Cross-Sectional and Longitudinal Analysis of Age-Associated Changes in Body Composition of Male Brown Norway Rats: Association of Serum Leptin Levels With Peripheral Adiposity

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Aging-associated alterations in body composition are accompanied by changes in the endocrine system. We evaluated, in male Brown Norway rats, the effects of aging on body composition and the association with serum levels of leptin, insulin, and testosterone. Body composition was assessed cross-sectionally in male rats (3, 8, 17, and 29 months) by a combination of dual energy x-ray absorptiometry (DEXA) and dissection of specific muscles and adipose depots. Longitudinal changes in body composition were quantified by DEXA before and after 3 months of ad-libitum feeding. Body weight, lean mass, absolute and percentage fat increased with age, whereas percentage of lean mass decreased. Leptin and insulin levels increased with age in proportion to adiposity; the increase in leptin with age was related to increased total and peripheral, but not visceral, fat. Testosterone decreased with age, and was associated with decreased lean and skeletal muscle mass. These findings suggest that alterations in body composition with age may be due to decreased trophic and increased lipogenic hormones. Relative to other rodent models, Brown Norway rats undergo shifts in body composition and in the hormonal milieu that are consistent with changes seen in aging humans.

Aging is associated with altered body composition (fat and lean mass and bone mineral content) in humans (1–6), non-human primates (7), rats (8–10), and other animals, and with adverse physiological consequences related to changes in the body habitus. Adipose tissue mass increases from middle to old age, and is associated with insulin resistance and impaired glucose tolerance, which contribute to development of Type 2 diabetes (NIDDM) and its sequelae (11–14). An age-associated decrease of lean mass, specifically skeletal muscle mass (sarcopenia), results in weakness, poor immune function, and impaired thermoregulation (2,6,15). In humans, decreased bone mass is a hallmark of aging. Bone mineral density and content decrease in women after menopause, but also in aged men, and may lead to osteoporosis and an increased rate of debilitating fractures (3,16). The mechanisms underlying these changes in body composition are unclear. However, alterations in the endocrine system are implicated, including decreased levels of trophic hormones (estrogen, testosterone, and growth hormone/insulin-like growth factor-1 [IGF-1]) and increased insulin levels (1,17).

We have been investigating the neuroendocrine mechanisms of age-related alterations in reproductive function, and energy (food intake and body weight) regulation in the male Brown Norway rat, and have found it to be a very good model of human aging. Compared to outbred strains, the genetic homo­geneity of this inbred rat model ensures greater uniformity of responses to experimental manipulations, with smaller numbers of animals needed. The National Institute of Aging maintains a breeding colony of Brown Norway BN/Bi (BN) rats in a specific pathogen-free environment, ensuring a long disease-free survival rate; this colony has a 50% survival age of 32 months for males (18). The use of this strain of rat as a model of human aging has several advantages over other strains that are commonly used in biomedical research. Unlike Fischer 344 (F344) or F344/BN hybrid rats (18,19), Brown Norway rats do not develop pituitary tumors that result in gonadotropin deficiency and/or hyperprolactinemia, or testicular tumors producing excessive amounts of progesterone, nor do they develop the extreme obesity that is common to many inbred strains of rats—all conditions that would confound studies of reproduction and energy balance. In contrast to other aging rat models, glomerulonephritis and pneumonitis are rare in Brown Norway rats, and testicular function is maintained until old age when serum testosterone levels decline (20).

We reported previously that aging Brown Norway rats exhibited reduced rates of food intake and body weight gain, accompanied by decreased expression of neuropeptide Y (NPY, a potent neuropeptide stimulator of food intake) in the arcuate nucleus, both under baseline ad-libitum feeding conditions and during refeeding following a 72-hour fast (21). A similar attenuation of food intake and body weight recovery following underfeeding has been found to occur in older men (22). Therefore, our findings suggest that the Brown Norway rat may be a useful model to investigate the mechanisms of age-related alterations in food intake and body weight regulation, and the anorexia of aging, in humans.

In addition to these important changes in overall body weight regulation, there are clearly significant age-related changes in adiposity, fat distribution, and lean body mass which have been reported in humans and experimental animals, as discussed above. However, such changes in body composition have not been re-
ported previously in male Brown Norway rats. Furthermore, longitudinal changes in body composition within the same animal, regional age-related differences in fat deposition, and their association with serum leptin levels have not been reported in any aging rat model.

In this study we sought to determine the effects of aging on body composition, and the relationship of age-related alterations in body composition to those in serum levels of leptin, insulin, and testosterone, in male Brown Norway rats from 3 to 29 months of age. Body composition was determined noninvasively by dual-energy x-ray absorptiometry, which permitted for the first time a longitudinal assessment of the rate of change of fat and lean mass in young, middle-aged, and old Brown Norway rats. Specific visceral and peripheral fat depots were dissected and weighed to directly assess age-related changes in regional fat distribution; muscles from the rear leg were dissected and weighed to evaluate the degree of sarcopenia with aging. Serum leptin, insulin, and testosterone levels were measured and related to the changes in body composition.

**Materials and Methods**

**Animals**

Adult male inbred, specific pathogen-free Brown Norway BN/Bi (BN) rats were purchased (Harlan Sprague-Dawley colony, Indianapolis, IN) and housed in an Association for Assessment of Accreditation of Laboratory Animal Care (AAALAC)-accredited facility at the VA Puget Sound Health Care System, American Lake Division in Tacoma, WA, in a room containing no other rats. After 2 months of acclimation, the initial age groups (n = 10 per group) were 5 months (young), 14 months (middle-aged), and 26 months (old), with a subsequent group of 3-month-old rats (very young; n = 10) added after 3 months. Two of the animals in the old group died early in the study. There was no apparent pathology and their data were removed from the analyses. Animals were housed in polycarbonate rat cages containing corn cob bedding, in a light- and temperature-controlled room on a 12-hour light-dark cycle. Throughout the experiment, animals were allowed ad-libitum access to Purina rodent chow (5001, Ralston Purina, St. Louis, MO) and tap water. All animal experiments were conducted in accordance with the principles and procedures outlined in the National Institute of Health (NIH) Guide for the Care and Use of Laboratory Animals.

**In Vivo Body Composition**

Body composition (fat mass, lean mass, and bone mineral content [BMC] and density [BMD]) was measured either once (very young) or twice (all other groups) by dual-energy x-ray absorptiometry (DEXA; QDR 1500, Hologic, Inc., Waltham, MA). DEXA has been described extensively elsewhere (23-25), and provides a noninvasive method for quantification of whole body and regional body composition and bone mineral content. The system is based on the differential attenuation of low (70 keV) and high (140 keV) energy x-rays by the tissues in the scan area. Energy is attenuated in proportion to tissue density, and this information is used by the detector and associated software, in conjunction with tissue calibration phantoms, to assess body composition. Soft tissues attenuate the energy beam less than bone; of the soft tissue mass, fat mass attenuates the beam less than lean mass. Fat mass consists primarily of adipose tissue, but lean mass includes organs, tendons, cartilage, blood, and body water in addition to skeletal muscle. Because the DEXA system is computer-based, it is possible to determine body composition and bone mineral content in specific regions as well as in the whole body. In the present study, fat and lean mass and bone mineral content are reported only for the whole body, but bone mineral density was assessed in specific bones (after ref. 23). The regions scanned for density (spine [L1-L4], proximal femur [not including the head of the femur], distal femur, and proximal tibia) were primarily trabecular rather than cortical bone.

Nonfasted rats were weighed and anesthetized with an intraperitoneal injection of 60 mg/kg pentobarbital. Animals were placed on the scanning platform in dorsal recumbency, positioned, and, when necessary, held in place with adhesive tape. Animals were first studied after 2 months of acclimation (scan 1 at 5, 14, and 26 months of age) and scanned using the Rat Whole Body software package (V5.67, Hologic Inc., Waltham, MA). Three months after the initial scans, the additional group of very young rats was included, and all animals (scan 2 at 3, 8, 17, and 29 months of age) were scanned with both the whole-body software, and with the Sub-Regional High Resolution software package (V4.66), for detailed analysis of BMD. A tissue calibration scan was performed each half-day before the animals were scanned, and a separate frozen rat carcass was scanned each day as a control for assay variations. Intra-assay coefficients of variation (CVs) were 5.8% (scan 1) and 5.6% (scan 2) for fat, 0.6% for lean (scan 1 and 2), 1.2% and 0.6% for BMC, and 1.7% and 0.7% for BMD. The interassay CVs for fat and lean mass, BMC, and BMD were 6.0%, 1.0%, 0.8%, and 1.5%, respectively. The sum of individual animals' fat mass, lean mass, and BMC was highly correlated (r = .999) with direct measurement of body weight.

**Sacrifice and Dissection**

One month after the second DEXA scan, nonfasted rats were killed by decapitation; brains were quickly removed and frozen on dry ice for further studies. Trunk blood was collected in serum separation tubes, and removed after clotting, for assay of leptin, insulin, glucose, and testosterone (see Hormone Assays, below, for details). Visceral and peripheral fat pads were freshly dissected and weighed. For dissection, an incision was made at the ventral midline, exposing the abdominal cavity. The gastrointestinal tract was severed above the stomach and at the base of the colon, and removed, exposing the deep visceral fat pads. The omental fat depot was carefully separated from the pancreas, and the mesenteric fat depot was removed from the length of the gut, with blood vessels and connective tissue included. Paired retroperitoneal, epididymal, and perirenal fat pads were removed, with kidneys, adrenals, testes, and epididymis dissected away. Peripheral axillary fat was removed from beneath both forelegs, and the interscapular brown fat depot was removed. Three muscles (unpaired) were removed from the left rear leg of each animal. These included the adductor longus (located in the upper thigh), the quadriceps, and the gastrocnemius, with soleus removed. Muscles were weighed immediately after dissection. Due to an initial error in muscle identification, the gastrocnemius was not collected in three animals (one 8 months and two 17 months old) and no muscles were collected from another 8-month-old animal.
**BODY COMPOSITION IN AGING MALE BN RATS**

### Hormone Assays

Serum was stored at -70°C until hormone assays were performed on duplicate samples. Glucose levels were determined by the glucose oxidase method (Beckman, Brea, CA). Leptin and insulin levels were determined by double antibody radioimmunoassay kits (rat leptin: RL-83K; rat insulin: RI-13K; Linco, St. Louis, MO). The sensitivity of the leptin assay is 0.5 ng/mL; intra-assay variability was 7.2%. The limit of sensitivity of the rat insulin assay is 0.1 ng/mL, and the intra-assay variability was 3%. Testosterone was measured by radioimmunoassay using reagents provided by the World Health Organization (WHO) Matched Reagent Program (20). Assay sensitivity is 0.1 ng/mL and intra-assay variability was 5.1%.

### Statistical Analyses

Body composition (fat and lean mass, BMC, and BMD), expressed as absolute weight (in grams) and percentage of total body weight from the DEXA scans, were compared between age groups by analysis of variance (ANOVA). Statistical comparisons were done separately for DEXA scan 1 (3 age groups) and scan 2 (4 age groups). Fat pad and muscle weights were compared between age groups by ANOVA as absolute weight (g) and as percentage of DEXA values for total fat (for fat pads) or as percentage of total lean mass (for muscles). Estimates of visceral and peripheral fat were calculated the following way: visceral fat pad weights (omental, mesenteric, retroperitoneal, perirenal, and epididymal) were summed and reported as “visceral fat.” This figure was subtracted from the total body fat reported by the DEXA analysis for an estimate of “peripheral fat.” Total visceral and peripheral fat mass estimates were used to normalize leptin levels in order to determine the variables influencing age-related differences in leptin. Leptin and other hormone levels were compared between age groups by ANOVA. Results are reported as mean ± SEM. Stringency for statistical tests was set at $p < .008$ (from the Bonferroni-Dunn correction), due to the interrelated nature of the body composition variables under study. Post hoc testing by Sheffe F test was performed for between-age differences where ANOVA determined significant age effects. The Sheffe test is a stringent procedure that is used when numbers are unequal and variances heterogeneous. The statistical software package used was StatView Version 4.57 for Windows (Abacus Concepts, Inc., and SAS Institute, Inc., Berkeley, CA).

### Results

#### Body Weight and Composition

Animals were larger and heavier with increased age. Figure 1 shows representative DEXA scans from a young, a middle-aged, and an old animal. Body weights (Figure 2 and Table 1) were significantly different from each other at scan 1 (young, 5 months; middle-aged, 14 months; old, 26 months), although 3 months later at scan 2, the middle-aged animals were no longer lighter in weight than the old. Weights of all components measured by DEXA (fat and lean mass and bone mineral content) were greater as age increased, due to the continuous growth of rats throughout their life span (26). Thus, results are shown in

![Figure 1. DEXA scans (digitized and color-inverted) of representative animals from this study.](image)

### Table 1. Body Composition From DEXA of All Age Groups of BN Rats

<table>
<thead>
<tr>
<th>Group</th>
<th>Age (mo)</th>
<th>n</th>
<th>Animal weight (g)</th>
<th>DEXA sum weight (g)</th>
<th>DEXA fat (g)</th>
<th>% Fat</th>
<th>DEXA lean (g)</th>
<th>% Lean</th>
<th>DEXA BMC (g)</th>
<th>% BMC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Very young</td>
<td>3</td>
<td>10</td>
<td>272 ± 6.6**</td>
<td>272 ± 6.1**</td>
<td>23.9 ± 0.6**</td>
<td>8.8 ± 0.2**</td>
<td>241.8 ± 5.7**</td>
<td>88.8 ± 0.2**</td>
<td>6.2 ± 0.1**</td>
<td>2.27 ± 0.02</td>
</tr>
<tr>
<td>Young</td>
<td>5</td>
<td>10</td>
<td>323 ± 4.2*</td>
<td>323 ± 4.2*</td>
<td>31.5 ± 1.1*</td>
<td>9.8 ± 0.3*</td>
<td>284.1 ± 3.8*</td>
<td>87.9 ± 0.3*</td>
<td>7.5 ± 0.1*</td>
<td>2.32 ± 0.02</td>
</tr>
<tr>
<td>Young</td>
<td>8</td>
<td>10</td>
<td>350 ± 5.9**</td>
<td>355 ± 6.1**</td>
<td>40.4 ± 1.3**</td>
<td>11.4 ± 0.3**</td>
<td>306.4 ± 5.4**</td>
<td>86.2 ± 0.3**</td>
<td>8.5 ± 0.1**</td>
<td>2.39 ± 0.02</td>
</tr>
<tr>
<td>Middle-aged</td>
<td>14</td>
<td>10</td>
<td>417 ± 10.0*</td>
<td>424 ± 9.7*</td>
<td>56.6 ± 2.5*</td>
<td>13.4 ± 0.5*</td>
<td>356.8 ± 8.7</td>
<td>84.2 ± 0.5*</td>
<td>10.5 ± 0.3</td>
<td>2.47 ± 0.02</td>
</tr>
<tr>
<td>Middle-aged</td>
<td>17</td>
<td>10</td>
<td>446 ± 9.2</td>
<td>452 ± 9.1</td>
<td>71.8 ± 3.5†</td>
<td>15.9 ± 0.8†</td>
<td>369.3 ± 8.8</td>
<td>81.7 ± 0.8†</td>
<td>10.9 ± 0.2</td>
<td>2.41 ± 0.02</td>
</tr>
<tr>
<td>Old</td>
<td>26</td>
<td>8</td>
<td>466 ± 12.4</td>
<td>475 ± 13.0</td>
<td>93.4 ± 6.8</td>
<td>19.5 ± 1.0</td>
<td>370.4 ± 7.8</td>
<td>78.1 ± 1.0</td>
<td>11.3 ± 0.3</td>
<td>2.38 ± 0.03</td>
</tr>
<tr>
<td>Old</td>
<td>29</td>
<td>8</td>
<td>475 ± 13.6</td>
<td>482 ± 14.0</td>
<td>96.5 ± 6.3</td>
<td>19.9 ± 0.8</td>
<td>373.7 ± 8.3</td>
<td>77.7 ± 0.8</td>
<td>11.5 ± 0.3</td>
<td>2.38 ± 0.03</td>
</tr>
</tbody>
</table>

### Notes:

Data (mean ± SEM) represent body composition from dual-energy x-ray absorptiometry (DEXA) at two scan time points. Young, middle-aged, and old rats were scanned initially (scan 1) and again in 3 months (scan 2); very young rats were scanned once only (scan 2). Body weights are reported as directly measured and as the sum of DEXA-derived values for fat mass, lean mass, and BMC. DEXA sum weights were used to calculate % fat, % lean, and % BMC. ANOVA was performed separately by scan time point. Statistical significance is set at $p < .008$ (Bonferroni-Dunn correction). Post hoc levels of significance (Sheffe F test) for scan 1: *$p < .0001$; †$p < .001$; ‡$p < .008$ (relative to old group [26 mo]); for scan 2: **$p < .0001$; ††$p < .001$ (relative to old group [29 mo]).
Age (months) and reported in Table 1 both as absolute weight (g) and as the percentage of total body weight (%). The DEXA weight (sum of DEXA values for fat and lean mass and BMC) was used for calculation of percentages of fat, lean, and BMC.

**Fat mass.**—Body fat mass increased from 24 g (9%) at 3 months to 96.5 g (20%) at 29 months of age. Both the absolute (g) and relative (%) amounts of fat were greater in the old group compared to all other ages.

**Lean mass.**—Absolute lean mass (g) was greater as age increased. Lean mass of the middle-aged group was not significantly different from the old at either scan 1 or scan 2; all other age comparisons were significant (p < .0001). Lean body mass, expressed as a percentage of total body weight, decreased from 89% at 3 months to 78% at 29 months. Old animals had a significantly lower percentage of lean mass when compared to the other age groups.

**Bone mineral content.**—Total bone mineral content (g) was lowest in very young (3 months) animals; highest in the larger and longer old animals. BMC of the middle-aged group was not significantly different from the old at either scan 1 or scan 2; all other between-age comparisons were significant (p < .0001). BMC (expressed as a percentage of weight) was highest for middle-aged animals; between-age comparisons were significant only for middle-aged versus young at scan 1, and versus very young at scan 2.

**Bone Mineral Density**

BMD (g/cm²) of the final four age groups (3, 8, 17, and 29 months) is shown in Figure 3 for whole body as well as specific skeletal regions. BMD was greater as age increased, globally and in all regions sampled (p = .0006 for distal femur; p < .0001 for all other sites); the density of bones in the very young group

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Figure 2. Body composition analysis by compartments determined from DEXA (fat and lean mass), and expressed as absolute weight (upper; g; mean ± SEM) and as percentage of total weight (lower; %; mean ± SEM). Data presented are from two separate scans: scan 1 (open symbols) was performed on 3 groups of rats (at 5, 14, and 26 months of age) and scan 2 (closed symbols) was done 3 months later, with a fourth group of very young animals (3 months of age) added at that time. Each age group is represented by a unique symbol (•, very young; ▲, young; ■, middle-aged; ○, old); solid lines indicate longitudinal changes within age groups. Error bars are present but difficult to resolve due to scale. See Table 1 for values and level of significance, relative to the old group.

Figure 3. Regional bone mineral density (g/cm²; mean ± SEM) of selected regions as determined by DEXA, using Regional High Resolution software and QDR-1500 scanner (Hologic, Inc, Waltham, MA), on four groups of anesthetized animals (very young, 3 mo; young, 8 mo; middle-aged, 17 mo; and old, 29 mo). Statistical significance from post hoc comparisons (Sheffe test) is indicated relative to the old (29 mo) group of animals. *p < .0001; †p < .001.
was consistently significantly lower than all other age groups in all areas except distal femur ($p < .0001$ for other sites). Density of bones in middle-aged and old rats (17 and 29 months) was not different for any region. There was no evidence of decreased BMD with advancing age in any region studied.

Longitudinal Changes Over 3 Months

Longitudinal changes between DEXA scan 1 and scan 2, which were performed on the same animals 3 months apart, are shown in Figure 2 (solid lines). Animals were initially 5, 14, and 26 months of age (young, middle-aged, and old, respectively).

Figure 4. Weights of dissected fat depots, expressed as absolute weight (upper; g, mean ± SEM) and as percentage of total fat from DEXA (lower; %, mean ± SEM) for the four age groups (very young, 3 mo; young, 8 mo; middle-aged, 17 mo; and old, 29 mo). Statistical significance from post hoc comparisons (Sheffe test) is indicated relative to the old (29 mo) group of animals. *$p < .0001$; †$p < .001$.

Table 2. Muscle Weights and Ratio of Muscle to Lean Body Mass From DEXA

<table>
<thead>
<tr>
<th>Age</th>
<th>n</th>
<th>Adductor longus (g)</th>
<th>Adductor longus/lean</th>
<th>Quadriceps (g)</th>
<th>Quadriceps/lean</th>
<th>n</th>
<th>Gastrocnemius (g)</th>
<th>Gastrocnemius/lean</th>
</tr>
</thead>
<tbody>
<tr>
<td>3 mo</td>
<td>10</td>
<td>.085 ± .003*</td>
<td>.035 ± .001</td>
<td>2.80 ± .06</td>
<td>1.160 ± .026*</td>
<td>10</td>
<td>2.02 ± .08</td>
<td>.836 ± .022*</td>
</tr>
<tr>
<td>8 mo</td>
<td>9</td>
<td>.104 ± .006†</td>
<td>.034 ± .002</td>
<td>3.27 ± .11</td>
<td>1.073 ± .031*</td>
<td>8</td>
<td>2.25 ± .08</td>
<td>.732 ± .016†</td>
</tr>
<tr>
<td>17 mo</td>
<td>10</td>
<td>.138 ± .013</td>
<td>.038 ± .003</td>
<td>3.62 ± .11*</td>
<td>.981 ± .025*</td>
<td>8</td>
<td>2.48 ± .09</td>
<td>.660 ± .018</td>
</tr>
<tr>
<td>29 mo</td>
<td>8</td>
<td>.147 ± .013</td>
<td>.039 ± .003</td>
<td>2.73 ± .15</td>
<td>.733 ± .042</td>
<td>8</td>
<td>2.21 ± .10</td>
<td>.590 ± .021</td>
</tr>
<tr>
<td>ANOVA</td>
<td></td>
<td>$p &lt; .0001$</td>
<td>$p = NS$</td>
<td>$p &lt; .0001$</td>
<td>$p = .0065$</td>
<td>$p &lt; .0001$</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Notes: Muscles (mean ± SEM) were dissected from left rear leg at sacrifice and weighed immediately. Ratios are relative to lean body mass derived from DEXA. Statistical significance is set at $p < .008$ (Bonferroni-Dunn correction). Levels of significance (relative to old group; Sheffe F test): *$p < .0001$; †$p < .001$.

Table 3. Estimated Levels of Visceral and Peripheral Fat and as Percentage of Total Fat Mass From DEXA

<table>
<thead>
<tr>
<th>Age</th>
<th>n</th>
<th>Total fat (g)</th>
<th>Visceral (g)</th>
<th>Visceral (% total fat)</th>
<th>Peripheral (g)</th>
<th>Peripheral (% total fat)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3 mo</td>
<td>10</td>
<td>23.9 ± 0.6*</td>
<td>5.7 ± 0.4*</td>
<td>23.9 ± 1.5</td>
<td>18.2 ± 0.6*</td>
<td>76.1 ± 1.5</td>
</tr>
<tr>
<td>8 mo</td>
<td>10</td>
<td>40.4 ± 1.3*</td>
<td>8.4 ± 0.8†</td>
<td>21.2 ± 2.7</td>
<td>32.0 ± 1.8*</td>
<td>78.8 ± 2.7</td>
</tr>
<tr>
<td>17 mo</td>
<td>10</td>
<td>71.8 ± 3.5†</td>
<td>13.1 ± 1.1</td>
<td>18.7 ± 1.7</td>
<td>58.7 ± 3.7†</td>
<td>81.3 ± 1.7</td>
</tr>
<tr>
<td>29 mo</td>
<td>8</td>
<td>96.5 ± 6.3</td>
<td>14.8 ± 1.0</td>
<td>15.5 ± 0.8</td>
<td>81.7 ± 5.6</td>
<td>84.5 ± 0.8</td>
</tr>
<tr>
<td>ANOVA (age)</td>
<td></td>
<td>$p &lt; .0001$</td>
<td>$p &lt; .0001$</td>
<td>$p = NS$ (.026)</td>
<td>$p &lt; .0001$</td>
<td>$p = NS$ (.026)</td>
</tr>
</tbody>
</table>

Notes: Fat depots (mean ± SEM) are presented in g and as percentage of total fat (from DEXA). Visceral fat was defined as the sum of visceral fat depots. Peripheral fat was defined as the difference between total fat (from DEXA) and the sum of visceral fat depots. Statistical significance is set at $p < .008$ (Bonferroni-Dunn correction). Post hoc level of significance (relative to old group; Sheffe F test): *$p < .0001$; †$p < .001$. 
Muscle weights of three leg muscles sampled in the final four age groups (3, 8, 17, and 29 months).

The ratio of adductor longus to lean body mass was virtually identical across age groups, with no significant differences.

Young and middle-aged animals gained similar amounts of weight (25 ± 3 g) over the 3 months, whereas the mean weight of the old animals did not change (p < .001 for old compared with young and middle-aged). Middle-aged animals gained five times more fat mass than old rats, whereas the young animals gained seven times more lean mass than the old rats.

Absolute amounts of fat mass gained over 3 months were 9 g in young, 15 g in middle-aged, and 3 g in old rats (ANOVA age, p = .003; old vs middle-aged, p = .003). As a percentage of total weight, fat mass increased nonsignificantly; the old animals gained less (0.4%) than young (1.6%) or middle-aged animals (2.5%).

Lean mass was greater as age increased, with young gaining more (22.3 g) than middle-aged (12.5 g) or old animals (3.4 g; ANOVA age, p = .0023; old vs young, p < .0024). Lean mass (as a percentage of total weight) decreased in all groups, as percentage fat increased. There were no significant differences among age groups in percentage of lean mass lost.

A significant effect of age was seen in BMC and percent BMC (p < .0001 and p = .0002, respectively), with young animals gaining significantly more (1 g; 0.8%) than middle-aged (0.4 g, -0.06%; p < .001) or old rats (0.2 g, p < .0001; 0.003%; nonsignificant).

Table 2 summarizes the analysis of absolute and relative weights of three leg muscles sampled in the final four age groups (3, 8, 17, and 29 months).

The adductor longus was heavier as age increased; old animals had significantly larger muscles than very young or young animals. The ratio of adductor longus to lean body mass was virtually identical across age groups, with no significant differences.

Quadriceps weight was significantly affected by age. From a peak at 17 months, mean weight was significantly lower (25%) at 29 months, with an average weight below that of the very young (3 months) animals. When expressed as a ratio, quadriceps to lean body mass significantly decreased with age, with the greatest decrease (25%) of muscle weights between middle-aged and old animals.

Gastrocnemius weight was lowest at 3 months, and highest at 17 months; decreasing by 11% in the old animals. When expressed as a ratio, gastrocnemius to lean body mass decreased significantly with age. Old animals had significantly lower relative gastrocnemius size than very young or young animals.

Adipose Tissue Distribution

Absolute weights of all fat depots (Figure 4) were significantly (p < .0001) heavier with increasing age. Fat pad weights of very young rats were all significantly lower than those of old animals, whereas for middle-aged animals, only the omental fat depot weighed significantly less than in the old animals. These age-related differences in fat pad weight disappeared when normalized to total fat mass (from DEXA) except for the epidydimal (ANOVA age, p < .0001) and mesenteric (ANOVA age, p < .0021) depots, which decreased in relative size with age.

Visceral fat depot weights were summed and used as an estimate of total “visceral fat mass,” while “peripheral fat mass” was estimated as the difference between total fat mass determined by DEXA and the sum of the visceral fat depots (Table 3). Total, “visceral,” and “peripheral” fat mass (g) were all greater with increasing age. Old animals had significantly more total and “peripheral” fat than other age groups, but levels of “visceral” fat were not different from those in middle-aged animals. “Visceral” fat accounted for 24% of total fat in 3 months old animals, and decreased as age increased, to a low of 15.5% in 29 months old animals. Concomitantly, “peripheral” fat increased with age from 76% in very young to 85.5% in old animals.

Table 4. Leptin Levels and Leptin Normalized to Body Weight and Fat Mass

<table>
<thead>
<tr>
<th>Age</th>
<th>n</th>
<th>Leptin (ng/mL)</th>
<th>Leptin/weight</th>
<th>Leptin/visceral</th>
<th>Leptin/peripheral</th>
</tr>
</thead>
<tbody>
<tr>
<td>3 mo</td>
<td>10</td>
<td>2.24 ± .19*</td>
<td>0.007 ± .001*</td>
<td>0.094 ± .008</td>
<td>0.390 ± .021*</td>
</tr>
<tr>
<td>8 mo</td>
<td>10</td>
<td>3.81 ± .40*</td>
<td>0.010 ± .001*</td>
<td>0.093 ± .008</td>
<td>0.487 ± .048</td>
</tr>
<tr>
<td>17 mo</td>
<td>10</td>
<td>8.71 ± .89</td>
<td>0.020 ± .002</td>
<td>0.123 ± .014</td>
<td>0.699 ± .084</td>
</tr>
<tr>
<td>29 mo</td>
<td>8</td>
<td>10.57 ± 1.08</td>
<td>0.023 ± .002</td>
<td>0.109 ± .009</td>
<td>0.700 ± .034</td>
</tr>
</tbody>
</table>

Notes: Leptin (mean ± SEM) was assayed from trunk blood of ad-libitum fed, nonfasted rats; sacrifice was between 1000 to 1500 h. Leptin is normalized either to body weight at time of sacrifice, to total fat (from DEXA), or to estimated visceral and peripheral fat. Statistical significance is set at p < .008 (Bonferroni-Dunn correction). Post hoc levels of significance (relative to old group, Sheffe F test): *p < .0001; †p < .001; ‡p < .008.

Leptin.—Leptin (Table 4) levels were greater as age increased; old animals had significantly higher serum leptin than very young or young groups. Similar patterns and levels of significance were seen for comparisons of leptin levels when normalized to body weight or “visceral fat mass.” In contrast, when leptin was normalized to total fat, or “peripheral fat mass,” significant between-age differences were abolished. Leptin and insulin were positively correlated (r = .62, p < .0001; n = 38); leptin was positively correlated (p < .0001 for all correlations) with...

Table 5. Serum Insulin, Glucose, and Testosterone Levels

<table>
<thead>
<tr>
<th>Age</th>
<th>n</th>
<th>Insulin (µU/mL)</th>
<th>Glucose (mg/dL)</th>
<th>Testosterone (ng/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3 mo</td>
<td>10</td>
<td>24.5 ± 4.2*</td>
<td>123.5 ± 2.3</td>
<td>2.71 ± .68</td>
</tr>
<tr>
<td>8 mo</td>
<td>10</td>
<td>27.3 ± 3.1*</td>
<td>113.9 ± 2.2</td>
<td>2.14 ± .42</td>
</tr>
<tr>
<td>17 mo</td>
<td>10</td>
<td>43.2 ± 2.1</td>
<td>116.7 ± 2.0</td>
<td>2.01 ± .30</td>
</tr>
<tr>
<td>29 mo</td>
<td>8</td>
<td>58.4 ± 13.7</td>
<td>125.0 ± 4.3</td>
<td>0.88 ± .13</td>
</tr>
</tbody>
</table>

Notes: Hormones and glucose (mean ± SEM) were assayed from trunk blood of ad-libitum fed, nonfasted rats; sacrifice was between 1000 and 1500 h. Statistical significance is set at p < .008 (Bonferroni-Dunn correction). *p = .01–.02. Post hoc comparisons (relative to old group) by Sheffe F test did not reach significance level as corrected by Bonferroni-Dunn.
body weight \( r = .80 \) and all measures of adiposity (fat \( r = .89 \)), percent fat mass \( r = .87 \), visceral fat \( r = .85 \), peripheral fat \( r = .84 \), and all fat pads \( r = .69 -.84 \)).

**Insulin, glucose, and testosterone.**—Insulin levels (Table 5) were greater with advancing age; levels of old animals were twice those of young, although there were no significant between-age comparisons. Insulin was positively correlated with all measures that correlated with leptin (total body weight and all fat measures), although at lower levels of significance than leptin \( p < .001 \). Nonfasted glucose levels were not significantly different across age groups. Testosterone (T; Table 5) levels were nonsignificantly lower with increasing age. The largest decrease in T levels (66%) occurred between 17 and 29 months of age. T was positively correlated with percent lean mass \( r = .41, p = .01 \) and adductor muscle weight \( r = .43, p = .01 \) and negatively correlated with peripheral fat \( r = -.41, p = .01 \).

**DISCUSSION**

In this study, body weight increased with age in male Brown Norway rats. Although this finding was expected, it indicates that the older animals were not ill, nor had they begun the wasting that would be expected in very old, senescent rats. The median life expectancy of male Brown Norway rats is 32 months, and maximum life span for males in this colony is 40 months (18). The age-related increase of body weight in these animals was due to increases in both absolute fat and lean body mass across the 26-month age span (3 to 29 months) in this study. When normalized to total body weight, however, percent fat mass increased, but percent lean mass decreased with age, a pattern similar to that seen in humans.

These male rats ranged from 9% to 20% fat and from 89% to 79% lean mass, in marked contrast to numerous studies of aging in other strains of rats, including F344, Long-Evans, and various strains of Sprague-Dawley rats, where body fat content is generally about twice as high in male rats of similar ages (8-11, 27-29). The increased percentage of fat we report is accompanied by decreased percentages of lean body mass, although again the Brown Norway rats in this study maintained higher levels of lean body mass than rats of similar age but different strains (8-11, 27-29), lending support to our belief that they represent a better model of healthy aging than these other strains of rats.

BMC and BMD of Brown Norway rats did not decline with advancing age as in humans (16,30,31). This may be because most rats grow throughout their life span and their epiphyses either do not close, or close late in life (26). We report that whereas BMC increased with age, differences became less pronounced between middle and old age, which is reflected by the relatively stable BMC as a percentage of body weight after 14 months. High-resolution DEXA scans of selected bone regions indicate that density (which is corrected for the size of the area scanned) increased most dramatically between 3 and 8 months, then remained relatively stable through adulthood (8-29 months). Despite the sedentary lifestyle of these individually housed rats, spine and proximal femur BMD did not decrease with age, in contrast to humans and larger animals that suffer from osteoporosis during senescence or inactivity. BMD of the distal femur and tibia was slightly but not significantly lower in the 29-month-old rats; it is not known if a significant loss of bone density would be detectable in these areas if studied at an older age. The continuous weight gain and moderate obesity with aging in rats may protect these animals from osteopenia by stimulating bone formation.

Longitudinal changes over the 3 months between DEXA scans indicate that young rats gain relatively more lean body mass until middle age, when fat mass increases rapidly. Old animals maintain a relatively stable weight, with lean mass decreasing as a percentage of total weight. This age-related decrease of lean body mass has been termed sarcopenia (32,33), indicating a specific loss of skeletal muscle. We dissected and weighed three muscles of the rear leg in order to determine if muscle wasting occurs with age in the male Brown Norway rat. The adductor longus was reported to be a muscle that does not suffer wasting with advancing age (34); this was verified in our study. The adductor longus was the only one of the three muscles that increased in size across all the ages studied, and the ratio of adductor longus weight to lean body mass was stable across the age groups. Weight-bearing muscles such as the quadriceps (thigh) and gastrocnemius (calf) decreased in absolute weight in the older animals, with the largest decreases between middle-aged and old groups, in parallel with the greatest decreases in testosterone levels. The ratio of the weights of these muscles to lean mass was dramatically decreased from 3 to 29 months of age. Age-dependent inactivity is believed to increase wasting in gastrocnemius and quadriceps (35). It may be that allowing access to exercise (e.g., swimming, treadmill, or running wheel) might protect against sarcopenia. Whereas there is evidence that exercise can slow the age-associated decrease in lean body mass (8,29,36), it must be acknowledged that responses of specific individuals or specific muscles vary according to the type and intensity of exercise and to concomitant alterations in food intake (34).

It is also possible that decreased testosterone levels in the aging animals contributes to muscle wasting. Testosterone levels were greatly decreased in the old animals, and we have consistently found lower testosterone levels in old male Brown Norway rats (20). Low testosterone is a hallmark of human aging and chronic illness (3,26,36), and is correlated with increased body fat and decreased lean body and skeletal muscle mass. Testosterone replacement may protect against muscle wasting and prevent fat accumulation with aging.

The distribution of body fat deposition shifts with aging in humans (2,4,5,37-40) and other animals (7,11,28,41). We dissected all discrete internal fat depots and two peripheral depots in order to examine differences in fat distribution in aging male Brown Norway rats. We were able to estimate the relative levels of visceral and peripheral fat mass, using a combination of DEXA for total fat and anatomical removal of specific fat depots, and we believe that our estimates of "peripheral" and "visceral" fat mass reflect relative visceral and peripheral adiposity. Young animals, although having significantly smaller amounts of fat than older animals, stored a larger proportion of their fat viscerally. With aging, the proportion of peripheral to total fat mass increased, while the proportion of visceral fat decreased.

In humans, the age-associated increases in body fat are primarily due to increased intra-abdominal fat throughout middle-age (2,39), whereas subcutaneous fat depots shift towards upper body accumulation and away from gluteofemoral accumulation in men (4,6,38,42,43). Few studies assess intra-abdominal fat mass by computed tomography (CT) in older people, and the number of subjects is necessarily small (39), although DEXA...
has been used to assess fat patterning in larger numbers of old and very old men (4–6). These studies suggest that the levels of absolute fat (20–36%), truncal fat (23–30%), and abdominal fat (14–16%) are similar to those measured in our old animals. It is likely that the generally high fat diet (> 30% kcal from fat) of industrialized societies favors the accumulation of visceral fat (12,44). The rats in the current study were eating rodent chow with 12% of kcal from fat, and it is possible that increasing the percentage of calories from fat would have increased their visceral adiposity towards levels seen in aging humans.

Leptin is the recently described (45,46) protein produced by adipocytes and believed to regulate adipose levels through central effects on food intake and energy expenditure (47–49). It is clear that leptin levels increase with adiposity. However, it is unclear whether levels of leptin increase with age. We found that absolute leptin levels increase with age in male Brown Norway rats, but when normalized to total body fat mass, these age-related differences are no longer present, suggesting that increases in leptin with age are related mostly to increases in fat mass. We used our estimates of visceral and peripheral fat to further examine the contribution of the fat pools to leptin production, by analyzing the ratio of leptin levels to visceral and to peripheral fat. When leptin levels are normalized to visceral fat mass, age-related increases persist, but when normalized to peripheral fat mass, no significant age-related differences are found. These findings suggest that increased peripheral (subcutaneous) fat deposition with increasing age is the major contributor to the higher leptin levels observed in our older animals. Reports that leptin protein and mRNA are higher in subcutaneous than visceral tissue (50,51) and that leptin levels are better correlated with subcutaneous than visceral fat mass in humans (52–54) support these observations. It is also likely that the increased levels of leptin that have been observed in females (55–58) are related to their higher levels of subcutaneous fat.

Both leptin and insulin levels increase with age, whereas testosterone levels decline. Age-related increases in serum insulin are generally due to increased insulin resistance in peripheral target tissues and are associated with increased adiposity in humans (14) and animals (11). On the other hand, centrally administered insulin has been shown to decrease food intake and body weights of young adult experimental animals (59,60), and to inhibit fasting-induced NPY content and mRNA expression when administered to hypothalamic sites involved in energy regulation (61,62). In previous (20,21) studies we have found that aged male Brown Norway rats have lower food intake relative to body weight during free feeding, and show blunted compensatory feeding responses to a 72-hour fast. Regaining of body weight after fasting is also impaired in old compared to younger animals. These physiological responses are associated with lower NPY content and mRNA in the arcuate nucleus of the hypothalamus, and with increased serum insulin levels (21). We speculate that the increased insulin (and perhaps leptin) in our old animals may contribute to a dysregulation of energy balance that occurs with age, leading to impairment of fasting-induced refeeding.

In summary, we have shown, using a combination of DEXA, anatomical measurements, and hormone measurements, that aging male Brown Norway rats undergo shifts in body composition and the hormonal milieu, that are mostly consistent with those seen in aging humans. Although increases in fat as a percentage of body weight occur between middle and old age both in humans and the rats in this study, we have shown that the deposition of this fat is predominantly peripheral in male Brown Norway rats. Concomitant with these changes in fat distribution are losses of lean body mass and muscle, and these are observed as early as middle age (14–17 months), whereas testosterone levels do not decline dramatically until after middle age in this model of aging. We have also observed that leptin levels, in addition to insulin, increase with age in this rat model, and that this increased leptin is likely to be due to increased levels of peripheral fat. These findings suggest that alterations in body composition with aging may be due to decreased trophic (e.g., testosterone, growth hormone) and increased lipotropic (e.g., insulin) hormones. We conclude that the aging Brown Norway rat is a good model in which to further investigate the mechanisms underlying age-related alterations in the regulation of body composition.

REFERENCES
