Dietary Restriction Does Not Adversely Affect Bone Geometry and Mechanics in Rapidly Growing Male Wistar Rats

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ABSTRACT

The present study assessed the effects of dietary restriction on tibial and vertebral mechanical and geometrical properties in 2-mo-old male Wistar rats. Two-month-old male Wistar rats were randomized to the *ad libitum* ($n = 8$) or the 35% diet-restricted (DR) feeding group ($n = 9$) for 5 mo. Tibiae and L_6 vertebrae were dissected out for microcomputed tomography (μCT) scanning and subsequently fractured in biomechanical testing to determine geometrical and mechanical properties. The DR group had significantly lower mean tibial length, mass, area, and cross-sectional moment of inertia, as well as vertebral energy to maximal load. After adjustment for body mass, however, DR tibial mean maximal load and stiffness, and DR vertebral area, height, volume, and maximal load were significantly greater, relative to *ad libitum* means. No significant differences were found between the DR and *ad libitum* mineral ash fractions. Because the material properties of the tibiae between the two groups were not significantly different, presumably the material integrity of the bones was not adversely affected as a consequence of DR. The similar material characteristics were consistent with mineral ash fractions that were not different between the two groups. Vertebral maximal load and stiffness were not significant between the DR and *ad libitum* animals. Importantly, we show that a level of dietary restriction (35%) that is less severe than many studies (40%), and without micronutrient compensation does not adversely affect tibial and vertebral mechanical properties in young growing male rats when normalized for body mass. (*Pediatr Res* **57: 227–231, 2005**)

Abbreviations
BMD, bone mineral density
DR, diet-restricted
L_6, 6th lumbar
μCT, microcomputed tomography

DR has emerged as an important avenue of investigation in the area of aging—specifically the prevention of aging. DR can increase both mean and maximal life span in a variety of animals, including rodents and primates (1). Whereas some studies of DR have focused singularly on reducing total calories, without compensation for micronutrient intake (2–4), others have maintained adequate and equal vitamin and mineral status for both DR and *ad libitum* animals (5,6). Study-to-study variations exist in method and degree of restriction,

period of dietary restriction, and the age at onset of restriction, and contribute to the lack of consensus on the effects of dietary restriction on bone adaptation.

Talbott *et al.* (7) used "younger" (3-mo-old) and "older" (10-mo-old) female Sprague-Dawley rats, matched for body mass and assigned to one of four diet groups representing two levels of energy (referred to as "caloric") intake and two levels of calcium intake. All rats had the same baseline diet (protein, fat, fiber, vitamins, and other minerals), but differed in caloric intake (normal *versus* 40% restriction) by reduced carbohydrate content or levels of calcium intake (normal = 78 mg/d and low = 15 mg/d). Rats were, therefore, restricted in calcium only, calories only, or calcium and calories, for a period of 9 wk. Dietary restriction of calcium or energy resulted in an elevated rate of bone turnover in both younger and older rats. Dietary restriction of energy alone had no significant influence on final BMD in younger rats. In older rats, however, final BMD was negatively influenced by DR, suggesting that although calcium or DR increased bone turnover in a similar

fashion in both younger and older rats there were still age-related differences of the influence of DR on BMD.

In a subsequent study, Talbott *et al.* (8) looked at the interaction of age and DR in relation to bone biomechanical properties. "Mature" (5-mo-old) and "aged" (12-mo-old) female Sprague-Dawley rats, approximately equivalent to 20 and 50 human years, respectively, were fed *ad libitum* or at 40% restriction for 9 wk. Both age groups experienced a 15% loss of body weight and decreased femoral bone density (32–35%). Despite small reductions in tibial and humeral density in both groups, biomechanical properties were reduced in aged but not mature rats. Clearly, the age at onset of DR in rats carried important implications for bone adaptation.

Additional support for an age-sensitive component of changes in bone metabolism comes from work by LaMothe *et al.* (5), who compared DR and *ad libitum* male Fischer 344 X Brown-Norway F1-hybrid rats. Rats were studied at ages representing "young adult" (8 mo old), "late middle aged" (28 mo old), and "senescence" (36 mo old). A group of rats 28 mo old was calorically restricted at 40% since 14 wk of age. Relative to age-matched *ad libitum* rats, the 28-mo-old DR rats had significantly shorter tibial lengths, and reduced total bone cross-sectional area, cortical shell area, mineral ash fraction, cross-sectional moment of inertia, load at proportional limit, maximal load, stiffness, and flexural rigidity. Relative to age-matched *ad libitum* rats, DR rats also had significantly shorter L_6 height and lower loads at the proportional limit and maximum. They concluded that DR adversely affected bone geometry and mechanics in aged male F344BN rats.

Body weight is recognized as a consistent predictor of BMD with body weight loss resulting in reductions in bone mass (6,7,9–13). Although Talbott *et al.* (7) failed to detect a significant reduction in BMD in young (3 mo) dietary-restricted rats, the effects on skeletal biomechanical and geometrical properties are not known. Given the significant effects of DR on age-related processes (14,15) and the questions arising regarding the age at which restriction is best initiated, it is important to address what effects DR-induced reductions in growth and accrual of total body mass have on bone strength in young rats.

A variety of levels of DR have been investigated but restricting food intake to 50–70% of that eaten by *ad libitum* rats is most commonly associated with increases in longevity and delays in age-associated physiologic deterioration (16–20). As such, the present study sought to determine the effect of DR (65% of *ad libitum*) on mechanical and structural properties of bone in young male Wistar rats. Importantly, this study examined how a period of DR longer than those of Talbott *et al.* (8) but shorter than those of LaMothe *et al.* (5) affected skeletal properties of young male Wistar rats. A restriction of 35% without nutritional supplementation was used, and we hypothesized that the growth and accrual of body weight in young rats would counterbalance the DR-related, deleterious effects in tibial and vertebral geometrical and mechanical properties seen in aged rats.

MATERIALS AND METHODS

Animals. Seventeen 2-mo-old male Wistar rats were randomly assigned to one of two groups: one group (*n* = 8) was fed standard rat chow *ad libitum* (Prolab RMH 2500, PMI Nutrition International Brentwood MO; 24% protein, 6% fat, 5.3% fiber, 6.9% ash, 0.95% calcium, 0.69% phosphorus), whereas the other group (*n* = 9) received 65% of the amount of food consumed by their respective *ad libitum*–fed controls on the previous day (*i.e.* 35% DR). Both were housed in standard rat cages with a 12:12 light-dark cycle. On the day of sacrifice, rats were anesthetized with halothane and killed by cervical dislocation. Carcasses were frozen (−80°C) until the time of dissection. After thawing and on the day of dissection, tibiae and the L_6 vertebrae were dissected and cleaned of nonosseous tissue for each rat. Bones were individually wrapped in saline-soaked gauze, sealed hermetically in plastic bags, and frozen until μCT scanning. On the day of scanning, bones were thawed in room-temperature PBS. Freezing and thawing do not have adverse effects on rat bone mechanical properties (21,22). All procedures were ethically approved by the University of Calgary Animal Care Committee and conformed to procedures set forth for the Care and Use of Laboratory Animals.

Tibial bone geometry. Randomly, either the right or left tibia from each rat was imaged using μCT. Once thawed, tibiae were scanned (Skyscan 1073, SkyScan, Aartselaar, Belgium) at a magnification of 14.07× (resolution of 19.73 μm). Scanning generated a series of planar transverse grayscale bit-mapped images. After scanning, the bones were again wrapped in saline-soaked gauze and refrozen (−20°C) until biomechanical testing. Bone geometries were determined by inputting the bitmapped images into custom software (Matlab, Natick, MA), which thresholded images and calculated geometric parameters such as total cross-sectional area, distances from centroid to the desired edge of the cross-section, and cross-sectional moment of inertia.

L_6 bone geometry. L_6 were thawed in room temperature (RT) PBS. Transverse processes were removed with dissection scissors, and the neural spine was removed with a diamond wafer saw (Buehler Isomet, Lake Bluff, IL). Vertebral height was measured with calipers (Model 599–578–1, Brown and Sharpe, Irvine, CA). L_6 were subsequently scanned with μCT (Skyscan 1073) at a magnification of 30× (resolution of 12.73 μm). Bitmapped images were generated, and the vertebral centrum was prepared for biomechanical testing. Caudal and rostral surfaces were cut to clean them of intervertebral discs and to ensure parallel surfaces for axial crush testing. After processing, L_6 length was measured and then re-wrapped in saline-soaked gauze and frozen (−20°C) until biomechanical testing. Bone geometries were determined by inputting the bitmapped images into custom software (Matlab), which thresholded images and calculated geometric parameters such as total cross-sectional area, cortical bone area, and trabecular area.

Tibial biomechanical testing. Tibiae were thawed in RT saline solution for at least 1 h. Tibial length was measured, and the midshaft marked. For testing, a round-surfaced cross-head probe of a servocontrolled electromechanical testing system (Model 1122, Instron Corp., Canton, MA) contacted the medial tibial surface at the longitudinal midpoint and applied a preload of 1 N. Tibiae were fractured in three point-bending (loading span of 13.3 mm) at 25.4 mm/min (5). Testing order was stratified between groups to eliminate any testing-order effects. Load-deformation curves were generated (RC Computerscope A/D Board, RC Electronics, Santa Barbara, CA). From these, the load at proportional limit, maximal load, and stiffness were calculated. Stiffness was defined as the linear region of the load-deformation curve. Proportional limit was the point where the linear region of the extended load-deformation curve deviated by >5% from the experimental curve [percentage difference = (predicted load value − experimental load value) · 100%/experimental load value]. Among the properties calculated were stress and strain at the proportional limit and maximal load, and flexural rigidity (22a).

L_6 biomechanical testing. L_6 centra were thawed in RT saline for at least 1 h. The caudal surface of the centrum was placed on a stainless steel plate lightly coated with mineral oil to facilitate unconstrained compression. The flat-surfaced crosshead probe of the testing system was also coated with mineral oil and contacted the rostral surface of the centrum with a preload of 5 N. The centrum was cycled with a load from 5 to 10 N, 20 times at 0.001%/s to obviate the viscoelastic creep of trabecular bone (23) and was stopped at a preload of 10 N. L_6 were then compressed at a fast strain rate (50%/s) (5). Structural properties were calculated (*e.g.* load at proportional limit, maximal load, and stiffness), as well as L_6 material stress and strain.

Ash analysis. Biomechanically tested tibiae and vertebrae were defatted in acetone for 7 d, dehydrated at 100°C (Thermolyne F62700, Barnstead International, Dubuque, IA) for 48 h in ceramic crucibles. Dehydrated samples were

measured (± 0.01 mg; Mettler AE 163, Mettler-Toledo, Inc., Columbus, OH) to determine dry bone mass. The samples were then incinerated at 600°C for 48 h, and the ash was measured (24). The ash mass divided by the dry mass was determined as the mineral ash fraction.

Statistics. Means and standard deviations were calculated for all variables. Means between the *ad libitum* and DR groups were compared with a nonparametric two independent samples test (Mann-Whitney U test; SPSS version 11.0, SPSS Inc., Chicago, IL). A significance level of $p \leq 0.05$ was used for all statistical tests.

RESULTS

Body mass. Final body mass was significantly lower in the DR group by almost 30% (Fig. 1), relative to *ad libitum* mass. Growth or increase in body mass over the 5-mo study period was >260% in *ad libitum* rats and >190% in DR rats, with DR rats achieving approximately 70% of the final body mass of the *ad libitum* rats. Bone structural properties were analyzed before and after adjustment for body mass. Bone mineral density and bone size are related to total body mass, and bone mineral density influences bone strength (25). Therefore, structural properties were reported without and with adjustment for body mass (26). Correction for body mass was achieved by dividing the geometrical or structural property of interest by body mass (26).

Tibial geometrics and mechanics. Tibial geometrical parameters, including tibial mass, length, area, and cross-sectional moment of inertia were significantly lower in the DR group (Table 1). DR tibial load and stress at proportional limit were reduced about ~20% but that was not significantly different from *ad libitum* rats. When adjusted (normalized) to total body mass, however, DR tibial values (*e.g.* maximal load, stiffness, flexural rigidity, mass, length, and area) were significantly greater than for the *ad libitum* rats (Table 2). The tibial mineral ash fraction was not different between the two groups.

L₆ geometry and mechanics. No significant differences were found between the two groups except for energy to maximal load, which was significantly (~20%) lower in the DR group (Table 3). Although DR stiffness and strain at the proportional limit were reduced by 20–25% when compared with the *ad libitum* rats, those differences were not statistically significant
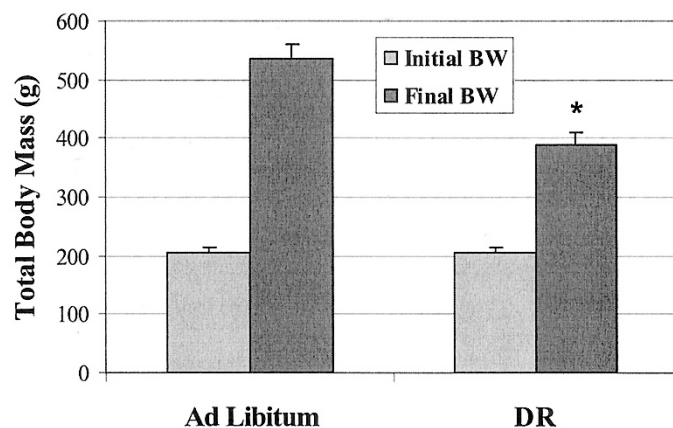
Table 1. *Tibial geometry and mechanics*

	Ad libitum ($n = 8$)	DR ($n = 9$)
Geometrical		
Tibial length (mm)	49.2 ± 9.0	44.3 ± 1.0*†
Tibial mass (g)	1.4 ± 0.1	1.2 ± 0.1*†
Total cross-sectional area (mm²)	5.6 ± 0.5	4.8 ± 0.3*†
Cross-sectional moment of inertia (mm⁴)	3.7 ± 0.8	2.7 ± 0.2*
Structural		
Maximal load (N)	166.7 ± 30.3	143.0 ± 27.6†
Load at proportional limit (N)	155.5 ± 32.7	126.3 ± 54.4
Stiffness (kN/mm)	378.0 ± 67.4	359.0 ± 20.9†
Displacement at maximal load (mm)	0.5 ± 0.1	0.5 ± 0.1
Displacement at proportional limit (mm)	0.5 ± 0.1	0.4 ± 0.2†
Energy to proportional limit (kN·mm)	17.2 ± 5.0	16.0 ± 7.6
Energy to maximal load (kN·mm)	22.4 ± 11.2	23.6 ± 9.2
Material		
Stress at maximal load (MPa)	175.8 ± 21.0	191.3 ± 37.6
Stress at proportional limit (MPa)	163.0 ± 19.9	190.5 ± 38.9
Strain at maximal load (%)	41.0 ± 9.0	36.0 ± 10.0
Strain at proportional limit (%)	36.0 ± 5.5	30.0 ± 1.2
Mineral ash fraction (%)	73.0 ± 0.40	73.4 ± 0.39
Flexural rigidity (kN·mm²)	18.5 ± 3.3	17.6 ± 1.0†

Values are mean ± SD. * Significant difference, $p < 0.05$. † Significant when corrected for body mass ($p < 0.05$), values presented in Table 2.

Table 2. *Body-mass adjusted tibial geometry and mechanics*

	Ad libitum ($n = 8$)	DR ($n = 9$)
Geometrical		
Tibial length (μm/g)	92.0 ± 18.0	110.0 ± 5.0†
Tibial mass (μg/g)	2.6 ± 0.2	3.1 ± 0.1†
Tibial length/mass (μm/g)	67.0 ± 16.0	94.0 ± 10.0†
Total cross-sectional area (μm²/g)	10.0 ± 1.0	12.0 ± 1.0†
Cross-sectional moment of inertia (μm⁴/g)	7.0 ± 1.0	9.0 ± 1.0
Structural		
Maximal load (N/g)	0.31 ± 0.05	0.37 ± 0.08†
Stiffness (kN/mm/g)	0.71 ± 0.13	0.92 ± 0.04†
Displacement at max load (μm/g)	1.0 ± 0.2	1.3 ± 0.4
Displacement at proportional limit (μm/g)	0.9 ± 0.0	1.1 ± 0.4†
Load at proportional limit (N/g)	0.29 ± 0.05	0.33 ± 0.14
Energy to proportional limit (N·mm/g)	32.0 ± 8.7	41.5 ± 19.7
Energy to maximal load (N·mm/g)	41.3 ± 19.4	60.2 ± 23.5
Flexural rigidity (N·mm²/g)	34.6 ± 6.6	45.1 ± 1.8†

Values are mean ± SD. † Significant when corrected for body mass, $p < 0.05$.

(Table 3). After adjusting for body mass, the following were significantly greater in the DR L₆ compared with the *ad libitum* rats: maximal load, energy to proportional limit, area, height, volume, trabecular area, and cortical area (Table 4). The L₆ mineral ash fraction was not different between the two groups.

DISCUSSION

When normalized for differences in total body mass, the geometrical, structural, and mechanical properties of the tibia and L₆ in growing male DR Wistar rats were not adversely affected, compared with *ad libitum* fed rats. Consistent with previous data (7,8,27), final body mass of DR animals was significantly lower than the *ad libitum* animals at the end of the study, but growth continued throughout the 5-mo study. After adjusting for body mass, DR produced changes in tibial geometry but did not significantly alter the structural and material

Figure 1. Total body mass of *ad libitum*–fed and diet-restricted male Wistar rats. Values are presented for the body mass at the beginning of the experimental period and after 5 mo of DR. *Significantly different from final body weight of *ad libitum* rats, $p < 0.05$ ($n = 8$–9).

Table 3. *Rat L_6 vertebral geometry and mechanics*

	Ad libitum (n = 8)	DR (n = 9)
Geometrical		
L_6 cross-sectional area (mm^2)	6.3 ± 0.4	5.8 ± 0.4†
L_6 height (mm)	6.1 ± 0.5	6.0 ± 0.7†
L_6 volume (mm^3)	38.2 ± 4.3	34.7 ± 4.3†
Trabecular area (mm^2)	2.0 ± 0.3	2.1 ± 0.1†
Cortical area (mm^2)	3.6 ± 0.3	3.6 ± 0.2†
Structural		
Maximal load (N)	461.3 ± 38.0	396.5 ± 87.3†
Load at proportional limit (N)	353.5 ± 70.5	300.0 ± 125.6
Stiffness (kN/mm)	527.6 ± 222.4	397.1 ± 240.6†
Displacement at maximal load (mm)	3.6 ± 2.8	3.4 ± 3.1
Displacement at proportional limit (mm)	1.1 ± 0.5	1.3 ± 1.0
Energy to proportional limit (kN·mm)	40.8 ± 13.1	45.1 ± 22.9†
Energy to maximal load (kN·mm)	22.4 ± 77.4	12.9 ± 64.0*
Material		
Stress at maximal load (MPa)	73.5 ± 6.8	67.7 ± 13.5
Stress at proportional limit (MPa)	56.1 ± 9.9	51.6 ± 20.9
Strain at maximal load (%)	60.8 ± 48.5	56.6 ± 52.8
Strain at proportional limit (%)	17.2 ± 8.8	22.5 ± 18.5
Mineral ash fraction (%)	63.8 ± 1.5	64.3 ± 1.5

Values are mean ± SD. * Significant difference, $p < 0.05$. † Significant when corrected for body mass ($p < 0.05$), values presented in Table 4.

Table 4. *Body-mass-adjusted L_6 vertebral geometry and mechanics*

	Ad libitum (n = 8)	DR (n = 9)
Geometrical		
L_6 cross-sectional area (μm^2/g)	12.0 ± 0.7	15.0 ± 1.4†
L_6 height (μm/g)	11.0 ± 0.8	15.0 ± 2.3†
L_6 volume (μm^3/g)	71.0 ± 7.0	89.0 ± 14.0†
Trabecular area (μm^2/g)	3.7 ± 0.6	5.4 ± 0.4†
Cortical area (mm^2/g)	7.0 ± 0.8	9.2 ± 1.0†
Structural		
Maximal load (N/g)	0.87 ± 0.08	1.03 ± 0.23†
Stiffness (kN/mm/g)	0.85 ± 0.54	1.03 ± 0.63†
Displacement at maximal load (μm/g)	6.8 ± 5.2	8.8 ± 8.3
Displacement at proportional limit (μm/g)	2.0 ± 1.0	3.4 ± 2.6
Load at proportional limit (N/g)	0.66 ± 0.14	0.78 ± 0.33
Energy to proportional limit (N·mm/g)	76.5 ± 24.0	116.9 ± 59.6†
Energy to maximal load (N·mm/g)	419.2 ± 141.7	334.6 ± 165.3

Values are mean ± SD. † Significant when corrected for body mass, $p < 0.05$.

properties compared with the *ad libitum* group. Indeed, when adjusted for total body mass, some DR tibial structural properties (maximal load and stiffness) were significantly greater than the *ad libitum* group.

L_6 geometry and structural and material properties were not significantly different between the two groups before adjustment for body mass. After adjusting for total body mass, all L_6 geometrical values and several structural properties were significantly greater in the DR group, compared with the *ad libitum* group. The general absence of detrimental effects on bone geometric and mechanical properties in DR rats contrasts with typical findings from several studies with mature or aged rats (5,7,8). The current results support the findings of Talbott *et al.* (7), in which DR did not adversely affect BMD in young rats. Our results provided evidence that DR at a level of 35% restriction without micronutrient supplementation in young

growing rats did not adversely affect bone geometry or mechanics. This is an important finding given that our understanding of the dose response effects of DR on bone mechanics is limited, including at which level of restriction micronutrient compensation is critical or necessary to maintain optimum bone mechanical properties.

Diverse protocols have been used to assess the impact of DR on bone, including studies restricting energy from 4 wk (28) to 4 mo (28a), matched (5) or not matched (2,3) micronutrient intake, using rats of various ages, sex, and strains (6,28). Our study involved a moderate level of restriction (35%), longer period of restriction (5 mo), and initiated the restriction period at 2 mo of age in male Wistar rats. Peak bone mass is achieved by approximately 10 mo in rats (29–31), thus, the Wistar rats in the current study were growing throughout the entire study. As several studies demonstrate adverse effects of DR on bone in mature or aged rats (5,7,8), it is possible that an early adaptive response related to growth in the young rats confers protection from the deleterious effects of DR on bone. Our work suggests that despite DR without micronutrient compensation, there were no adverse effects on bone geometry or mechanics in young, growing rats. This protective mechanism requires further investigation.

In the present study, tibiae from DR rats were significantly smaller in length, mass, and area when compared with *ad libitum* tibiae, which suggested a possible inhibitory effect on bone growth. Stunted bone growth has been demonstrated in several other studies (5,27). When normalized for total body mass, however, DR tibiae length, mass, and area were significantly greater than the *ad libitum* rats, suggesting that bone growth per unit body mass was not diminished with our DR paradigm.

Independent of body mass, DR did not alter any of the L_6 geometry or material properties, and only energy to maximal load was significantly lower in structural properties. Normalizing these properties for body mass resulted in a consistent improvement in all L_6 geometries and several structural properties with DR. Our findings were in contrast to the negative effects of DR seen in L_6 bone responses in mature and aged rats (5,8,27).

Why normalized geometry and mechanical properties were not adversely affected by DR in our young rats was not entirely clear but may be linked to gains in body mass. Our DR rats continued to gain mass throughout the 5-mo restriction period, although at a significantly lower rate of increase than the *ad libitum* animals. In contrast to the current rats that were in their rapid growth phase, the majority of studies examining DR in mature or aged rats reported a loss of body mass over the course of DR (5,7,8,27). The effects of adult-onset weight loss on bone structural and material biomechanical properties are not completely understood, but Talbott *et al.* (8) reported that whereas weight loss due to DR was detrimental to some bone parameters in mature rats, only aged rats had consistent reductions in biomechanical properties. Thus, the effects of DR may interact with the aging process to compromise adaptation with increasing age.

In agreement with the findings of Talbott *et al.* (7) demonstrating no negative influence of DR on BMD in young rats,

mineral ash fraction was not significantly different between the two groups in the present study. As the material properties of the tibiae and L_6 vertebra between the two groups were not significant in this study, it can be assumed that the material integrity of the bones was not adversely affected by the DR paradigm. Our study used a slightly lower level of DR (35%) compared with the 40% of Talbott *et al.* (7) and we did not compensate for vitamin and mineral intake. It is noteworthy that studies that have specifically assessed energy restriction *versus* calcium restriction severely limit the calcium content of the diet. For example, Talbott *et al.* (7) compared a control diet with 0.9% calcium phosphate to a 0% level in the calcium-restricted diet and 0.65% calcium carbonate control to 0.25% in the calcium-restricted diet. The reduction in micronutrient intake in our study was much less severe given that the restricted rats still received 65% of the calcium and mineral intake of the *ad libitum* fed rats (0.95% calcium and 0.69% phosphorus). Therefore, although there was reduced mineral intake in our restricted rats it was not as severely limiting as that of many studies addressing the effects of mineral restriction. Although these differences in study protocol existed, we were still able to show a positive effect of DR on bone change when normalized to body mass. The most likely protective factor in young rats is likely to be the influence of growth and the endocrine environment associated with it. Future studies will need to include detailed analysis of endocrine factors related to growth and anabolism.

In summary, changes in body mass due to DR accounted for minimal differences in L_6 and tibial mechanics. When normalized for total body mass, DR did not adversely affect tibial and L_6 mechanics in spite of a 35% restriction in caloric intake in the young, rapidly growing male Wistar rats. The mechanisms of this effect remain unknown but may involve alterations in the endocrine environment associated with growth. The looming public health crisis related to obesity and an increasingly aged population dictates that we fully understand one of the most effective and powerful interventions known to mitigate age-associated disease, DR. Our work suggests that early initiation of DR at a level of 35% does not adversely affect bone geometry and mechanics in young, growing rats. In the future, it will be important to fully understand the benefits and risks associated with DR.

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