Food restriction suppresses muscle growth and augments osteopenia in ovariectomized rats

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Food restriction suppresses muscle growth and augments osteopenia in ovariectomized rats. J. Appl. Physiol. 88: 265–271, 2000.—We examined effects of 4 wk of food restriction on ovariectomy-related changes in muscle, bone, and plasma insulin-like growth factor I (IGF-I). Female Sprague-Dawley rats (7 mo old) were assigned to freely eating groups: sham-operated (Sham), ovariectomized (Ovx-AL), and estrogen (estradiol)-replaced Ovx (Ovx-E2). Ovx rats were also pair fed with Sham (Ovx-PF) or weight matched with Sham by food restriction (Ovx-FR). Ovx-AL and Ovx-PF rats had similar estrogen status and body weight; therefore, the groups were combined (group: Ovx). After treatment, body weight was ~10% greater in Ovx than in Sham rats (P < 0.05), and muscle weight-to-body weight ratios were comparable among all groups. Bone mineral contents of whole tibiae in Ovx-FR and Ovx were ~15% (P < 0.05) and ~6% lower than in Sham rats (P < 0.05), respectively. Plasma IGF-I was ~30% higher in Ovx than in Sham (P < 0.05) but was similar between Sham and Ovx-FR. IGF-I was highly correlated with body weight and muscle mass. Within non-estrogen-replaced Ovx rats, IGF-I explained ~19% of variance in bone mineral content after accounting for variance attributable to body weight. Findings suggest that estrogen acts indirectly on skeletal muscle and bone in rats through regulation of body growth by factors such as IGF-I.

estradiol; myofibrillar protein; food restriction; insulin-like growth factor I

THE ESTROGEN RECEPTOR has been identified in skeletal muscle (12, 33), but its role in the control of muscle gene expression has not been elucidated. In humans, both cross-sectional and longitudinal studies have found an association between menopause and an accelerated decline in lean body mass, implicating a role of the decline in estrogen production at menopause in the failure to maintain lean tissues (1, 31). In addition, there is evidence that hormone replacement therapy (HRT) in postmenopausal women may help preserve muscle strength (21, 29), although muscle mass was not measured in these studies.

Subsequent to ovariectomy in rats, increased food intake, decreased physical activity, and elevated plasma growth hormone (GH) and insulin-like growth factor I (IGF-I) concentrations (7, 22, 38) contribute to an increase in body weight and organ weights, including muscle mass (6, 32). Increased muscle mass in ovariectomized (Ovx) animals has led some investigators to suggest that estrogen inhibits skeletal muscle growth (25, 34). However, muscle weight-to-body weight ratios were similar in control rats, Ovx animals, and Ovx rats with estrogen replacement (6, 32, 42). This suggests that the increased muscle mass in Ovx animals compared with controls (3, 25) was related to increased IGF-I/GH activity rather than to the direct effects of sex hormone deficiency on skeletal muscle.

Bone mineral content (BMC) and bone mineral density (BMD) have been shown to decline in Ovx rats in comparison to control animals, because bone resorption exceeds formation (23). It has been reported that freely eating Ovx rats suffered less bone loss than did food-restricted Ovx animals, suggesting that freely eating Ovx animals were partially protected from bone loss by their greater body weight (39).

The purpose of this study was to examine the effects of ovarian hormones on hindlimb muscle mass, muscle protein content, tibial BMC and BMD, and plasma IGF-I concentration. On the basis of preliminary findings, we expected that fractional muscle protein content would be similar in control and Ovx animals. We therefore hypothesized that there would be no change in muscle mass in Ovx animals if ovariectomy-related increase in body weight were prevented by food restriction. Furthermore, we postulated that body weight, muscle mass, and bone measurements would be related to plasma IGF-I concentrations.

Methods

Animals. Adult (7-mo-old) female Sprague-Dawley rats (Harlan Bioproducts, Indianapolis, IN) were allowed 1 wk to acclimate to the animal facility after their delivery. Rats were divided into five groups: sham-operated controls (Sham; n = 12); ovariectomized and pair fed with weight-matched Sham rats (Ovx-PF; n = 5); ovariectomized and fed ad libitum (Ovx-AL; n = 6); ovariectomized and food restricted to match body weight of Sham (Ovx-FR; n = 7); and ovariectomized, freely eating, estrogen (estradiol) replaced (Ovx-E2; n = 9). Rats were housed one animal per cage in a light-controlled (12:12-h light-dark cycle), constant temperature (22°C), virus-free room. Food for all animals was standard, pelleted rat chow (PicoLab Rodent Diet 20). Rats were allowed free access to chlorinated water. Animals were free to move about their cages, but their activity was not monitored.

All animal procedures were approved by the Animal Use Review Board of Washington University and were performed...
Food restriction prevents ovariectomy-related growth

According to the recommendations of the Guiding Principles in the Care and Use of Animals from the American Physiological Society.

Pair feeding and food restriction. Sham, Ovx-AL, and Ovx+E₂ animals were fed ad libitum, and food intake was monitored approximately every 3 days. Ovx-PF animals were paired with Sham animals of similar body weight before surgery, and the amount of food eaten by the Sham animals was fed to their Ovx-PF partners. Ovx-FR animals were initially given ~80% of the amount of food that Sham rats ate, and the amount of food given was adjusted approximately every 3 days to keep the mean body weight of Ovx-FR similar to that of Sham animals.

Ovariectomy. Animals were anesthetized (80 µl/100 g body wt) with a mixture of ketamine HCl (75 mg/ml) and promazine HCl (12.5 mg/ml). Ovariectomy was performed through bilateral flank incision. Ovarian blood supply was ligated, and ovaries were sectioned from the uterine horns and removed. For sham operation, ovaries were accessed through flank incision, lifted out of the animal, and replaced. Muscle and skin incisions were sewn separately with 4.0 silk.

Estrogen injections. Ovx+E₂ rats were injected subcutaneously with estradiol benzoate in olive oil (5 µg/E₂/100 µl oil, 1 µl solution/g body wt) on days 7, 14, and 21 postsurgery, a dose similar to that of Gaumet et al. (18). All other animals were injected weekly with 1 µl/g body wt of vehicle.

Tissue analyses. Four weeks postsurgery, animals were anesthetized with pentobarbital sodium (50 mg/ml, 0.15 ml/100 g body wt). Soleus (Sol), plantaris (Pla), peroneus longus (Per), extensor digitorum longus (EDL), gastrocnemius (Gast), and quadriceps (Quad) were quickly removed, weighed (wet weight), and frozen in liquid nitrogen. Animals were killed by removal of the heart. The uterus and heart of each animal were then weighed. Tissues were stored at −80°C until analysis.

Muscle samples (~25 mg) from representative antagonists (Sol), locomotor (Pla), and nonpostural muscles (Per, EDL) were diluted 1:20 in ice-cold buffer (250 mM sucrose, 100 mM KCl, and 5 mM EDTA, pH 6.8) and homogenized with a Kontes glass tissue grinder. Myofibrillar proteins were isolated as described by Thomason et al. (35) with slight modifications. Homogenates were centrifuged for 5 min at 7,000 × g, and supernatants were discarded. Pellets were twice resuspended in a washing solution (175 mM KCl, 2 mM EDTA, and 0.5% Triton X-100, pH 6.8) and spun to remove mitochondrial proteins. Triton X-100 was removed from pellets with two further washes and spins (150 mM KCl and 20 mM Tris, pH 7.0). Final pellets were dissolved in 40 mM Na₃P₂O₇, pH 8.8. SDS-PAGE analysis showed that discarded supernatants from the myofibrillar protein isolation procedure did not contain myosin heavy chain (data not shown). Total protein and myofibrillar protein were assayed with the bicinchoninic acid method (Sigma Chemical, St. Louis, MO).

IGF-I. Blood samples were obtained from the abdominal aorta and centrifuged in heparin-coated tubes. Blood samples were taken from only 27 of the 39 animals (see Fig. 2 for numbers of animals in each group). Plasma was stored at −80°C until analysis. Plasma IGF-I concentrations were determined by competitive radioimmunoassay with a modified method of Daughaday et al. (13). Plasma samples were diluted 1:5 in acid ethanol (0.25 N formic acid in ethanol), left at room temperature for 30 min, and centrifuged. Supernatants were diluted with 0.5 M phosphosaline buffer, pH 7.4, and samples were assayed in duplicate. Samples were incubated in phosphosaline buffer with ¹²⁵I-labeled IGF-I and rabbit anti-IGF-I antibodies (UB3–189, National Hormone and Pituitary Program, Baltimore, MD) for 18 h at 4°C. Goat anti-rabbit IgG (Linco Research, St. Charles, MO) and normal rabbit serum (Pel-Freeze Biologicals, Rogers, AR) were added, followed by a 2-h incubation at 4°C, centrifugation, and determination of radioactivity in pellets by an Isomedic Systems 10/600 Gamma Counter. Standard curves were prepared by assay of known concentrations of purified IGF-I (R&D Systems, Minneapolis, MN).

Bone measurements. After the animals were killed, tibiae were disarticulated at the ankle and knee, separated from fibulae, cleared of soft tissue, and stored in Ringer solution at −20°C until analysis. Bones were scanned with a Hologic QDR-1000w dual-energy X-ray absorptiometry machine at high resolution (point resolution 0.02 mm). Bones were scanned while in a rectangular, Plexiglas trough filled to a depth of ~2.5 cm with distilled water. Whole bones were scanned, and bones were positioned such that the anterior side faced down and the tibial head was scanned first. Bone measurements obtained were BMC (g) and BMD (g/cm²). The ratio of BMC to muscle mass was computed by using the combined muscle mass of all six skeletal muscles examined. All of these muscles either attach to (Sol, Per, Quad) or span (Pla, Gast, EDL) the tibia.

Statistics. Differences between group means were analyzed with a one-way ANOVA, α = 0.05. If the ANOVA was significant, post hoc comparisons were made between Sham and all other groups with a one-tailed Dunnett’s test (α = 0.05). Body weights were analyzed as change scores from initial to final weights.

The relationship of body weight and mass of each muscle to IGF-I was determined by regression analysis (α = 0.05). The independent relationship of IGF-I to muscle mass was determined by an analysis of covariance (α = 0.05), with muscle mass as the dependent variable, IGF-I concentration as the independent variable, and animal group and body weight as covariates. The relationship between IGF-I and bone variables within non-estrogen-replaced Ovx rats was determined by analysis of covariance with body weight as the covariate (α = 0.05).

All data are presented as means ± SE.

Results

Uterine weight. Uterine weights were higher in Sham animals (785 ± 41 mg; P < 0.05) than in all other groups, including Ovx+E₂ (513 ± 47 mg). Success of ovariectomy was confirmed by low uterine weights in Ovx-AL (248 ± 58 mg), Ovx-PF (221 ± 63 mg), and Ovx-PF rats (189 ± 54 mg).

Body weight and food intake. The increase in body weights in Ovx-AL (52 ± 4 g) and Ovx-PF (44 ± 4 g) were greater (P < 0.05) than the weight gain in Sham rats (20 ± 3 g; Fig. 1). The weight gains in Ovx-FR (21 ± 4 g) and Ovx+E₂ (14 ± 3 g) were not different from the change in Sham rats.

Even though body weights were comparable, food eaten by Ovx-FR (13.4 ± 0.4 g/day) was 80% that of Sham (16.7 ± 0.3 g/day; P < 0.05). There were no significant differences in food intake between Sham and Ovx-PF (16.8 ± 0.5 g/day), Ovx+E₂ (16.8 ± 0.4 g/day), or Ovx-AL rats (17.7 ± 0.5 g/day).

Muscle mass and muscle weight-to-body weight ratio. Muscle weights were expressed as the average weight of the left- and right-side muscles from each animal (Table 1). We expected that differences in muscle weight and bone measurements would be related to differences in body weight. Because body weights and ovarian...
hormone status were similar in Ovx-PF and Ovx-AL rats, we combined the data for these two groups under the group heading Ovx.

Pla, Per, and heart mass were significantly larger in Ovx than in Sham rats (P < 0.05). There were no other statistically significant differences in muscle mass between Ovx and Sham rats. Muscle mass was similar to Sham in both Ovx-FR and Ovx+E2 rats. The ratio of muscle mass to body weight was comparable between Sham and all other groups for all muscles (Table 2).

Muscle protein content. There were few differences between Sham and other groups for Sol, Pla, Per, and EDL total (mg/g) or myofibrillar fractional protein content. However, Pla protein content (Table 3) was lower in Ovx than in Sham rats (P < 0.05), and Per total protein and myofibrillar protein content were lower in Ovx-FR than in Sham rats (P < 0.05).

Table 1. Muscle mass

<table>
<thead>
<tr>
<th></th>
<th>Ovx</th>
<th>Sham</th>
<th>Ovx-FR</th>
<th>Ovx+E2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soleus</td>
<td>130 ± 3</td>
<td>126 ± 3</td>
<td>125 ± 4</td>
<td>122 ± 4</td>
</tr>
<tr>
<td>Plantaris</td>
<td>363 ± 8*</td>
<td>330 ± 7</td>
<td>320 ± 10</td>
<td>321 ± 9</td>
</tr>
<tr>
<td>Gastrocnemius</td>
<td>1,698 ± 40</td>
<td>1,607 ± 37</td>
<td>1,526 ± 48</td>
<td>1,535 ± 43</td>
</tr>
<tr>
<td>Peroneus</td>
<td>59 ± 4*</td>
<td>148 ± 4</td>
<td>136 ± 5</td>
<td>144 ± 4</td>
</tr>
<tr>
<td>EDL</td>
<td>121 ± 3</td>
<td>115 ± 3</td>
<td>118 ± 3</td>
<td>111 ± 3</td>
</tr>
<tr>
<td>Quadriceps</td>
<td>2,748 ± 80</td>
<td>2,591 ± 75</td>
<td>2,612 ± 98</td>
<td>2,479 ± 86</td>
</tr>
</tbody>
</table>

All 6 skeletal muscles combined

| Heart    | 903 ± 22*    | 829 ± 21    | 805 ± 27    | 807 ± 24    |

Values are means ± SE given in mg. Ovx, combined freely eating and pair-fed, ovariectomized groups; Sham, sham-operated controls; Ovx-FR, food restricted, ovariectomized; Ovx + E2, ovariectomized, estrogen (estradiol) replaced; EDL, extensor digitorum longus. *Significantly greater mass than Sham, P < 0.05.

Table 2. Muscle weight-to-body weight ratios

<table>
<thead>
<tr>
<th></th>
<th>Ovx</th>
<th>Sham</th>
<th>Ovx-FR</th>
<th>Ovx+E2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soleus</td>
<td>0.442 ± 0.014</td>
<td>0.460 ± 0.014</td>
<td>0.459 ± 0.018</td>
<td>0.456 ± 0.016</td>
</tr>
<tr>
<td>Plantaris</td>
<td>1.198 ± 0.041</td>
<td>1.202 ± 0.039</td>
<td>1.155 ± 0.052</td>
<td>1.159 ± 0.046</td>
</tr>
<tr>
<td>Gastrocnemius</td>
<td>5.527 ± 0.206</td>
<td>5.852 ± 0.199</td>
<td>5.489 ± 0.260</td>
<td>5.659 ± 0.230</td>
</tr>
<tr>
<td>Peroneus</td>
<td>0.518 ± 0.018</td>
<td>0.536 ± 0.017</td>
<td>0.494 ± 0.022</td>
<td>0.530 ± 0.020</td>
</tr>
<tr>
<td>EDL</td>
<td>0.394 ± 0.008</td>
<td>0.418 ± 0.008</td>
<td>0.432 ± 0.011</td>
<td>0.411 ± 0.009</td>
</tr>
<tr>
<td>Quadriceps</td>
<td>9.034 ± 0.259</td>
<td>9.259 ± 0.251</td>
<td>9.846 ± 0.328</td>
<td>9.276 ± 0.289</td>
</tr>
</tbody>
</table>

All 6 skeletal muscles combined

| Heart | 16.83 ± 0.324 | 17.76 ± 0.308 | 17.67 ± 0.404 | 17.35 ± 0.356 |

Values are means ± SE given in mg/g. There were no significant differences among groups.

Fig. 1. Body weights of rats. Ovx, ovariectomized; Ovx+E2, estrogen (estradiol)-replaced Ovx. Bars, SE. *Significantly greater mass than Sham, P < 0.05.

Bone measurements. BMC was ~15% lower in Ovx-FR than in Sham (P < 0.05) but only ~6% lower (not statistically significant) in Ovx than in Sham (Table 4). BMD was ~8 and ~5% lower in Ovx-FR and Ovx, respectively, than in Sham rats (P < 0.05). The ratio of BMC to combined muscle mass was 11.6 and 13.9% lower in Ovx and Ovx-FR, respectively, than in Sham rats (P < 0.05). There were no differences in bone measurements between Sham and Ovx+E2 groups.

IGF-I. As previously mentioned, estrogen status, body weight, and food eaten by Ovx-AL and Ovx-PF rats were similar, so IGF-I data were pooled for these groups under the heading Ovx. (Plasma IGF-I concentrations were similar in Ovx-AL and Ovx-PF animals.) Plasma IGF-I concentrations (Fig. 2) were ~30% higher in Ovx than in Sham animals (P < 0.05). Plasma IGF-I concentrations were not different between Sham and Ovx-FR or Ovx+E2.

Plasma IGF-I concentration was significantly correlated (P < 0.05) with body weight (r = 0.67) and muscle mass for Pla (r = 0.65), Gast (r = 0.58), EDL (r = 0.48), Quad (r = 0.42), heart (r = 0.55), and the combined mass of all six skeletal muscles studied (r = 0.55, Fig. 3). Correlations between IGF-I and muscle mass for Sol.

Table 3. Muscle total protein, myofibrillar protein, and myofibrillar protein as percentage of total protein

<table>
<thead>
<tr>
<th></th>
<th>Ovx</th>
<th>Sham</th>
<th>Ovx-FR</th>
<th>Ovx+E2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soleus</td>
<td>173 ± 3</td>
<td>174 ± 3</td>
<td>167 ± 4</td>
<td>169 ± 4</td>
</tr>
<tr>
<td>Myofibrillar, mg/g</td>
<td>84 ± 4</td>
<td>82 ± 3</td>
<td>79 ± 4</td>
<td>81 ± 4</td>
</tr>
<tr>
<td>%Total</td>
<td>49 ± 2</td>
<td>47 ± 2</td>
<td>47 ± 2</td>
<td>48 ± 2</td>
</tr>
<tr>
<td>Plantaris</td>
<td>186 ± 3*</td>
<td>200 ± 3</td>
<td>195 ± 4</td>
<td>198 ± 3</td>
</tr>
<tr>
<td>Myofibrillar, mg/g</td>
<td>100 ± 3</td>
<td>101 ± 2</td>
<td>93 ± 3</td>
<td>98 ± 3</td>
</tr>
<tr>
<td>%Total</td>
<td>54 ± 1</td>
<td>51 ± 1</td>
<td>48 ± 2</td>
<td>50 ± 1</td>
</tr>
<tr>
<td>Peroneus</td>
<td>195 ± 4</td>
<td>201 ± 4</td>
<td>184 ± 5*</td>
<td>203 ± 5</td>
</tr>
<tr>
<td>Myofibrillar</td>
<td>107 ± 3</td>
<td>111 ± 3</td>
<td>94 ± 4*</td>
<td>110 ± 3</td>
</tr>
<tr>
<td>%Total</td>
<td>55 ± 1</td>
<td>55 ± 1</td>
<td>51 ± 1</td>
<td>54 ± 1</td>
</tr>
<tr>
<td>EDL</td>
<td>211 ± 4</td>
<td>205 ± 4</td>
<td>205 ± 5</td>
<td>208 ± 5</td>
</tr>
<tr>
<td>Myofibrillar, mg/g</td>
<td>105 ± 3</td>
<td>106 ± 3</td>
<td>103 ± 4</td>
<td>108 ± 4</td>
</tr>
<tr>
<td>%Total</td>
<td>50 ± 2</td>
<td>52 ± 2</td>
<td>50 ± 2</td>
<td>52 ± 2</td>
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</table>

Values are means ± SE. *Significantly less than Sham, P < 0.05.
and Per (r = 0.23) were not statistically significant. IGF-I did not explain any variance in muscle mass beyond that explained by animal group and body weight.

Within non-estrogen-replaced Ovx rats, IGF-I explained ~19% more variance in BMC beyond that explained by body weight alone (P < 0.05, Table 5). IGF-I did not explain a statistically significant portion of variance in BMD beyond that explained by body weight.

**DISCUSSION**

The presence of estrogen receptors in skeletal muscle suggests that skeletal muscle is a target tissue for estrogen (12, 33). Whether estrogen plays a role in the regulation of muscle mass is controversial (9, 17, 21). For example, estrogen has an anabolic effect on muscle in growing farm animals (36), but it appears to inhibit muscle development in growing (3-mo-old) rats (25, 34). In humans, there appears to be an accelerated rate of decline in lean body mass at menopause (1, 31) that may be attenuated but is not reversed by HRT (2, 17). The present study was undertaken to examine the effects of estrogen on skeletal muscle mass in rats while controlling for the influence of body weight. When we prevented the usual increase in body weight that occurs in response to ovariectomy, there were no differences in muscle mass between Sham and Ovx rats.

Muscle mass and body weights were larger in Ovx-AL rats than in Sham animals, but muscle mass-to-body weight ratios were not different. Other investigators have found that body weight increases after ovariectomy without a change in percentage of body fat in young adult (up to ~4-mo-old) animals (11). Ovariectomy may therefore promote general growth of the entire rat body. For example, Booth and Tipton (6) found that muscle mass and organ weights increased approximately in parallel with body weight in Ovx animals (~3 mo old). It therefore seems likely that differences that we and others have observed in muscle mass between Ovx and Sham animals (6, 25, 32, 34, 42) are related to differences in body growth rate.

Despite a similar food intake, Ovx-PF animals gained markedly more weight than did Sham animals and nearly as much as Ovx-AL animals. Therefore, factors other than food intake must be responsible for weight gain in Ovx animals. A drop in spontaneous cage activity in Ovx rats not receiving estrogen replacement has been reported by others (38) and is probably partially responsible for differences in energy balance between Ovx and Sham animals. An additional factor that might influence energy balance is the reported

<table>
<thead>
<tr>
<th>Table 4. Tibia measurements</th>
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<tr>
<td><strong>BMC, g</strong></td>
</tr>
<tr>
<td>Ovx</td>
</tr>
<tr>
<td>Sham</td>
</tr>
<tr>
<td>Ovx-FR</td>
</tr>
<tr>
<td>Ovx + E₂</td>
</tr>
</tbody>
</table>

Values are means ± SE. BMC, bone mineral content; BMD, bone mineral density; BMC/muscle mass, ratio of BMC to combined muscle mass. Muscle mass in the BMC/muscle mass ratio was the combined muscle mass of all six skeletal muscles examined. All of these muscles either attach to (soleus, peroneus longus, quadriceps) or span (plantaris, gastrocnemius, EDL) the tibia.* Significantly lower value than in Sham, P < 0.05.

<table>
<thead>
<tr>
<th>Table 5. Relationship of plasma IGF-I concentration to tibial bone mineral content and bone mineral density within non-estrogen-replaced Ovx rats</th>
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<tr>
<td><strong>R²</strong></td>
</tr>
<tr>
<td>BMC</td>
</tr>
<tr>
<td>BMD</td>
</tr>
</tbody>
</table>

Values are from 15 rats. IGF-I, insulin-like growth factor I; R², portion of variance explained in multiple regression analysis. *IGF-I accounted for a significant portion of variance beyond that explained by body weight (P < 0.05, analysis of covariance).
downregulation of the Na\(^+\)-K\(^+\) pump by ovariectomy and its upregulation by estrogen (14). Because energy expenditure by the Na\(^+\)-K\(^+\) pump has been suggested to be an important contributor to resting metabolic rate (30), it seems plausible that metabolic rate in rats declines after ovariectomy. Energy conserved by decreases in cage activity and resting metabolic rate could contribute to the pool of energy available for growth in Ovx rats.

Plasma IGF-I concentrations, which were measured as an index of stimulation of the growth hormone/IGF-I axis, were ~30% higher in Ovx rats than in controls. However, the increase in plasma IGF-I concentration in Ovx rats was markedly attenuated by food restriction. Long-term food restriction in rats has previously been shown to reduce plasma IGF-I and GH concentrations (4, 8). Because Ovx-PF had higher plasma IGF-I levels than did controls in this study, blood IGF-I levels may be more related to energy balance than to food intake per se. Estrogen, therefore, may downregulate IGF-I expression through two pathways: through direct influence on tissues such as bone and liver (5, 7) or indirectly through regulation of energy balance. IGF-I may be only one of several possible factors, such as greater insulin levels in Ovx rats compared with controls (19), that might contribute to differences in body weight in estrogen-sufficient and estrogen-deficient animals. However, given the suggested importance of the GH/IGF-I axis in the regulation of growth rate (20), it appears possible that the prevention of increase in plasma IGF-I by food restriction in the present study may also have prevented the usual increase in growth rate in Ovx rats.

Apparent species differences for the effects of estrogen on lean mass (e.g., skeletal muscle) (17, 34, 36) are probably dependent on differences in response of the GH/IGF-I axis or sensitivity of tissues to changes in GH/IGF-I with alterations in circulating estrogen. Estrogen replacement causes a decrease in plasma concentrations of IGF-I in both postmenopausal women and Ovx rats (7, 15). However, estrogen treatment has been reported to increase GH release in prepubertal girls and postmenopausal women (15, 28), whereas upregulation of GH expression in the rat occurs after removal of estrogen by ovariectomy (22). In healthy humans, neither GH nor IGF-I treatments have been found to affect muscle protein synthesis (40). However, the greater mean and maximum circulating levels of growth factors in Ovx rats compared with controls may channel energy toward body growth (22), as opposed to fat storage.

Net bone loss in Ovx rats and postmenopausal women is qualitatively similar (23). However, because GH release appears to be regulated in opposite directions by estrogen in postmenopausal women and Ovx rats (15, 22), there may also be differences in bone metabolism between postmenopausal women and Ovx rats. Estrogen and GH effects on bone are additive and act by inhibition of resorption (estrogen) and stimulation of formation (GH) (41). In the Ovx rat, increased GH could directly and indirectly (through increased body weight) counteract the deleterious effects of estrogen removal on bone. In postmenopausal women, on the other hand, estrogen insufficiency and the related decline in GH release could have additive, negative effects on BMC. Conversely, HRT in postmenopausal women and the corresponding increase in GH release (15) could each have a positive impact on bone metabolism.

BMC was statistically lower in Ovx-FR than Sham but not in Ovx compared with Sham animals. Other investigators have suggested that the higher body weight in Ovx controls compared with Ovx-FR animals provided partial protection against bone loss (39). However, we have shown in the present study a second possible explanation for the partial protection against bone loss in Ovx animals. Plasma IGF-I concentrations and presumably GH concentrations (22) were higher in Ovx than in Ovx-FR animals. Because both GH and IGF-I have been found to stimulate osteoblast activity in vitro (10), increased IGF-I and/or GH concentrations in Ovx rats compared with Ovx-FR rats may have been responsible for the seemingly different rate of bone mineral loss between the two groups. In fact, within non-estrogen-replaced Ovx animals, plasma IGF-I concentration accounted for ~19% more variance in BMC than that explained by body weight. Whereas other investigators have previously reported that IGF-I and GH treatments both help prevent osteopenia in Ovx rats, the reported effects of IGF-I and GH were probably partially mediated by the increased body weights observed in those IGF-I- and GH-supplemented Ovx rats (37). In the present study, there appears to be a positive association with IGF-I and BMC, separate from any effects of differences in body weight on BMC.

We must acknowledge that whole tibia measurements might obscure site-specific changes in bone, such as the greater ovariectomy-related bone loss in cancellous bone compared with cortical bone (24). However, even in cancellous bone, bone formation and bone mineral apposition have been found to increase after ovariectomy in rats, despite net trabecular bone loss (42). This finding is consistent with an increase in GH in Ovx rats and the anabolic effect of GH on cancellous bone (41). For example, supplemental GH was found to completely abolish bone loss in proximal tibial trabecular bone of Ovx rats (37) and increase cancellous bone volume in hypophysized Ovx rats (41). Of particular relevance to the present study, Wronsiki et al. (39) compared proximal tibial trabecular bone loss in control, Ovx, and food-restricted Ovx animals. They found that, after 14 wk, Ovx rats lost ~30% less trabecular bone volume than did Ovx-FR rats (compared to ~60% less whole bone BMC loss in Ovx than in Ovx-FR rats in the present study). Thus our whole bone measurements may underestimate changes in cancellous bone metabolism after Ovx with or without food restriction, but the underlying mechanisms (increased resorption secondary to estrogen deficiency; increased formation, possibly related to increased GH in Ovx rats but not food-restricted Ovx rats) are probably reflected by changes in whole bone measurements.
A weakness of the present study is the possibility that tissue-level and systemic IGF-I expressions are different. Fortunately, however, the pattern of systemic IGF-I expression in the present study (Sham = Ovx+<sub>E2</sub> < Ovx) is the same as the reported femur IGF-I mRNA expression 24 h after vehicle or E<sub>2</sub> injections (5). In the same study (5), after 3 wk of vehicle treatment, there was a 40% greater plasma IGF-I expression in Ovx rats than in Sham rats (very similar to the magnitude of difference in the present study). However, plasma IGF-I was only 10% lower in Ovx to the magnitude of difference in the present study). In conclusion, we prevented the usual ovariectomy-related growth of BMC to muscle mass was 11.6 and 13.9% lower in Ovx-AL and Ovx-FR rats, respectively, compared with sham-operated controls. It has been suggested that, in humans, postmenopausal decline in bone mass may be secondary to changes in muscle (29); i.e., strength may decline at menopause such that muscle exerts less mechanical stimulus on bone. In rats, by contrast, decline in bone density has been observed subsequent to ovariectomy even though muscle mass increased (42). In the present study, there appears to be a similar, estrogen-related separation between muscle mass and BMC. It has been proposed by Frost (16) that estrogen lowers the set point of load necessary to promote bone accumulation or prevent bone loss. Frost's mechanostat hypothesis is supported by data from postmenopausal women that suggest that the combination of weight-bearing exercise and estrogen replacement increases whole body BMD more than does the sum of the separate effects of estrogen and exercise (26). Lack of estrogen in the present study is related to a lower BMC-to-muscle mass ratio compared with controls and is consistent with the idea that estrogen may alter the load required to prevent bone loss.

In conclusion, we prevented the usual ovariectomy-related body weight gain and corresponding increase in muscle mass by food restriction. The increased weight gain in Ovx animals appears to be related to changes in the GH/IGF-1 axis. Ovariectomy led to an increase in plasma IGF-I concentration, except not in Ovx-FR animals. IGF-I was correlated with both body weight and muscle mass. The increased IGF-I in Ovx animals, but not in Ovx-FR rats, also appeared to attenuate whole tibia bone loss in addition to the partial protection against bone loss provided by increased body weight.

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