Effects of Aging and Caloric Restriction on Bone Structure and Mechanical Properties

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This study examined the effects of caloric restriction on structural and material properties of tibiae and sixth lumbar vertebrae in F344BN male rats. Rats were divided into two dietary groups, ad libitum or calorie restricted. Caloric restriction commenced at 14 weeks of age, with 40% restriction and micronutrient supplementation by 16 weeks maintained until death. Rats were studied at 100% (8 months), 70% (30–35 months), and 35% (35–40 months) survival rates. Specimens were assessed using microcomputed tomography, mechanical testing, and ash analysis. A calorically restricted diet resulted in a significant decrease in total body mass when compared to ad libitum diet. Generally, direct comparisons between same-aged groups showed no significant changes in material properties, with significantly greater normalized-to-body-mass structural properties under caloric restriction. These results suggested a possible beneficial response to the calorically restricted diet where bone quality was maintained with bone quantity improved per unit body mass.

Key Words: Caloric restriction—Bone—Biomechanics—Aging.

Caloric restriction (CR) can impact mean life expectancy in mammals. McCay and colleagues (1) were among the first to demonstrate that a diet reduced in calories resulted in prolonging the life span of rats. Since then, much of the research in CR has sought to explain why and how a reduced caloric intake influences the mechanisms of aging. It is also important, however, to examine the impact that reduced caloric intake can have on other processes and structures of the body to have an integrated understanding of this life-extending paradigm.

CR results have demonstrated several positive effects on health that occur during dietary restriction and appear to have a direct link to increasing longevity. As summarized by Heilbronn and Ravussin (2), CR reduces metabolic rate and oxidative stress, alters neuroendocrine and sympathetic nervous system function, and improves insulin sensitivity. Although there may be other CR-influenced processes, the specific effect each has on the length of life span is unknown. However, it is believed that no single factor, but rather a combination of factors, is responsible for the observed effects of CR on aging (3).

There are also negative effects associated with CR. These include growth retardation, incessant hunger, reduced reproductive function, and cold intolerance (4). From a cost–benefit point of view, however, it is widely accepted that the overall physical effects of CR outweigh the negative effects, at least in laboratory animals. To date, how the adaptations of CR interact with the various environmental challenges in an “outside the laboratory” setting are unknown, limiting evaluation of the benefits and risks of CR.

Aside from prolonging life span, CR can influence mechanical, material, and geometrical characteristics of bone. McCay and colleagues (1) reported that some of the femurs of CR rats were “crumbling during dissection” and “appearing only as thin cylinders of bone.” These results suggested that CR had a negative influence on bone, but these results may have been caused by extremely reduced food consumption with a likely insufficient vitamin and mineral intake to maintain proper bone health (5). Furthermore, the results from McCay and colleagues (1) underlined the caution when making comparisons between studies and drawing conclusions on the effects of CR. Results on CR are varied, as initial conditions chosen in the design of the study often differed. Particularly, there is variability in the degree of restriction, age at onset of restriction, duration of restriction, as well as the use or disuse of supplementation of micronutrients, all of which can influence the impact of CR (4). When studying CR, it is important to consider carefully each of these parameters and understand their influence on bone properties.

Aging and CR can influence the material and mechanical properties of bone. LaMothe and colleagues (6) found that from 8 to 36 months of age, the structural properties of the tibiae in ad libitum (AL) fed Fischer 344 × Brown-Norway (F344BN) rats remained constant, except for an increase in cross-sectional moment of inertia (Ixx) with a corresponding increase in age. The increase in Ixx simultaneously compensated for an age-related decrease in bone material properties, resulting in no net change in skeletal strength as bone material properties decreased with aging. Furthermore, sixth lumbar vertebrae (L6) geometry did not change with increasing age for the AL group. The same study examined 28 month old (mo), 40% CR rats, restricted from 14 weeks of age, with micronutrient supplementation. When comparing
the 28 mo CR rats to same-aged AL fed rats, the reduced diet adversely affected bone geometry and mechanics. For the appendicular skeleton, CR animals had a significant reduction in the length of the tibia and a reduction in total bone cross-sectional area (CSA), cortical shell area, mineral ash fraction, $I_{xx}$, load at proportional limit, maximal load, stiffness, and flexural rigidity. For the axial skeleton, CR animals had significantly shorter L6 height and lower loads at the proportional limit and maximal load. The authors concluded that CR adversely influenced axial and appendicular bones in 28 mo CR F344BN rats.

To examine the effects of CR and aging on the mechanical and structural properties of bone, Lambert and colleagues (7) applied a dietary restriction of 35% (65% of AL diet without micronutrient supplementation) on young growing male Wistar rats. The CR regimen commenced at 2 months of age, and the animals were killed prior to the attainment of full skeletal maturity, at approximately 7 months of age. A relationship was found between age and onset of CR, where CR implementation in young growing rats did not adversely affect bone geometry and mechanics.

To determine the effects of reduced bone mineral density on fragility, Talbott and colleagues (8) examined mature (5 mo) and aged (12 mo) female Sprague-Dawley rats under control (AL fed) and CR conditions (40% fewer calories than AL). CR commenced at 5 and 12 months of age for the mature and aged groups, respectively. Dietary controls were maintained for 9 weeks, when the animals were killed. CR significantly reduced biomechanical properties of the aged group, with no significant effects on the mature group. These results demonstrated that CR had an age-dependent relationship between the time of initiation of CR and bone adaptation.

The purpose of the current study was to examine the interactive effects of diet and aging on the mechanical and material properties of bone. We investigated how an AL diet and 40% CR with nutrient supplementation beginning at 14 weeks of age interacted with the aging process through the changes in bone geometry and bone mechanics in the appendicular and axial skeleton in young adult and senescent F344BN rats. We hypothesized that CR would not negatively influence bone mechanics or geometry in young growing rats, but that CR would negatively influence bone mechanics and geometry in adult and senescent rats (6). The results indicate that, in general, with direct comparisons between same-aged groups based on percentage survival rate, CR does not result in significant changes in material properties, but a CR diet does result in significantly greater normalized-to-body-mass structural properties when compared to the AL diet.

**Materials and Methods**

**Animals**

F344BN rats were obtained from the National Institute on Aging in two dietary groups, the AL group ($n = 21$) and the CR group ($n = 21$), using a protocol approved by the University of Calgary Animal Care Committee. F344BN rats were chosen because this strain has been shown to exhibit fewer age-related pathologies than F344 rats (9). The AL group represented young adult (8 mo) and aged (30–35 mo) rats. The CR group represented 8 mo and 35–40 mo rats. AL and CR groups were matched for comparison based on the percent survival rate (SR) (10). Group comparisons were based on percent SR as follows: 8 mo AL and CR groups served as a baseline measurement with 100% SR ($n = 16$), 30 mo AL and 35 mo CR had a SR of 70% ($n = 15$), and 35 mo AL and 40 mo CR had a SR of 35% ($n = 11$). Vertebral samples for the 35% SR animals were erroneously disposed of before commencing the study, thereby eliminating these samples from the study (i.e., no vertebral samples for 35% SR CR). CR commenced at 14 weeks of age, with a net dietary restriction of 40% (60% caloric intake of AL group) achieved by 16 weeks of age, up until being killed. The CR group received a fortified food mixture (NIH 31/NIA fortified; National Institute on Aging, Baltimore, MD) to supplement and maintain a proper nutritional balance. All diets remained constant until the animals were killed.

Rats were housed at the University of Calgary Biological Sciences vivarium (two per cage) for a minimum of 1 week prior to experimentation. The rats followed a 12-hour light/dark cycle, and temperature was held constant at 22°C. The AL group was fed with rat chow (NIH31; National Institute on Aging), whereas the CR group was given supplemented rat feed (NIH31/NIA fortified; National Institute on Aging, Baltimore, MD). All rats had an unlimited supply of water. Post experiment necropsies were performed and did not detect any abnormalities or lesions within any animal. Two animals, one 35 mo CR animal and one 35 mo AL fed animal were excluded from the analysis as they died of heart failure prior to the protocol completion.

**L6 and Tibia Preparation for Testing**

After extraction, tibiae were individually wrapped in phosphate-buffered saline (PBS)-soaked gauze (pH = 7.2–7.4) and aluminium foil, hermetically sealed in plastic bags, and frozen at –30°C until testing. L6 vertebræ remained frozen in the body until extraction for testing. After being removed, L6 were prepared for storage in the same fashion as the tibiae. Before testing, bones were allowed to thaw for a minimum of 1 hour in PBS solution. Previous research by Pelker and colleagues (11) and Peng and colleagues (12) revealed that freezing and thawing of bone did not adversely affect bone mechanical properties.

**Testing Protocol**

To examine the effects of CR and age on bone geometry and mechanics, a series of experiments were performed that included geometry analysis, biomechanical testing, and ash analysis (6).

**Tibia bone geometry.—**Tibiae were subjected to microcomputed tomography (μCT) scanning (Skyscan 1073; Aartselaar, Belgium) at a magnification of 16× (resolution of 19.39 μm). Using reconstruction software (NRecon; Skyscan), bitmap images were generated from scanning that represented longitudinal cross-sectional slices of each tibia. The selected cross-sectional slices (one per tibia) were...
analyzed with custom software (Image J; National Institutes of Health, Bethesda, MD, and Moment Macro; M. Warfel, Cornell University, Ithaca, NY) that performed a threshold on the images and calculated geometrical parameters including total bone CSA, cortical bone CSA, and moment of inertia (I_{xx}) with respect to the test bending axis. Tibial length was measured using digital callipers (±0.01 mm; model 14-648-17; Fisher Scientific, Pittsburgh, PA).

L6 bone geometry.—Vertebrae were subjected to μCT scanning (Skyscan 1073) at a magnification of 30× (resolution of 13.59 μm). Bitmap images generated from scanning of the vertebrae region (one image per vertebrae) were measured using custom software (Image J) that determined total CSA. Vertebral height was measured with callipers (±0.01 mm; model 14-648-17; Fisher Scientific). From these measurements, the total volume of the centrum was calculated.

Tibial biomechanical testing.—Tibiae were tested in three-point bending with the lateral cortex downward on a round surfaced 13.3 mm loading span. A round-surfaced crosshead probe of a servo-controlled electromechanical testing system (model 1122; Instron Corp., Canton, MA) contacted the medial tibial cortex at its longitudinal midpoint. A preload of 6 N was applied, and, subsequently, a load speed of 25.4 mm/min was applied until complete failure (6). Load deformation curves were acquired (200 Hz, Windaq; Datqa Instruments Inc., Akron, OH), from which stiffness, load at proportional limit, and maximal load were determined using Excel (Microsoft Office XP suite, Bellevue, WA). Proportional limit was the region where the slope of the force-deformation curve deviated by ≥5% from the slope of the linear region (13). Results from the force-deformation curves were used in conjunction with geometrical properties to calculate stress and strain at proportional limit and maximal load, modulus of elasticity, and flexural rigidity. Material properties were calculated using the methods described by Turner and Burr (14). Flexural rigidity was determined as the product of I_{xx} and the elastic modulus.

L6 biomechanical testing.—Prior to testing, endplate tissue was removed from the vertebrae. Using a diamond wafer saw (Buehler Isomet; Lake Bluff, IL), transverse processes and the neural spine were removed and the caudal surfaces were cut to create a parallel surface (6). The caudal surface of the vertebrae was placed on a stainless steel plate thinly coated with mineral oil to provide unconstrained compression. The flat-surfaced crosshead of a servo-controlled electromechanical testing system (model 1122; Instron Corp.) was also lubricated and contacted the rostral surface of the vertebrae with a preload of 5 N. The load was cycled from 5–10 N 20 times at a strain rate of 0.001 %/s (0.5 mm/min). After 20 repetitions, cycling was stopped at a preload of 10 N, and samples were compressed at a strain rate of 50%/s (229 mm/min) (6). Load-deformation curves were acquired (200 Hz; Windaq, Dataq Instruments Inc.). Stiffness, load at proportional limit, and maximal load were determined (Excel, Microsoft Office XP suite), and the proportional limit was the region where the slope of the force deformation curve deviated by ≥5% from the slope of the linear region (13). Results from the force-deformation curves, along with the geometrical parameters, were used to calculate stress and strain at proportional limit and maximal load and modulus of elasticity (14).

Ash analysis—tibia and vertebral.—After biomechanical testing, each whole, fractured tibia was defatted in a 100% acetone solution for 9 days, and each vertebra was defatted in a 100% acetone solution for 7 days. After defatting, two 4 mm sections of the tibia diaphysis immediately distal and proximal to the fracture site were removed from the tibia using a diamond wafer saw (Buehler Isomet) (13). Samples were dehydrated at 100°C for 48 hours (model F62700; Barnstead/Thermolyne, Dubuque, IA). Following dehydration of the bone samples, they were weighed to determine dry bone mass (±0.1 mg; XS 104; METTLER TOLEDO, Greifensee, Switzerland). Samples were incinerated at 600°C for 48 hours and reweighed to determine ash mass. Mineral ash fraction was calculated and defined as the ratio of ash mass divided by dry mass multiplied by 100%.

Statistics
As conducted in previous studies (6,7) means between diet groups were compared with Mann–Whitney U tests, which revealed significant differences between same-aged percent SR groups (SPSS version 13.0; Chicago, IL). A significance level of p ≤ .05 was used for all statistical analyses. Data are presented as mean ± standard deviation.

RESULTS

Body Mass
Normalization of data according to body mass (15) was performed because of significant differences in body mass between the AL and CR diet groups across all age categories (Figure 1).
ally showed a significant decrease with the CR diet, except

Table 1. Tibia and L6 Vertebral Material Property Comparisons According to Percent Survival Rate for F344BN AL and CR Diet Groups

<table>
<thead>
<tr>
<th>Material Property</th>
<th>100% Survival Rate</th>
<th>70% Survival Rate</th>
<th>35% Survival Rate</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>8 mo AL</td>
<td>8 mo CR</td>
<td>30 mo AL</td>
</tr>
<tr>
<td>Tibia</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Elastic modulus, MPa</td>
<td>12,855.0 ± 958.2</td>
<td>13,199.3 ± 985.0</td>
<td>9803.6 ± 1075.3</td>
</tr>
<tr>
<td>Stress at proportional limit, MPa</td>
<td>168.7 ± 21.2</td>
<td>162.9 ± 22.4</td>
<td>129.8 ± 12.9</td>
</tr>
<tr>
<td>Strain at proportional limit, %</td>
<td>1.3 ± 0.1</td>
<td>1.24 ± 0.17</td>
<td>1.33 ± 0.10</td>
</tr>
<tr>
<td>Stress at maximum load, MPa</td>
<td>265.3 ± 12.2</td>
<td>248.3 ± 45.3</td>
<td>208.9 ± 26.1</td>
</tr>
<tr>
<td>Strain at maximum load, %</td>
<td>2.7 ± 0.4</td>
<td>2.5 ± 0.7</td>
<td>3.0 ± 0.7</td>
</tr>
<tr>
<td>Mineral ash fraction, %</td>
<td>69.2 ± 1.5</td>
<td>69.4 ± 2.0</td>
<td>70.9 ± 3.0</td>
</tr>
<tr>
<td>L6 Vertebral</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Elastic modulus, MPa</td>
<td>326.0 ± 34.8</td>
<td>262.1 ± 48.3*</td>
<td>326.0 ± 66.3</td>
</tr>
<tr>
<td>Stress at proportional limit, MPa</td>
<td>24.0 ± 3.1</td>
<td>22.7 ± 5.8</td>
<td>20.4 ± 4.8</td>
</tr>
<tr>
<td>Strain at proportional limit, %</td>
<td>7.4 ± 1.2</td>
<td>8.9 ± 3.2</td>
<td>6.4 ± 1.4</td>
</tr>
<tr>
<td>Stress at maximum load, MPa</td>
<td>32.5 ± 2.5</td>
<td>30.8 ± 1.9</td>
<td>27.6 ± 3.8</td>
</tr>
<tr>
<td>Strain at maximum load, %</td>
<td>11.8 ± 1.3</td>
<td>14.6 ± 3.2</td>
<td>10.1 ± 1.2</td>
</tr>
<tr>
<td>Mineral ash fraction, %</td>
<td>64.0 ± 1.1</td>
<td>64.4 ± 1.2</td>
<td>62.4 ± 1.9</td>
</tr>
</tbody>
</table>

Notes: All values are mean ± standard deviation.
*Significant from 8 mo AL (p ≤ .05).
AL = ad libitum; CR = calorie restricted.

Tibia and L6 Material Properties: CR Versus AL

Of all the material properties measured, there were no significant differences between CR and AL diet groups of similar percent SR, except for L6 elastic modulus in the 100% SR comparison (Table 1). Otherwise, all material properties for tibia and L6 showed no significant changes among all age group comparisons.

Tibia and L6 Structural Properties: CR Versus AL

Prior to normalization, tibial structural properties generally showed a significant decrease with the CR diet, except in the 35% SR group, which showed no significant changes except for maximal load and stiffness with the CR diet (Table 2). L6 structural properties all showed a significant decrease except for load at proportional limit, with the CR diet, for the 100% SR diet group. In the 70% SR diet group, CR showed a significant decrease in structural properties except for stiffness, load at proportional limit, and maximal load (Table 2).

After normalization, tibial structural properties generally showed a significant increase in the CR diet group over the AL diet group. Of all the structural properties, only load at

Table 2. Tibia and L6 Vertebral Structural Property Comparisons According to Percent Survival Rate for F344BN AL and CR Diet Groups

<table>
<thead>
<tr>
<th>Structural Property</th>
<th>100% Survival Rate</th>
<th>70% Survival Rate</th>
<th>35% Survival Rate</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>8 mo AL</td>
<td>8 mo CR</td>
<td>30 mo AL</td>
</tr>
<tr>
<td>Tibia</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stiffness, N/mm</td>
<td>473.5 ± 33.1</td>
<td>386.5 ± 28.4*</td>
<td>670.3 ± 52.8</td>
</tr>
<tr>
<td>Tibia length, mm</td>
<td>43.1 ± 0.6</td>
<td>41.5 ± 0.5*</td>
<td>44.6 ± 0.3</td>
</tr>
<tr>
<td>Total bone CSA, mm²</td>
<td>5.9 ± 0.2</td>
<td>5.4 ± 0.3*</td>
<td>7.9 ± 0.4</td>
</tr>
<tr>
<td>Cortical bone CSA, mm²</td>
<td>4.6 ± 0.1</td>
<td>4.1 ± 0.2*</td>
<td>5.7 ± 0.3</td>
</tr>
<tr>
<td>Cross-sectional moment of inertia, mm³</td>
<td>1.8 ± 0.2</td>
<td>1.5 ± 0.2*</td>
<td>3.2 ± 0.3</td>
</tr>
<tr>
<td>Flexural rigidity, kN/mm²</td>
<td>22.8 ± 1.4</td>
<td>19.1 ± 1.4*</td>
<td>31.5 ± 2.2</td>
</tr>
<tr>
<td>Load at proportional limit, N</td>
<td>93.6 ± 11.0</td>
<td>76.8 ± 8.0*</td>
<td>105.9 ± 7.8</td>
</tr>
<tr>
<td>Maximal load, N</td>
<td>147.7 ± 12.1</td>
<td>116.4 ± 15.6*</td>
<td>170.5 ± 17.6</td>
</tr>
<tr>
<td>L6 Vertebral</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stiffness, N/mm</td>
<td>481.4 ± 104.0</td>
<td>349.5 ± 73.4*</td>
<td>669.7 ± 121.5</td>
</tr>
<tr>
<td>Vertebral body height, mm</td>
<td>7.5 ± 0.4</td>
<td>7.2 ± 0.3*</td>
<td>8.2 ± 0.3</td>
</tr>
<tr>
<td>Total CSA, mm²</td>
<td>9.9 ± 0.6</td>
<td>8.9 ± 0.3*</td>
<td>13.2 ± 1.3</td>
</tr>
<tr>
<td>Total volume, mm³</td>
<td>74.7 ± 7.7</td>
<td>63.6 ± 4.3*</td>
<td>108.5 ± 13.7</td>
</tr>
<tr>
<td>Load at proportional limit, N</td>
<td>235.7 ± 31.1</td>
<td>201.1 ± 49.6</td>
<td>264.9 ± 52.1</td>
</tr>
<tr>
<td>Maximal load, N</td>
<td>318.9 ± 19.4</td>
<td>273.3 ± 16.1*</td>
<td>359.9 ± 34.7</td>
</tr>
</tbody>
</table>

Notes: All values are mean ± standard deviation.
*Significant from 8 mo AL (p ≤ .05).
#Significant from 30 mo AL (p ≤ .05).
$Significant from 35 mo AL (p ≤ .05).
AL = ad libitum fed; CR = calorie restricted; CSA = cross-sectional area.
proportional limit, maximal load (both at 100% SR), and maximal load at 35% SR showed no significant changes (Table 3).

Similarly, after normalization of the L6 structural properties, there was a significant increase in all structural properties except for load at proportional limit and stiffness at the 100% SR comparison, which showed no significant change (Table 4).

**DISCUSSION**

Similar to the findings of LaMothe and colleagues (6) and Lambert and colleagues (7), in the current study, CR significantly reduced body mass at all ages when compared to the AL group. With aging, peak body mass was achieved at 70% SR in the AL diet group, with a subsequent significant decrease from 70% to 35% SR, which was in agreement with Turturro and colleagues (10). However, between 28 and 36 mo AL, LaMothe and colleagues (6) found no significant change in body mass. Meanwhile, the CR group showed a slight (but insignificant) increase in body mass across 100% to 35% SR, with the highest mean mass achieved at 35% SR.

When comparing the 100% SR diet groups, normalized tibial structural properties all significantly increased except for the load at proportional limit and maximal load. Similar results were found in studies by Lambert and colleagues (7), in which all comparable normalized properties were significantly larger in the CR group. The only exception was cross-sectional moment of inertia, which showed no significant difference, and maximal load, which was significantly larger in the Lambert and colleagues (7) study only. Furthermore, both studies showed no significant change in load at proportional limit, and no significant differences were found in tibial material properties in both studies.

Results similar to those of Lambert and colleagues (7) were also obtained when comparing L6 structural and material properties among the 100% SR diet groups. Again, both studies showed similar trends with a significant increase in normalized structural properties in the CR diet group. The only exception was stiffness, which did not increase in the current study, and load at proportional limit, which showed no significant changes in both studies. Also, as with the tibia, both studies found no significant changes in material properties, except for elastic modulus, which significantly decreased in the CR diet group.

Normalized data between 100% SR AL and CR showed significantly greater structural properties of CR tibiae except for load at proportional limit and maximal load with no change in CR and AL material properties. Similarly, normalized data between 100% SR AL and CR showed significantly greater structural properties of CR L6 vertebrae with only a significant decrease in CR elastic modulus. That finding was in agreement with the results from Lambert and colleagues (7). Thus, the first hypothesis was tested and, in the aggregate, CR did not negatively impact bone geometry or mechanics in growing, male F344BN rats. Furthermore, the chosen CR regimen of 35% CR in 7 mo rats, without micro-nutrient supplementation, and earlier onset of CR (2 mo) as chosen by Lambert and colleagues (7), when compared to the current study (8 mo, 40% CR with micronutrient supplementation), appeared to affect bone adaptation in similar fashion.

In previous studies examining CR and aging, LaMothe and colleagues (6) found normalized tibial structural properties in 28 mo CR F344BN rats to be significantly larger when compared to the same-aged AL diet group. An increase occurred in normalized properties including tibial length, total bone CSA, cortical bone CSA, and stiffness in 28 mo CR over the same-aged AL group, with only load at proportional limit and maximal load remaining significantly smaller. Comparing these findings to the current study, at 70% SR (30 mo AL vs 35 mo CR) all structural properties were found to be significantly higher in the CR diet group. Material properties measured between 70% SR diet groups showed no significant changes. However, between 28 mo AL and 28 mo CR, LaMothe and colleagues (6) found a significant decreases in mineral ash fraction.

From the L6 results comparing 28 mo AL and CR diet groups, LaMothe and colleagues (6) found no changes in material properties or normalized structural properties. That contrasted partly with the results of the current study, where in the 70% SR group results showed a significant increase in all structural properties with no changes in material properties. When observing the changes in material and structural properties in the appendicular and axial skeleton between 70% SR AL and CR diet groups, normalized results showed
significantly greater CR diet group structural properties over the AL rats with no significant changes in material properties. Thus, in contrast to our hypothesis and results of LaMothe and colleagues (6), we found that between 70% SR AL and CR diet groups, CR did not adversely affect bone adaptations in aged male F344BN rats.

When examining the 35% SR diet group, we found that all normalized tibial structural properties except maximal load were significantly higher in the CR diet group. There were no significant differences in material properties between AL and CR diet groups. Therefore, as with the two younger-age-group comparisons, in later senescence, the tibial material and normalized structural properties of male F344BN rats were not negatively impacted with CR. Talbott and colleagues (8) found that a CR regimen of 40% in Sprague-Dawley rats, commenced at 5 months, with a CR diet for 9 weeks, did not significantly affect non-normalized bone biomechanical properties. However, in the same study the authors found that with the given CR regimen, when starting at 12 months, there was a significant decrease in non-normalized bone biomechanical properties. Because the current study found no significant negative effects on bone biomechanical properties with the current CR regimen, the present results confirmed those found by Talbott and colleagues (8). However, as demonstrated by Talbott and colleagues (8), the age of onset of CR appeared to be an important factor in the outcome of future bone adaptation, with an earlier onset being more advantageous than onset at a later age. Further investigation is warranted into the role of CR and age of onset.

To gain a more holistic understanding of the impacts of a CR diet on bone, those processes or factors that drive bone adaptation to CR need be identified. In particular, Brochmann and colleagues (16) examined the impact of CR on total body, femoral, and vertebral bone in SENCAR, C57BL/6, and DBA/2 mice. The authors found CR to impact femoral bone mineral density and bone mineral content through changes in lean body mass, and found CR to improve vertebral bone mineral density and bone mineral content under a high oxidative state. Therefore, CR appears to affect the appendicular and axial skeleton in different manners, with biochemical processes having an impact on trabecular bone in the vertebrae. Further experimentation, examining the effects of CR on the biomechanical properties of the axial skeleton under increased oxidative stress, would help provide further insight into the relationship between CR and bone.

The results of the current study showed that a CR diet resulted in a significant decrease in total body mass when compared to the AL diet group. Generally, direct comparisons between same-aged groups based on percent survival rate showed no significant changes in material properties, with significantly greater normalized structural properties with CR. The significantly greater structural properties in the CR group than in the AL diet group after normalization suggested a potential beneficial response to the CR diet (where bone quality is maintained with bone quantity improved per unit body mass). A thorough understanding of the CR-induced mechanisms on bone development will require further investigation. The current study demonstrated that a CR diet with 40% restriction at 14 weeks (with nutrient supplementation) did not negatively impact tibial geometrical and mechanical properties in young and senescent male F344BN rats.

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