), or their chemical composition (RS1, RS2, and RS3) [8-10]. RS5 is the subtype most widely used commercially. We selected it for this investigation because a number of prior studies from other laboratories provide considerable evidence demonstrating that dietary RS improves absorption and balance of calcium, magnesium, and several trace minerals in experimental animals, particularly the rat [12-19]. This study is based on the hypothesis that RS5, by reducing gastrointestinal loss of calcium, magnesium, and other bone minerals, may help to maintain bone mass subsequent to weight cycling.

Because of the difficulty of controlling the various factors that could influence the effects of weight cycling on bone mass in humans, we conducted a study in rats so that diet composition, weight cycling patterns, age, and other relevant variables could be tightly controlled. The primary objective of this investigation was to determine if a diet high in natural RS5 from high-amylose cornstarch also termed "malted starch" can reduce the present loss of femur BMC and BMD subsequent to weight cycling in female rats. The female Sprague-Dawley (SD) rat was chosen as the experimental animal based on prior studies in our laboratories in this species; these studies suggest that the female SD rat is an appropriate model for the study of relationships between weight loss and bone composition [20,24]. A second goal was to analyze bone for other minerals and trace elements that are known or suspected to be required for optimal bone strength, specifically calcium, copper, iron, magnesium, manganese, and zinc [25]. This was done to obtain insight into specific bone metals that may be involved in the effects of weight cycling and/or dietary resistant starch on bone mass. A specific diet with RS5 was used to identify selected non-caloric organs (liver and kidney) with high metabolic activity for the same metals in order to compare effects of weight cycling and dietary resistant starch on bone versus soft tissues.

Methods

Diet preparation

Diet were formulated and prepared by Research Diets, Inc., New Brunswick, NJ. The composition of these diets is described in Table 1. Carbohydrate in each diet is prepared by waxy comcast, pregelatinized waxy cornstarch, and sucrone. A mixture of waxy comcast, pregelatinized waxy cornstarch, and sucrose was used for the control
Table 1: Composition of calcium-deficient normal and resistant starch pelleted diets fed during weight maintenance and during AR energy restriction

| Ingredients | NS | RS | NS | RS | NS | RS |
|-------------|----|----|----|----|----|----|
| Carbohydrate | 47.3 | 48.8 | 57.3 | 58.1 | 47.3 | 48.1 |
| Fat | 4.2 | 15.9 | 15.9 | 15.9 | 4.2 | 15.9 |
| Total | 51.5 | 103.4 | 51.4 | 103.4 | 51.5 | 103.4 |

Energy

| Ingredient | Amount | Amount |
|------------|--------|--------|
| Carbohydrate | 3.74 kcal | 3.54 kcal |
| Protein | 2.69 kcal | 2.49 kcal |

Experimental details:

Male rats were chosen for this study because low BMI and BMC, as well as a higher risk of osteoporosis and osteo-

Arthritis Society, National Office, 222 E. Chicago Ave., Chicago, IL 60611. 

Dietary Carbohydrate:

Dietary Fiber:

Dietary Fat:

Dietary Protein:

Dietary Calcium:

Dietary Phosphorus:

Dietary Magnesium:

Dietary Sodium:

Dietary Potassium:

Dietary Chloride:

Dietary Zinc:

Dietary Copper:

Dietary Iron:

Dietary Manganese:

Dietary Iodine:

Dietary Selenium:

Dietary Fluoride:

Dietary Vitamin A:

Dietary Vitamin D:

Dietary Vitamin E:

Dietary Vitamin C:

Dietary Vitamin B1:

Dietary Vitamin B2:

Dietary Vitamin B3:

Dietary Vitamin B6:

Dietary Vitamin B12:

Dietary Folic Acid:

Dietary Vitamin K:

Dietary Chromium:

Dietary Cobalt:

Dietary Fluoride:

Dietary Iodine:

Dietary Selenium:

Dietary Copper:

Dietary Zinc:

Dietary Manganese:

Dietary Iron:

Dietary Magnesium:

Dietary Calcium:

Dietary Phosphorus:

Dietary Sodium:

Dietary Chloride:

Dietary Potassium:

Dietary Zinc:

Dietary Copper:

Dietary Iron:

Dietary Magnesium:

Dietary Calcium:

Dietary Phosphorus:

Dietary Sodium:

Dietary Chloride:

Dietary Potassium:

Dietary Zinc:

Dietary Copper:

Dietary Iron:

Dietary Magnesium:

Dietary Calcium:

Dietary Phosphorus:

Dietary Sodium:

Dietary Chloride:

Dietary Potassium:

Dietary Zinc:

Dietary Copper:

Dietary Iron:

Dietary Magnesium:

Dietary Calcium:

Dietary Phosphorus:

Dietary Sodium:

Dietary Chloride:

Dietary Potassium:

Dietary Zinc:

Dietary Copper:

Dietary Iron:

Dietary Magnesium:

Dietary Calcium:

Dietary Phosphorus:

Dietary Sodium:

Dietary Chloride:

Dietary Potassium:

Dietary Zinc:

Dietary Copper:

Dietary Iron:

Dietary Magnesium:

Dietary Calcium:

Dietary Phosphorus:

Dietary Sodium:

Dietary Chloride:

Dietary Potassium:

Dietary Zinc:

Dietary Copper:

Dietary Iron:

Dietary Magnesium:

Dietary Calcium:

Dietary Phosphorus:

Dietary Sodium:

Dietary Chloride:

Dietary Potassium:

Dietary Zinc:

Dietary Copper:

Dietary Iron:

Dietary Magnesium:

Dietary Calcium:

Dietary Phosphorus:

Dietary Sodium:

Dietary Chloride:

Dietary Potassium:

Dietary Zinc:

Dietary Copper:

Dietary Iron:

Dietary Magnesium:

Dietary Calcium:

Dietary Phosphorus:

Dietary Sodium:

Dietary Chloride:

Dietary Potassium:

Dietary Zinc:

Dietary Copper:

Dietary Iron:

Dietary Magnesium:

Dietary Calcium:

Dietary Phosphorus:

Dietary Sodium:

Dietary Chloride:

Dietary Potassium:

Dietary Zinc:

Dietary Copper:

Dietary Iron:

Dietary Magnesium:

Dietary Calcium:

Dietary Phosphorus:

Dietary Sodium:

Dietary Chloride:

Dietary Potassium:

Dietary Zinc:

Dietary Copper:

Dietary Iron:

Dietary Magnesium:

Dietary Calcium:

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Dietary Iron:

Dietary Magnesium:

Dietary Calcium:

Dietary Phosphorus:

Dietary Sodium:

Dietary Chloride:

Dietary Potassium:

Dietary Zinc:

Dietary Copper:

Dietary Iron:

Dietary Magnesium:

Dietary Calcium:

Dietary Phosphorus:

Dietary Sodium:

Dietary Chloride:

Dietary Potassium:

Dietary Zinc:

Dietary Copper:

Dietary Iron:

Dietary Magnesium:

Dietary Calcium:

Dietary Phosphorus:

Dietary Sodium:

Dietary Chloride:

Dietary Potassium:

Dietary Zinc:

Dietary Copper:

Dietary Iron:

Dietary Magnesium:

Dietary Calcium:

Dietary Phosphorus:

Dietary Sodium:

Dietary Chloride:

Dietary Potassium:
The rat femurs were analyzed for the whole bone "total" BMD and BMC, and at 2 other standard sites prone to fracture, specifically the femoral neck and the distal femur (20% from the distal end of the bone) [28]. After non-destructive analysis by DEXA, the four femurs were subsequently used for laboratory analyses of their mineral composition, which enabled measurement of mineral concentrations using the same bone that was used to determine BMD and BMC. The right femur and lumbar vertebra bone were analyzed for minerals and trace elements that are considered to be essential or beneficial for optimal bone strength, specifically calcium, copper, magnesium, manganese, and zinc [23]. Iron was also determined in these samples. For these analyses the entire right femur and a set of 3-4 lower lumbar vertebra were assayed; the bone samples were cleared of all non-calcified tissue before analysis. The non-calcified organs collected (liver and kidney) were also analyzed for the same metals. These analyses were conducted using flame or electrothermal atomic absorption spectrophotometry. Standards, blanks, and National Institute of Standards and Technology (NIST, Gaithersburg, MD) Standard Reference Materials (SRM # 1446-Booe Meal or SRM # 1577b-Bovine Liver) with certified values for their metal concentrations were analyzed with each set of daily analysis as a component of our quality control program [20, 21].

Data analysis
Data evaluation methods included descriptive and inferential statistical analyses. Two-way ANOVA was conducted to determine the separate effects of dietary resistant starch and weight cycling, as well as their interactions, on the various study outcome variables. Pairwise differences were evaluated subsequent to two-way ANOVA by comparison of least-squares means at p < 0.05. Associations between selected variables were determined by calculation of Pearson correlation coefficients. A limited number of multiple regression analyses were conducted with BMD or BMC as the dependent variable and final body weight and treatment group as the independent variables. Ethical treatment of animals
All applicable institutional and governmental regulations concerning the ethical care of animals were followed during the study. The study and its specific procedures were reviewed and approved by the Duke University Institutional Animal Care and Use Committee.

Results
Food consumption
Rats fed the normal diets and their counterparts fed the resistant starch diets had similar total food intakes (mean ± SD) during the 21-week study, specifically 2588 ± 87 g and 2585 ± 88 g for the rats not undergoing weight cycling, 2333 ± 59 g and 2424 ± 53 g for those rats subject to one round of weight cycling, and 2142 ± 51 g and 2118 ± 55 g for those rats undergoing two rounds of weight cycling.

Diet composition
Table 2 contains the results of our analyses of diet concentrations of iron and the 5 metals for which there is evidence of Ca (Ca, Mg, Mn, Zn) in the maintenance of bone mineral density and bone mineral content. Our target for diets moderately deficient in calcium for the rat was a concentration of 1.0 mg of calcium per gram of diet (25.0 μmol/g). Measured mean calcium concentrations for Diets ND and RD fed during periods of weight maintenance or weight gain were 1.09 mg/g (37.2 μmol/g) and 1.07 mg/g (37.2 μmol/g), and thus are within 10% of the target concentration. Calcium present in other food components besides the dibasic calcium phosphate used to add calcium to the diets likely contributed to the total calcium concentration of the diets. Diets NDWL and RSWL that

| Table 2: Essential mineral concentrations of custom calcium-deficient diets |
|-------------------|-------------------|-------------------|-------------------|-------------------|
| Diet              | Calcium (μg)      | Copper (μg)       | Iron (μg)         | Magnesium (μg)    |
| ND                | 27.20 ± 0.24      | 0.094 ± 0.024     | 0.775 ± 0.110     | 21.38 ± 0.23      |
| NDWL              | 41.17 ± 0.58      | 0.313 ± 0.004     | 1.074 ± 0.027     | 34.14 ± 0.40      |
| RS                | 37.95 ± 0.67      | 0.534 ± 0.001     | 1.104 ± 0.021     | 33.93 ± 0.16      |
| RSVL              | 129.7 ± 0.46      | 0.994 ± 0.63      | 20.6 ± 1.07       | 0.55             |

Laboratory analysis
After placing the excised femur on a Delphi block, BMD and BMC of the right femur were determined by dual energy x-ray absorptiometry (DEXA) using a GE-Lunar PIXimus densitometer with software version 1.4. The PIXimus densitometer uses a 144 degree stationary anode x-ray tube generator with a 0.25 mm × 0.25 mm focal spot that generates a zone beam x-ray (55/60 kVp at 400 μA).
were fed dietary periods of weight loss at 60% of ad lib intended to have calcium concentrations that were 40% (1.40-fold) greater than Diets ND and RS; measured values were 51% and 42% greater, respectively. The latter results are based on 8 replicate analyses, but even well mixed solid diets may have some degree of heterogeneity. Measured concentrations of Ca, Fe, Mg, Mn, and Zn in Diets ND and RS were near the target levels for the AOAC diet for rodents that are provided in Table 2.

**Body weights**

Figure 1 displays mean body weights for the 14 rats in each of the six treatment groups. These data demonstrate that we were able to successfully produce weight loss and regain in the groups of rats undergoing one round (ND1 and RS1D) or two rounds of weight cycling (ND2 and RS2D). The results further demonstrate that body weights in the normal diet and corresponding resistant starch diet groups (ND0 versus RS0D and ND2 versus RS2D) tracked closely with one another throughout the study. Final body weights differed slightly between the ND0 and RS0D groups (7.2 g = 2.7% difference in mean final body weight), and the ND2 and RS2D groups (8.4 g = 2.5% difference in mean final body weight). Final body weights differed more substantially between the ND1 and RS0D1 groups (20.5 g = 5.1% difference in mean final body weight). Despite random assignment of rats to the six treatment groups and virtually identical mean group body weights, substantial variance in body weight was observed in the six groups (see Figure 1, Day 0). The body weights of the latter two groups did not track as closely as the other groups described above (see Figure 1).

**Bone and mineral density & content**

Figure 2 illustrates the results for BMD and BMC for right femurs of the rats. The data are provided for the BMD and BMC of the entire femur, as well as for the distal femur and femoral neck for a total of six variables. Pairwise comparisons in Figure 2 reveal that the values for BMD and BMC were unchanged (total of 18 pairwise comparisons) for the rats that were fed the resistant starch diet groups such as the corresponding groups for the normal diet rats. This was in contrast to the rats that were fed the resistant starch diet that were not undergoing weight cycling (groups ND0 and RS0D). Rats undergoing weight cycling had substantially lower BMD-total than rats not undergoing weight cycling. Weight cycling did not significantly affect the other 5 measures of BMD and BMC, although the effect of weight cycling on BMD-total approached statistical significance (p = 0.05). Weight cycling and diet did not interact to significantly influence any measure of BMD or BMC.

Figure 3 shows that rats in the four treatment groups undergoing weight cycling (ND1, ND2, RS1D, RS2D) had mean bone weights that at their minimum values were about 80-120 g lower than those of rats not undergoing weight cycling (groups ND0 and RS0D). Rats undergoing weight cycling also had substantially lower final body weights. As expected, final body weights were significantly associated with BMD-total (r = 0.32, p < 0.005) and with BMC-total (r = 0.66, p < 0.0001). Therefore, in further studies on the relationship among BMD, final body weight, and treatment group, we conducted a multiple regression analysis. In this model BMD-total was the dependent variable, and final body weight and treatment group were the independent variables. Final body weight and treatment group were significant (p < 0.05) predictors of BMD-total, accounting for 18.7% of the variability in BMD-total (final body weight = 10.3% of variability, treatment group = 8.3% of variability).

**Mineral concentrations**

None of the kidney metal concentrations measured differed significantly among treatment groups. For some there were some differences in concentrations among treatment groups, but no consistent relationships to weight cycling or diet compositions were observed.

Table 4 contains mineral concentrations for femur and humerus trabecular bone samples. We evaluated these by differences in body weights between rats fed the resistant starch diets and their counterparts fed the normal diets because their body weights were very similar throughout the study (Figure 1).

We evaluated the results for BMD and BMC using 2-way ANOVA (Table 3) to determine the independent effects of diet (ND vs. RS diet) and weight cycling protocol (0, 1, or 2 rounds of weight cycling), as well as the effect of diet on weight cycling interactions, on these variables. Compared to rats fed the normal diet, rats fed the RS diet had significantly higher BMD-total, BMD-femoral neck, and BMD-crestal femur, and BMD-crestal neck; the corresponding values for BMC were also significantly higher in RS-fed rats. Rats undergoing weight cycling had significantly lower BMD-total than rats not undergoing weight cycling. Weight cycling did not significantly affect the other 5 measures of BMD and BMC, although the effect of weight cycling on BMD-total approached statistical significance (p = 0.05). Weight cycling and diet did not interact to significantly influence any measure of BMD or BMC.

**Figure 2**

Bone mineral density and bone mineral content. Bone mineral density (BMD) and bone mineral content (BMC) of the total femur, femoral neck, and distal femur for right femurs of rats in the six treatment groups after 21 weeks. N = 8-14 rats. Each bar in the figure is the mean ± standard error of 14 femurs. Mean BMD and BMC are consistently higher for rats fed the resistant starch diets than their counterparts fed the control diets; this result cannot be explained by differences in body weights between rats fed the resistant starch diets and their counterparts fed the normal diets because their body weights were very similar throughout the study (Figure 1).

We evaluated the results for BMD and BMC using 2-way ANOVA (Table 3) to determine the independent effects of diet (ND vs. RS diet) and weight cycling protocol (0, 1, or 2 rounds of weight cycling), as well as the effect of diet on weight cycling interactions, on these variables. Compared to rats fed the normal diet, rats fed the RS diet had significantly higher BMD-total, BMD-femoral neck, and BMD-crestal femur, and BMD-crestal neck; the corresponding values for BMC were also significantly higher in RS-fed rats. Rats undergoing weight cycling had significantly lower BMD-total than rats not undergoing weight cycling. Weight cycling did not significantly affect the other 5 measures of BMD and BMC, although the effect of weight cycling on BMD-total approached statistical significance (p = 0.05). Weight cycling and diet did not interact to significantly influence any measure of BMD or BMC.
Table 3: Bone mineral density and content of total femur, femoral neck and distal femur: P values for 2-way ANOVA

|                    | Diet Effect | Weight Cycling Effect | Diet/Weight Cycling Interaction |
|--------------------|-------------|-----------------------|---------------------------------|
| BMD Total Femur    | p < 0.01    | NS                    | NS                              |
| BPD Femoral Neck   | p < 0.01    | NS                    | NS                              |
| BMD Femoral Neck   | p < 0.01    | NS                    | NS                              |
| BMD Total Femur    | p < 0.05    | p < 0.05              | NS                              |
| BPD Femoral Neck   | p < 0.05    | NS                    | NS                              |
| BPD Total Femur    | p < 0.01    | NS                    | NS                              |

NS = n.s.
Results are for the right femur

Diet Effect = normal diet versus resistant starch diet
Weight Cycling Effect = 0, 1, or 2 rounds of weight cycling

Discussion
results for femur mineral concentrations using 2-way ANOVA (Table 5) to determine the independent effects of diet (BID versus ND diet) and weight cycling protocol (0, 1, or 2 rounds of weight cycling), as well as diet/weight cycling interactions, on these variables. Compared to controls fed the normal diet, rats fed the 1% diet had significantly higher femur calcium and magnesium concentrations, but did not show higher femur concentrations of copper, iron, manganese, and zinc. Weight cycling had no significant effect on femur metal concentrations of calcium, copper, iron, zinc, and magnesium. However, rats undergoing weight cycling had significantly lower femur zinc, and there were significant interactions between weight cycling and diet that influenced femur iron and zinc concentrations.

Compared to controls fed the normal diet, data evaluation using 2-way ANOVA demonstrated that rats fed the 1% diet had significantly higher femur calcium and magnesium concentrations. However, rats undergoing weight cycling had significantly lower femur zinc.

Rats fed the 1% diet had significantly higher lumbar vertebral calcium and magnesium concentrations, but did not show higher concentrations of copper, iron, manganese, and zinc. (Table 6). These results are consistent with the results for the femur metal concentrations. Weight cycling had no significant effect on any of the 6 lumbar vertebral metal concentrations measured. However, as observed for the femur, there was a significant interaction between weight cycling and diet that influenced lumbar vertebral iron concentrations.

Lumbar vertebral calcium and magnesium concentrations were significantly associated with BMC-total (r = 0.22, 0.30, and 0.20, p < 0.05), but femur copper, iron, and manganese were not (r = 0.04, 0.00, and 0.01, p > 0.05). Similarly, femur calcium, magnesium, and zinc concentrations were significantly associated with BMC-total (r = 0.22, 0.20, and 0.20, p < 0.05), but femur copper, iron, and manganese were not (r = 0.02, 0.00, and 0.01, p > 0.05).

Using the femur calcium concentrations and femur weights, we calculated the total calcium content of the right femur. These values were significantly associated with BMC-total of the right femur (r = 0.94, p < 0.001), demonstrating very good agreement between the DXA measurements of BMC-total done in the laboratory of one of the co-authors (SAS) and femur calcium concentrations measured by flame atomic absorption spectrophotometry in the laboratory of another co-author (SAS).

Discussion
The results provide the first evidence that a dietary component, in this case 1%, can prevent or reduce loss of BMD and BMC due to weight cycling. Rats fed 1% also had higher bone mass in the absence of weight cycling. Although the differences we found among treatment groups in BMD and BMC are small, no moderate in magnitude (2.8-18.2%), they are within the range of the decreases in BMD and BMC of about 10% that are associated in women with a substantially increased risk of fractures of the hip, wrist, or spinal column [29,30].

Although BMD and BMC for the total femur, distal femur, and femoral neck were consistently lower in rats undergoing weight cycling in comparison to those animals not being weight, the differences were significant (p < 0.05) only for BMC total, because we measured BMD and BMC only after regain of body weight, larger reductions in BMD and BMC with weight loss may have been partially restored by the rapid regain of body weight prior to wetting the femurs for determination of BMD and BMC.

In the current study rats fed the 1% diet had significantly higher femur calcium and magnesium concentrations. We also found significant pairwise associations between
femur BMD or BMC and femur calcium and magnesium concentrations. These data suggest that dietary RS may prevent loss of bone mass during weight cycling by enhancing absorption and retention of calcium and/or magnesium (as suggested by the Table 5 data for femur calcium and magnesium), but not the retention of copper, iron, magnesium, and zinc. This result is consistent with the results of prior studies [16,20] showing that RS increases calcium and magnesium absorption; a potential mechanism for this effect is hypothesized to be the cell wall heparan sulfate and colocalization of RS-induced fermentation products in the large intestine [19].

Lobo et al. [31] have studied the effect of a diet containing 5% fructooligosaccharides (FOS) on intestinal absorption of calcium and magnesium, bone mineral density, and bone strength parameters in growing, 6-week-old male rats. Compared to controls, rats fed 5% FOS had a higher apparent absorption of calcium and magnesium, higher femur and tibia calcium concentrations, and increased femur peak load and yield load. Thus, our study and that of Lobo et al. suggest that specific dietary carbo-

hetero acids may have important roles in building and maintaining bone calcium and strength during weight cycling or growth, and that the mechanism for this effect may be enhanced calcium and magnesium absorption and retention.

A recent study [32] used NIH/USC III data to estimate the mean intake of total starch in the diets of US residents age one year or older. The mean intake was 4.0 g/day, with a range of 3.5-5.7 g/day. The major sources of starch were bread (22%), cooked corn, and pasta (19%), vegetables other than legumes (16%), plantain (16%), and peas (9%). Legumes contained the most resistant starch per serving, as much as 8 g. Comparison of the above intakes for an adult to the RS dose administered to the rats in the current study suggests that higher intakes than those found for the above US adults studied may be needed to effect short-term improvements in BMD and BMC. However, it is possible that long-term consumption of intakes as high as these or at the end of the range of 5.2-7.0 g of total resistant starch daily may improve BMD and BMC.

In the current study 30-week-old female rats were fed diets that were deficient in calcium but that contained levels of copper, iron, magnesium, and zinc that were near ANRC and target values. Thus, the diets contained normal/adequate concentrations of the latter five essential metals that are required for bone health. The effects of RS on bone mineral and BMC might differ from the results found in the current study if the diet were deficient in calcium or deficient in copper, iron, magnesium, and/or zinc. The results might also differ if male rats of female rats of a different age were studied, because age and gender have substantial effects on BMD and BMC and on bone metabolism.

Conclusion
Weight cycling reduces bone mass. However, a diet high in RS can prevent or reduce loss of bone mass during weight cycling and may increase bone mass in the absence of weight cycling. The results provide the first evidence that a dietary component can help preserve BMD and BMC during weight cycling. If the above effects of dietary RS are found to apply to humans, then increased dietary RS may help to build and preserve BMD and BMC in men and women, especially during weight cycling.

Competing interests
Dr. Bfiber and Brown were employees of the National Starch and Chemical Company for a portion of the period during which this research was being done. Neither is currently employed by the company.

Authors' contributions
DB designed and secured funding for this study, performed some of the laboratory work, and prepared the manuscript. FSK did some of the laboratory work and performed most of the data quality control efforts and statistical analyses. ADEH, HAA, and S. H. did considerable portions of the laboratory work, SAS, IBD, and AMM contributed to study design modifications, data evaluation, and manuscript revision.

Acknowledgements
This study was supported by the National Science Foundation of China, USF, and the New Jersey Agricultural Experiment Station grants 9961854354. We acknowledge Friso, Heineken, and MB. Craig L. ATP contributed to the laboratory work done for this project.

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