Physiology of Aging
Selected Contribution: Bone adaptation with aging and long-term caloric restriction in Fischer 344 × Brown-Norway F1-hybrid rats

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LaMothe, Jeremy M., Russell T. Hepple, and Ronald F. Zernicke. Selected Contribution: Bone adaptation with aging and long-term caloric restriction in Fischer 344 × Brown-Norway F1-hybrid rats. J Appl Physiol 95: 1739–1745, 2003. First published June 13, 2003; 10.1152/japplphysiol.00079.2003.—Rodents are commonly used as models for human aging because of their relatively short life span, the ease of obtaining age-specific tissue samples, and lower cost. However, age-associated disease may confound inbred animal studies. For example, numerous physiologically significant lesions, such as chronic nephropathy, are more common in aged Fischer 344 (F344) rats than in other strains (Bronson RT, Genetic Effects of Aging, 1990). Conversely, F344 × Brown-Norway F1-hybrid (F344BN) rats, developed by the National Institute on Aging for aging research, live considerably longer and have fewer pathologies at any given age vs. inbred strains (Lipman RD, Chrise CE, Hazzard DG, and Bronson RT, J Gerontol A Biol Sci Med Sci 51: 54–59, 1996). To our knowledge, there are no data regarding the effect of age on bone geometry and mechanics in this strain of rat. Furthermore, caloric restriction (CR) extends the mean and maximal life span of animals and significantly reduces age-associated disease but may have adverse consequences for bone growth and mechanics. Thus we investigated the effects of age and CR on bone geometry and mechanics in the axial and appendicular skeleton of F344 Brown-Norway rats. Ad libitum fed rats were assessed at 8 mo (young adult; n = 6), 28 mo (late middle age; n = 5), and 36 mo (senescence; n = 6). CR rats were assessed at 28 mo (n = 6). Tibiae and the sixth lumbar vertebrae (L6) were dissected, scanned (micro-computed tomography) to determine geometry, and tested mechanically. From 8 to 36 mo, there were no significant changes in L6 geometry, and only the cross-sectional moment of inertia changed (increased) with the tibia. CR-induced body mass reductions accounted for changes in L6 load at proportional limit, maximal load, and stiffness (structural properties), but altered tibial structural properties were independent of body mass. In tibiae, geometric changes dominated alterations in structural properties. Those data demonstrated that, whereas aging in ad libitum-fed animals induced minor changes in bone mechanics, axial and appendicular bones were adversely influenced by CR in late-middle-aged animals in different manners.

tibia; vertebra; bone geometry; biomechanics

AGE-RELATED OSTEOPOROSIS adversely affects quality of life and the health care system. As of 1995, it was estimated that osteoporosis-related fractures cost the United States health care system $13.8 billion annually (32). With an increase in global life expectancy, osteoporosis-related fractures have the potential to become an even larger problem in the future (12) and strongly justify gerontological research investigating aging and bone health. Human studies are complicated by the difficulty of obtaining age-specific tissue samples, and longitudinal studies are complicated by the long lifespan of humans. Thus surrogate animals are commonly used.

Abundance, short lifespan, and cost render rodents desirable test subjects. Average longevity for healthy, pathogen-free inbred rats is strain dependent and lies between 750 and 950 days (31). However, age-associated disease, neoplastic lesions, and degenerative lesions may confound results obtained from inbred animal studies. For example, numerous physiologically significant lesions, such as chronic nephropathy, are more common in aged Fischer 344 (F344) rats than in other strains (6). In contrast, the Fischer 344 × Brown-Norway (BN) F1-hybrid rat, developed by the National Institute on Aging (NIA) for aging research, lives considerably longer (40) and has fewer pathologies for any given age vs. inbred strains.

Caloric restriction (CR) is one paradigm that consistently extends the lifespan of animals and significantly reduces the incidence of numerous age-related diseases and lesions regardless of species or sex (21, 42). Despite the beneficial aspects of CR on mortality and disease, diet affects bone health (1), and CR paradigms may adversely affect bone physiology and mechanics. The classic studies of McCay et al. (22) detailing the effect of CR on longevity also noted that bones became fragile after CR and that “some crumbled with the course of dissection.” Because of the severe CR that McCay et al. used, however, bone fragility was likely the result of extreme dietary deprivation including calcium insufficiency (11, 14).
With less severe CR paradigms (e.g., 60% of the ad libitum diet), Kalu et al. (15) reported that CR slowed bone maturation and prevented age-associated hyperparathyroidism in F344 rats. Sixteen weeks of 16-month-old male F344 rats being fed diets containing 60 and 80% of ad libitum dietary energy, while maintaining sufficient mineral and protein intake, decreased bone mineral content (18). Nnakwe (28) reported that when 3-wk-old F344 rats were fed a diet containing 60% of all ad libitum dietary components for 3 and 12 mo, ultimate bone strength declined significantly. When Lobund-Wistar rats were fed diets containing 50 and 65% of ad libitum calories enriched with proteins, vitamins, and minerals, femoral bone loss occurred, but the loss was entirely accounted for by the CR-associated reduction in body mass (35).

The NIA Biomarkers of Aging Program has primary objectives to identify rodent biomarkers associated with aging to determine the efficacy of aging interventions (40). NIA studies use BN, F344, and F1 F344 × BN hybrid rats (F344BN) fed 60% of the calories and the same quantity of proteins, vitamins, and minerals as age-matched ad libitum-diet rats. Because CR F344BN rats have the longest mean and maximal life span and the fewest pathologies of any strain of rat, we used CR F344BN rats to determine the interactive effects of aging and CR on bone geometry and mechanics.

Given that F344BN rats live considerably longer and have fewer pathologies for any given age vs. inbred strains, we hypothesized that age would affect F344BN bone mechanics less than age affects mechanics seen in prior studies of inbred strains. Moreover, because CR reduces the average age-associated disease and lesion burden (21), such as testicular atrophy (21), which may be harmful to bone material, and because the NIA supplements the diet of CR animals such that they receive the same vitamin and mineral intake as ad libitum fed animals, we hypothesized that when CR induced alterations in body mass as were seen in the tested centrum to 80% of testing. Previous studies suggest that freezing and thawing do not adversely affect rat bone mechanical properties (29, 30).

Tibial biomechanical testing. Tibial geometry permits more stable and repeatable placement of the diaphyseal shaft on the loading span than the femur, which is more circular in cross section. On the day of testing, tibiae were thawed in 22°C buffer (50 mM potassium phosphate buffer solution, pH = 7.4) for at least 1 h. Tibial length was measured by using callipers (model 599-578-1, Brown and Sharpe, Irvine, CA). When thawed, the round-surfaced cross head probe of a servocell所得際電機械力試験装置 (model 1122, Instron, Canton, MA) contacted the medial tibial surface at its longitudinal midpoint, between a 13.3-mm loading span, and applied a preload of 5 N; the medial surface was in compression and the lateral surface was in tension. Testing order was stratified on the basis of group, and load was applied at 25.4 mm/min until failure. Load-deformation curves were generated (RC Computerscope A/D Board, RC Electronics, Santa Barbara, CA). From the load-deformation curves, the following structural properties were determined: load at proportional limit, maximal load, and stiffness. As bone mineral density (BMD) and bone size are influenced by total body mass (3), and as BMD influences bone strength (10), structural properties were normalized (divided by body mass) as previously reported (e.g., Ref. 43). Material properties were calculated and included stress and strain at proportional limit, stress and strain at maximal load, and flexural rigidity (44).

\( L_0 \) biomechanical testing. On the day of testing, \( L_0 \) were thawed in 22°C buffer (50 mM potassium phosphate buffer solution, pH = 7.4) for at least 1 h. With the use of established methods (34, 44), caudal and rostral surfaces were cleaned of intervertebral discs and synovial joints. To isolate the vertebral centrum, the neural spine and transverse processes were removed with a diamond saw (Buehler Isomet, Lake Bluff, IL). Vertebral height was measured with callipers (model 599-578-1, Brown and Sharpe). To ensure parallel surfaces, a wafer was cut from the caudal surface, bringing the total length of the tested centrum to 80% of the centrum length in its entirety. The wafer was saved for micro-computed tomography scanning (see \( L_0 \) bone geometry).

The caudal surface of the centrum was placed on a stainless steel plate thinly coated with mineral oil to simulate unconstrained compression. The flat-surfaced cross head
of a servo-controlled electromechanical testing system (model 1122, Instron) was also lubricated and contacted the rostral surface of the centrum with a preload of 5 N. The centrum was cycled from 5 to 10 N 20 times at 0.001%/s to eliminate viscoelastic creep of trabecular bone (19). Cycling was stopped at a preload of 10 N, and samples were subsequently compressed at a fast strain rate (50%/s). Testing was stratified on the basis of group to prevent a testing-order effect. Load-deformation curves generated as output were used to determine load at proportional limit, maximal load, and stiffness. As with tibial data, structural properties were also normalized (divided by total body mass). Stress and strain at the proportional limit and stress and strain at maximal load were determined (34, 44). Apparent elastic modulus was the slope of the linear region of the stress-strain curve.

Tibial bone geometry. After failure in three-point bending, fractured tibial surfaces were fixed together with cyanoacrylate adhesive (Loctite 495 instant adhesive, Mississauga, ON). Fractured regions (cross head contact point) were subjected to micro-computed tomography scanning (SkyScan 1073, Aartselaar, Belgium) at a magnification of ×14 (resolution of 19 μm). Bitmap images generated from scanning were input to custom software (Matlab, Natick, MA) that thresholded images and calculated geometric parameters including total cross-sectional area, cortical bone area, trabecular area, distances from centroid to the desired edge of the cross section, and cross-sectional moment of inertia (Ixx).

To normalize for geometric changes associated with total body mass, tibial length, total cross-sectional area, and cortical bone area were divided by body mass.

A section of the tibial diaphysis immediately distal to the site of fracture was dehydrated at 100°C (Thermolyne F62700, Dubuque, IA) for 48 h in a ceramic crucible. Dehydrated bone samples were weighed (Mettler AE 163, Anaheim, CA; ±0.0001 g) to determine dry bone mass. After samples were weighed, they were incinerated at 600°C for 24 h, and then at 800°C for 24 h. The ash was weighed, and ash mass divided by dry mass was the mineral ash fraction.

L6 bone geometry. Wafers of bone cut from the caudal surface of the L6 centra were scanned with micro-computed tomography (Skyscan 1075) at a magnification of ×23.87 (resolution of 11 μm). Bitmap images generated from scanning of the region that was in contact with the body of the centrum were thresholded and subjected to pixel-counting routines to determine cross-sectional area (Scion Image, Frederick, MD). The product of cross-sectional area and centrum height defined L6 volume. Immediately after compression testing, L6 were discarded, thereby precluding any ash analysis.

Statistics. Means between groups were compared with Kruskal-Wallis nonparametric ANOVA. Mann-Whitney post hoc comparisons determined where intergroup significant differences were present (SPSS version 11.0, Chicago, Illinois). A significance level of P = 0.05 was used for all statistical tests. Values are means ± SD.

RESULTS

No tissue lesions were found in this set of animals. Total body mass. Body mass was significantly different among groups (Fig. 1). Twenty-eight-month-old and 36-mo-old rats were significantly heavier than 8-mo-old rats. Body mass in 28-mo-old CR animals was significantly lower than in the age-matched ad libitum-fed animals.

Tibial geometry. With one exception, no significant differences due to aging alone existed in any of the tibial geometric parameters. Ixx increased with age, and by 36 mo was significantly larger (≥32%) than the 8-mo Ixx (Table 1). Tibial length, total cross-sectional area, cortical shell area, and Ixx were significantly less in CR rats compared with age-matched ad libitum-fed rats (Fig. 2, Table 1). When adjusted for body mass, CR related reductions in tibial length, total cross-sectional area, and cortical bone area remained significant. Tibial mineral ash fraction did not change significantly

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**Table 1. Rat tibial geometry**

<table>
<thead>
<tr>
<th></th>
<th>8 mo</th>
<th>25 mo</th>
<th>28 mo CR</th>
<th>36 mo</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tibial length, mm</td>
<td>43.9 ± 1.3</td>
<td>44.0 ± 0.9</td>
<td>41.9 ± 0.4</td>
<td>44.1 ± 0.7</td>
</tr>
<tr>
<td>Tibial length/mass, mm/g</td>
<td>0.1 ± 0.01</td>
<td>0.09 ± 0.01</td>
<td>0.13 ± 0.01</td>
<td>0.09 ± 0.01</td>
</tr>
<tr>
<td>Total bone cross-sectional area, mm²</td>
<td>5.7 ± 0.4</td>
<td>6.0 ± 0.6</td>
<td>4.7 ± 0.4</td>
<td>6.0 ± 0.6</td>
</tr>
<tr>
<td>Total area/mass, mm²/g⁻¹·10⁻²</td>
<td>1.3 ± 0.1</td>
<td>1.2 ± 0.1</td>
<td>1.5 ± 0.1</td>
<td>1.2 ± 0.2</td>
</tr>
<tr>
<td>Cortical shell area, mm²</td>
<td>5.7 ± 0.5</td>
<td>5.9 ± 0.5</td>
<td>4.6 ± 0.3</td>
<td>5.9 ± 0.5</td>
</tr>
<tr>
<td>Cortical area/mass, mm²/g⁻¹·10⁻²</td>
<td>1.2 ± 0.2</td>
<td>1.2 ± 0.1</td>
<td>1.4 ± 0.1</td>
<td>1.2 ± 0.1</td>
</tr>
<tr>
<td>Cortical shell contribution, %area</td>
<td>99.1 ± 0.9</td>
<td>98.1 ± 1.9</td>
<td>97.2 ± 1.0</td>
<td>97.2 ± 2.3</td>
</tr>
<tr>
<td>Trabecular core area, mm²</td>
<td>0.05 ± 0.05</td>
<td>0.12 ± 0.12</td>
<td>0.14 ± 0.05</td>
<td>0.17 ± 0.14</td>
</tr>
<tr>
<td>Trabecular core contribution, %area</td>
<td>0.90 ± 0.95</td>
<td>1.88 ± 1.86</td>
<td>2.84 ± 1.01</td>
<td>2.80 ± 2.25</td>
</tr>
<tr>
<td>Mineral ash fraction, %</td>
<td>71.3 ± 0.3</td>
<td>70.9 ± 0.2</td>
<td>69.1 ± 1.7</td>
<td>70.5 ± 0.9</td>
</tr>
<tr>
<td>Cross-sectional moment of inertia, mm⁴</td>
<td>2.9 ± 0.6</td>
<td>3.5 ± 0.9</td>
<td>2.3 ± 0.3</td>
<td>3.8 ± 0.5</td>
</tr>
</tbody>
</table>

Values are means ± SD. *Significantly different from 8-mo mean (P < 0.05). †Significantly different from 36-mo mean (P < 0.05). ‡Significantly different from 28-mo mean (P < 0.05).
with age but decreased significantly with CR compared with that of age-matched ad libitum fed rats (Table 1).

**L6 geometry.** There were no significant differences in L6 cross-sectional area, height, or volume among 8-, 28-, and 36-mo-old rats (Table 3). When CR, L6 height from CR 28-mo-old animals was significantly less than either 28- or 36-mo-old ad libitum fed animals.

**Tibial mechanics.** Stiffness was the only structural property that changed significantly with age (Table 2). Tibial stiffness increased with age and was significantly different between 8- and 36-mo-old rats: 36-mo-old tibiae were stiffer (>11%). Flexural rigidity was the only material property significantly affected by aging. Flexural rigidity increased with age and became significant by 36 mo (>14%) compared with 8 mo.

With CR, values for tibial structural properties decreased significantly, including load at proportional limit (<20%), initial maximal load (<35%), and stiffness (<19%), compared with age-matched ad libitum-fed rats. When normalized for body mass, those structural differences remained significant. Associated with the observed decrease in mineral ash fraction, flexural rigidity was the only material property that showed significance due to CR (19% decrease compared with age-matched ad libitum-fed rats). Stress at maximal load (<14%) and strain at maximal load (<19%) were smaller in CR animals compared with age-matched rats, but those differences were not significant. Stress and strain at proportional limit were not different between age groups or with the introduction of CR.

**L6 mechanics.** Mechanical properties decreased with age (Table 4), but the decrements were not statistically significant in ad libitum-fed animals. With CR, load at proportional limit (<32%) and maximal load (<34%) decreased significantly compared with age-matched ad libitum-fed rats (Table 4). When load at proportional limit and maximal load were adjusted for body mass, significant differences disappeared. All CR L6 material properties showed a decrement but were not significant (Table 4).

## DISCUSSION

The present results are the first geometrical and mechanical data to reveal age- and CR-related changes in axial and appendicular bones in male F344BN rats. With ad libitum feeding, from 8 to 36 mo there were no significant changes in L6 height, cross-sectional area, or volume, and only the tibial Ixx changed (increased) with age. CR, however, stunted bone growth, including a significant reduction in L6 height, and decreased L6 and tibial bone structural properties. CR-induced whole body mass reductions accounted for significant changes in L6 bone mechanics, but altered tibial mechanics were independent of body mass. Tibiae showed significant decrements in load at proportional limit and maximal load in CR animals compared with age-matched animals when adjusted for body mass. As the corresponding material properties (stress at proportional limit and stress at maximal load) did not show significant decrements, changes in load at proportional limit and at maximal load must have been due to changes in bone geometry. Hence, geometrical changes dominated alterations in structural properties, with fewer significant alterations in material properties, including a decline in mineral ash fraction.

### Table 2. Rat tibial mechanics

<table>
<thead>
<tr>
<th></th>
<th>8 mo</th>
<th>28 mo</th>
<th>28 mo CR</th>
<th>36 mo</th>
</tr>
</thead>
<tbody>
<tr>
<td>Load at proportional limit, N</td>
<td>63.1±6.2</td>
<td>62.4±4.6</td>
<td>49.4±5.0**‡‡</td>
<td>60.3±9.1</td>
</tr>
<tr>
<td>Load at proportional limit/mass, N/g</td>
<td>0.14±0.02</td>
<td>0.12±0.01</td>
<td>0.10±0.01**‡‡</td>
<td>0.12±0.02</td>
</tr>
<tr>
<td>Stress at proportional limit, MPa</td>
<td>73.5±7.3</td>
<td>70.2±15.1</td>
<td>72.5±11.8</td>
<td>63.1±7.5</td>
</tr>
<tr>
<td>Strain at proportional limit, %</td>
<td>3.7±1.1</td>
<td>3.4±0.8</td>
<td>3.2±0.9</td>
<td>3.7±1.3</td>
</tr>
<tr>
<td>Maximal load, N</td>
<td>85.3±7.1</td>
<td>87.7±7.0</td>
<td>56.6±6.2**‡ ‡</td>
<td>87.7±5.2</td>
</tr>
<tr>
<td>Maximal load/mass, N/g</td>
<td>0.19±0.02</td>
<td>0.18±0.02</td>
<td>0.11±0.01**‡‡</td>
<td>0.18±0.02</td>
</tr>
<tr>
<td>Stress at maximal load, MPa</td>
<td>97.7±8.3</td>
<td>97.7±14.1</td>
<td>83.2±14.5*</td>
<td>86.0±15.4</td>
</tr>
<tr>
<td>Strain at maximal load, %</td>
<td>5.4±1.0</td>
<td>5.1±0.1</td>
<td>4.0±0.7†</td>
<td>5.9±1.3</td>
</tr>
<tr>
<td>Stiffness, kN/mm</td>
<td>292±23.0</td>
<td>311±33</td>
<td>249±26**‡‖</td>
<td>326±37*</td>
</tr>
<tr>
<td>Stiffness/mass, kN·mm⁻¹·g⁻¹</td>
<td>0.66±0.06</td>
<td>0.62±0.10</td>
<td>0.77±0.09*</td>
<td>0.67±0.11</td>
</tr>
<tr>
<td>Flexural rigidity, N·mm²</td>
<td>14.3±1.1</td>
<td>15.2±1.6</td>
<td>12.2±1.3**‡‖</td>
<td>16.3±2.2*</td>
</tr>
</tbody>
</table>

Values are means ± SD. †Significantly different from 8-mo mean (P ≤ 0.05). ‡Significantly different from 36-mo mean (P ≤ 0.05). *Significantly different from 8-mo mean (P ≤ 0.05). ‡‡Significantly different from 28-mo mean (P ≤ 0.05).
Table 4. Rat L6 vertebral mechanics

<table>
<thead>
<tr>
<th></th>
<th>8 mo</th>
<th>28 mo</th>
<th>28 mo CR</th>
<th>36 mo</th>
</tr>
</thead>
<tbody>
<tr>
<td>Load at proportional limit, N</td>
<td>341 ± 82</td>
<td>352 ± 88</td>
<td>240 ± 23‡¶</td>
<td>300 ± 27</td>
</tr>
<tr>
<td>Load at proportional limit/mass, N/g</td>
<td>0.78 ± 0.22</td>
<td>0.70 ± 0.17</td>
<td>0.74 ± 0.08</td>
<td>0.60 ± 0.08</td>
</tr>
<tr>
<td>Stress at proportional limit, MPa</td>
<td>16.6 ± 3.9</td>
<td>16.8 ± 3.9</td>
<td>12.9 ± 2.7</td>
<td>14.9 ± 2.2</td>
</tr>
<tr>
<td>Strain at proportional limit, %</td>
<td>26.1 ± 3.9</td>
<td>35.6 ± 10.2</td>
<td>30.7 ± 2.3</td>
<td>26.5 ± 3.0</td>
</tr>
<tr>
<td>Maximal load, N</td>
<td>404 ± 94</td>
<td>398 ± 118</td>
<td>261 ± 17†‡</td>
<td>342 ± 48</td>
</tr>
<tr>
<td>Maximal load/mass, N/g</td>
<td>1.26 ± 0.28</td>
<td>0.80 ± 0.23</td>
<td>0.80 ± 0.06*</td>
<td>0.69 ± 0.11*</td>
</tr>
<tr>
<td>Stress at maximal load, MPa</td>
<td>18.6 ± 4.5</td>
<td>18.2 ± 4.2</td>
<td>13.5 ± 2.6</td>
<td>16.3 ± 3.4</td>
</tr>
<tr>
<td>Strain at maximal load, %</td>
<td>29.1 ± 4.3</td>
<td>36.8 ± 9.1</td>
<td>32.6 ± 1.9</td>
<td>28.2 ± 2.4</td>
</tr>
<tr>
<td>Stiffness, kN/mm</td>
<td>1.41 ± 0.59</td>
<td>1.31 ± 0.98</td>
<td>0.78 ± 0.28</td>
<td>1.18 ± 0.30</td>
</tr>
<tr>
<td>Stiffness, kN·mm⁻¹·g⁻¹</td>
<td>3.23 ± 1.48</td>
<td>2.64 ± 1.95</td>
<td>2.42 ± 0.86</td>
<td>2.36 ± 0.59</td>
</tr>
<tr>
<td>Apparent elastic modulus, MPa</td>
<td>330 ± 129</td>
<td>308 ± 210</td>
<td>237 ± 53</td>
<td>296 ± 90</td>
</tr>
</tbody>
</table>

Values are means ± SD. *Significantly different from 8-mo mean (P ≤ 0.05). †Significantly different from 36-mo mean (P ≤ 0.05). ‡Significantly different from 28-mo mean (P ≤ 0.05).

Aging geometry and mechanics. From 8 to 36 mo, tibial length and cross-sectional area did not change. Previous results showed that F344 femoral length, cross-sectional area, BMD, and tibial BMD stabilized between 7 and 10 mo of age (14, 36), but rat tibial cross-sectional Ixx increased between 6 and 24 mo of age (4). With age, tibiae became stiffer and flexural rigidity increased (4). The age-related radial expansion of cortical bone, and hence greater cross-sectional Ixx, observed here was consistent with the observed greater bending stiffness. Human cortical bone also undergoes a radial expansion with age (2, 26, 33). In older human men, endosteal expansion was accompanied by periosteal bone apposition, thereby maintaining cross-sectional area (33). By redistributing bone material further from the cross-sectional centroid, bone strength can be maintained under conditions of decreased material strength (33).

Although there was an age-related decrease in tibial stress at proportional limit and stress at maximal load, those changes were not significant. Previous studies showed a significant decrease in maximal stress between 6 and 24 mo by 21% in F344 rats (4) and by 14% in Wistar rats (16). We observed a 12% decrease in ultimate stress in F344BN rats from 8 to 36 mo. Differences in rat strain may have influenced those discrepancies in results, because significant variability exists in BMD, structure, and bone mechanics among different rat strains (39). Our results support our first hypothesis that age affects F344BN bone mechanics less than age affects bone mechanics, as seen in prior studies of inbred strains. That suggests that F344BN rats conserved their material properties to a greater degree with age than either F344 or Wistar rats.

As with the tibia, L6 geometry did not change between 8 and 36 mo, indicating that F344BN vertebral growth plateaued by 8 mo of age. Structural and material properties, however, declined with age (Table 4). Maximal load/mass significantly declined with age. Thus lower material properties accounted for the age-related decline in bone mechanical properties. Similar to our observed decreases in stiffness and initial maximum load in L6 (Table 4), Sato et al. (36) reported a decrease in stiffness and maximal load between 6 and 17 mo in F344 rats.

CR: geometry and mechanics. CR has been the one intervention that consistently increased mean and maximal male and female life span in nearly all species tested to date (42). In addition to the life-extending effect of CR, McCay and colleagues’s (22, 23) seminal studies chronicled the effect of long-term dietary restriction on body growth retardation. As bone growth is influenced by diet and body mass (1, 3), bone mechanics are altered with CR. The severe dietary restriction in those studies, however, likely induced calcium insufficiency (17) that adversely affected bone (13). Subsequent studies, such as the present study, investigated the effects of food restriction while maintaining nutritional sufficiency.

Distal femoral bone mineral content decreased when restricted to 65 and 50% of ad libitum-fed calories, but changes in bone mineral content were entirely accounted for by CR-associated body mass reduction (35). L6 structural properties in the present study were consistent with those earlier findings for the distal femur. L6 load at proportional limit and maximal load declined significantly with CR, but when normalized for body mass, significant differences disappeared (Table 4). Body mass decreased 36% with CR, and load at proportional limit, maximal load, and stiffness decreased by 32, 35, and 40%, respectively (Fig. 1, Table 4). Both the distal femur and L6 are replete with trabecular bone. Most studies have found body mass a most significant predictor of BMD when trabecular bone sites, such as the vertebrae and femoral neck, are assessed (3, 24). Thus body mass-related changes in highly trabecular sites (e.g., distal femur and L6) should be similar. Alterations in femoral structural properties were also reported to parallel changes in body mass, whereas decreased material properties showed independent, less significant changes (8). That the values for CR and ad libitum-fed rats were similar was not consistent with our hypothesis that the mechanical properties of CR rats, when body mass was accounted for, would be greater than those of ad libitum-fed rats. Age-associated pathologies begin in male F344BN rats at mean ages ranging from 15.9 to 40 mo; different lesions start at dissimilar ages (see Ref. 20 for a detailed summary). Perhaps if the rats were assessed at a later age, significant age-associated disease would...
become more prevalent and affect bone properties. For example, adrenal gland medullary and testicular interstitial cell hyperplasias are relatively common in senescent male F344BN rats (20). Alternatively, because age-associated pathologies vary between rat strains (20), the pathologies most prevalent in male F344BN rats may not be detrimental to bone properties. Further studies are warranted.

In contrast to \( L_d \) properties, changes in tibial diaphyseal shaft structural properties and geometry were not fully accounted for by changes in body mass. That tibial material properties did not significantly decline with CR indicated that geometric changes accounted for the majority of the changes in structural properties observed. When older (57 wk) Sprague-Dawley rats were fed 60% of the calories that ad libitum-fed rats received, a slight decrease in tibial density was observed (37). Moreover, bone formation (27), and specifically bone turnover, increased dramatically with age and CR (38) possibly relating to the observed mineral ash fraction decrement. When C57BL/6 mice are fed a diet with an increase in stiffness. Age-associated increases in axial material properties could be attributed to declining bone material properties. Long geometry did not change from 8 to 36 mo; hence, the observed decrease in bone mechanical properties could be attributed to declining bone material properties. Thus age-related changes in axial and appendicular bone mechanics in male F344BN rats were driven by different mechanisms. Because CR reliably extends the mean and maximal life span of animals (42), CR paradigms are potentially useful for gerontological studies. Caution must be taken when aged CR animals are assessed solely to extract age-related changes in bone; we demonstrated that CR adversely affected bone geometry and mechanics independent of CR-related body mass reductions. CR-induced changes in body mass accounted for alterations in \( L_d \) mechanics but not tibial mechanics. In tibiae, geometric changes dominated alterations in structural properties with less significant changes in material properties, including a decline in mineral ash fraction.

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DISCLOSURES

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