Ovariectomy-Induced Hyperphagia Does Not Modulate Bone Mineral Density or Bone Strength in Rats\textsuperscript{1,2}

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Abstract

The ovariectomized (OVX) rat is a widely used animal model for the development of prevention and treatment strategies for postmenopausal osteoporosis. However, ovariectomy-induced hyperphagia results in weight gain and adiposity. To prevent potential protective effects of increased body weight on bone from confounding outcomes of preclinical studies, pair-feeding is used in some but not all studies to control food intake, but its importance is not well elucidated. We investigated if the type of feeding, pair-feeding vs. consumption of diet ad libitum, modulates bone mineral and bone strength in OVX rats. Three-month-old female Sprague-Dawley rats (n = 12/group) were randomized to 1) sham-operated control (SHAM); 2) OVX pair-fed (OVX-PF); and 3) OVX ad libitum (OVX-AL). For 14 wk, OVX-PF rats were pair-fed with the SHAM group and daily food intakes and weekly body weights were obtained. At necropsy, regional body composition was measured by dual energy X-ray absorptiometry. Bone mineral density (BMD) and biomechanical bone strength of femurs and lumbar vertebrae (LV) were also measured. OVX-AL rats had higher overall food intake (P < 0.01), final body weight (P < 0.01), weight gain (P < 0.01), and fat mass (P < 0.05) than either SHAM and OVX-PF rats. Conversely, SHAM rats had higher femur (P < 0.001) and LV1–3 BMD (P < 0.001) as well as LV4 peak load (P < 0.01) than both the OVX groups, whereas bone outcomes did not differ between the OVX-PF and OVX-AL groups. In summary, ovariectomy-induced hyperphagia and weight gain do not modulate BMD or biomechanical strength at 14 wk postovariectomy, suggesting that pair-feeding is not essential. J. Nutr. 138: 2106–2110, 2008.

Introduction

Osteoporosis is a skeletal disorder characterized by compromised bone strength leading to an increased susceptibility to fragility fractures (1). It is a debilitating disease associated with considerable medical, social, and financial consequences (2). Animal models play a crucial role in the development of prevention and treatment strategies for osteoporosis, allowing researchers to elucidate if a dietary and/or drug intervention modulates bone metabolism by altering bone mineral, bone strength (a surrogate measure of fracture risk), and/or bone structure (3–5).

The ovariectomized (OVX)\textsuperscript{3} rat is the FDA-recommended model for testing preclinical therapies for osteoporosis (6). Ovariectomy closely mimics the postmenopausal state in that endogenous estrogen levels are significantly reduced and the skeleton undergoes an increase in bone turnover, followed by accelerated bone loss, resulting in reductions in bone mineral at several skeletal sites (3–5). Similar to postmenopausal women, rats also experience micro-architectural alterations in the trabecular network, including osteoclastic perforation and the thinning of trabecular elements that, combined with the loss of bone mineral, weakens bones and increases susceptibility to fragility fractures (3–5).

Ovariectomy is also known to induce secondary effects of hyperphagia, increased weight gain, and adiposity (7) as estrogen regulates food intake via anorexigenic pathways of the central nervous system (8–16). Estradiol injection increases the central processing of the vagal cholecystokinin (CCK) satiation signal in OVX rats (11). CCK is a peptide released from the small intestine during meals and binds to receptors on vagal afferents of pylorus and proximal duodenum to initiate a negative-feedback satiation signal (11). Estrogen effectively enhances the satiating potency of CCK, leading to reductions in meal size and overall food intake (11). Similarly, estrogen is also thought to exert inhibitory effects on feeding by augmenting glucagon-mediated satiety signaling (12). Additionally, the complex interaction between estrogen and...
Body composition. The overall daily food intake was greater \((P < 0.01)\) in OVX-AL rats than in the SHAM and OVX-PF rats. By design, food intake did not differ between the SHAM and OVX-PF rats during the 14-wk study period (Fig. 1A). In the weeks immediately after ovariectomy (wk 1–3), food intake increased rapidly for all rats, followed by a gradual plateau in food intake from wk 4 to 14 (Fig. 1A). The OVX-AL rats consumed more food \((P < 0.05)\) than both the SHAM and the OVX-PF rats during the first 5 wk of study (Fig. 1A). At 6 wk, OVX-AL rats had greater food intake \((P < 0.05)\) than the OVX-PF rats (Fig. 1A). Food intake did not differ among groups from 6 wk onwards (Fig. 1A).

Body weight. Initial body weights were similar among all groups. Final body weight was higher \((P < 0.01)\) in OVX-AL rats than in SHAM and OVX-PF rats (Fig. 1B). OVX-AL rats had higher body weights \((P < 0.01)\) than both the SHAM and the OVX-PF rats by the 2nd wk postovariectomy (Fig. 1B). The differences in body weight were maintained until the end of study (Fig. 1B). Body weight difference between the SHAM and the OVX-PF rats at any time during the study (Fig. 1B). Total body weight gain was higher in OVX-AL rats than in SHAM and OVX-PF rats \((P < 0.01)\), but the SHAM and OVX-PF rats did not differ (data not shown).

Body composition. The OVX-AL rats had a higher fat mass \((P < 0.05)\) than the SHAM and OVX-PF rats but lean mass and bone mass did not differ among groups (Table 1).

Uterine weight. Uterine weights were higher \((P < 0.001)\) in SHAM rats than in OVX rats, confirming a cessation in gonadal estrogen production due to ovariectomy (Table 1). Uterine weights did not differ among the OVX rats (Table 1).

Femur and lumbar vertebra BMC and BMD. SHAM rats had higher femur BMD \((P < 0.001)\) than both of the OVX groups (Table 1). Femur BMC did not differ between the OVX rats (data not shown). LV1–3 BMC (data not shown) and BMD were higher in OVX-AL rats than in SHAM and OVX-PF rats \((P < 0.01)\).

Materials and Methods

Animals and diets. This study was conducted in accordance with the guidelines established by the Canadian Council on Animal Care (35) and all procedures were approved by the Animal Ethics Committee at the University of Toronto, Toronto, Canada. Three-month-old female virgin Sprague-Dawley rats \((n = 36)\) were obtained from Charles River Canada and housed in a light- and temperature-controlled environment \((12\text{-}\text{h}:12\text{-}\text{h}\text{-dark cycle} ; 23^\circ\text{C})\). After a 1-wk acclimatization period, rats were randomized to 1 of 3 treatment groups: sham-operated control (SHAM) \((n = 12)\), OVX pair-fed (OVX-PF) \((n = 12)\), and OVX ad libitum (OVX-AL) \((n = 12)\). Sham operations were performed by exposing the ovaries without excision whereas the ovariectomies \((n = 24)\) were performed by ligating and excising the ovaries. The rats were anesthetized using isoflurane inhalation \((3\%\text{ dissolved in oxygen})\) followed by a subcutaneous administration of the analgesic buprenorphine \((0.05\text{ mg/kg body weight})\).

The rats were fed a standard pelleted diet \((AIN93M) (36)\). Fresh diet was provided daily and distilled water was freely accessible. SHAM and OVX-AL rats consumed diet ad libitum whereas the OVX-PF rats were restricted to the average amount of food eaten by the SHAM group the previous day. We measured food intake daily by weighing the amount of food remaining in each cage every 24 h. Body weight was measured weekly using an electronic scale \((\text{Denver Instrument XP-1500})\). At the end of the 14-wk feeding period, rats were anesthetized using \(\text{CO}_2\) asphyxiation, and killed by cervical dislocation. At necropsy, uteri were excised, cleaned of soft tissue, and stored at \(-80^\circ\text{C}\) until analyses.

Body composition. Fat mass, lean mass, and bone mass were measured at the end of the 14-wk study period by dual energy X-ray absorptiometry \((\text{DEXA}) (\text{pSabre, Orthometrix})\) using a specialized software program \((\text{Host Software version: 3.9.4; Scanner Software version: 1.2.0})\). Following \(\text{CO}_2\) asphyxiation, the rats were placed directly on the DEXA. Because the scan window of the DEXA was not large enough to accommodate a whole-body scan, a region 11.4 cm wide and 4 cm long starting from the rat pelvis was consistently selected for scanning. All scans were performed at a speed of 10 mm/s and a resolution of \(0.5 \times 1.0\text{ mm}\).

Bone area, bone mineral content, and BMD. Bone area and bone mineral content \((\text{BMC})\) of the right femurs and LV1–3 were measured by DEXA \((\text{pSabre, Orthometrix})\) using a specialized software program \((\text{Host Software version: 3.9.4; Scanner Software version: 1.2.0})\) at a speed of 10 mm/s and a resolution of \(0.2 \times 0.2\text{ mm}\).

Biomechanical strength testing. The biomechanical strength properties of left femurs and LV4 were measured using a materials testing system \((\text{Model 4442, Instron})\) and a specialized software program \((\text{Instron Series IX Automated Materials Tester-version 8.15.00; Instron})\) as previously described (37).

Three-point bending tests were performed on left femurs to determine the yield load, resilience, ultimate stiffness, peak load, and toughness. Each femur was placed, on its posterior surface, on 2 supporting bars of a jig that were positioned 15 mm apart while a crosshead directly above lowered at a constant rate of 2 mm/min until the femur fractured. Compression tests were performed on LV4 to determine peak load as previously described (37) with a crosshead speed of 2 mm/min.

Statistical analyses. Statistical analyses were performed using Sigma Stat (version 2.0, Jandel Scientific). For data that followed a normal distribution, 1-way ANOVA followed by Student Newman-Keuls post hoc test was used to determine differences among treatment groups. For data that did not follow a normal distribution, a Kruskal-Wallis 1-way ANOVA on ranks followed by Student Newman-Keuls post hoc test was used to determine differences among groups \((\text{bone mass, uterine weights})\). Significance was set at \(P < 0.05\). Results are expressed as means \(\pm \text{SEM}\).
greater in the SHAM rats than in the OVX groups (P < 0.001), whereas the OVX-AL and OVX-PF rats did not differ (Table 1).

Bone dimensions and biomechanical strength properties.
The dimensions (weight, length, depth, and width) of the femur or LV4 did not differ among groups (Table 1). The biomechanical strength properties at the midpoint of the femur (yield load, resilience, peak load, toughness, or stiffness) did not differ among groups (Table 1). LV4 peak load was higher in SHAM rats than in OVX rats (P < 0.01), whereas the pair-fed rats had a similar peak load to OVX-AL rats (Table 1).

Discussion
Ovariectomy-induced hyperphagia and weight gain did not modulate bone mass or biomechanical bone strength at 14 wk postovariectomy. Regardless of whether rats were pair-fed to SHAM or consumed diet ad libitum, OVX rats had significantly lower BMD at both the femur and the LV compared with the SHAM rats with intact ovaries. Moreover, the peak load of the LV, a functional assessment of vertebral strength and a surrogate measure of fracture risk, was also significantly lower among the OVX rats. Therefore, increased weight gain as a result of estrogen deficiency did not offer any protection against the deterioration of bone tissue in OVX rats.

Pair-feeding was successful in preventing excess weight gain associated with ovariectomy-induced hyperphagia. By maintaining comparable food intake between the OVX-PF and the SHAM rats, similar body weight and weight gain were achieved. Although OVX rats consuming diet ad libitum had a greater overall food intake than both the SHAM and the pair-fed rats, hyperphagia disappeared by 7 wk postovariectomy. Similar observations of transient hyperphagia in OVX rats have been reported by others (7,38,39,40). Transient hyperphagia may be partially explained by the fluctuating levels of hypothalamic neuropeptide Y in the paraventricular nucleus of the hypothalamus postovariectomy (40). Neuropeptide Y levels increase in response to ovariectomy-induced estrogen deficiency but decrease to normal levels following a period of progressive weight gain (40). This coincides with the pattern of ovariectomy-induced hyperphagia in which food intake increases rapidly, followed by a period of gradual normalization (40).

Marked changes in body weight among the OVX-AL rats were also observed early in the study. Although the OVX-AL rats had significantly higher final body weights and total weight gain than both the SHAM and the OVX-PF rats, the rate of weight gain

<table>
<thead>
<tr>
<th>TABLE 1</th>
<th>Food intake and anthropometric measures during the 14-wk study period in intact (SHAM) rats and ovariectomized rats that were pair-fed to SHAM (OVX-PF) or consumed diet ad libitum (OVX-AL)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SHAM</td>
</tr>
<tr>
<td>n</td>
<td>12</td>
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<tr>
<td>Average food intake, g/d</td>
<td>11.75 ± 0.21*</td>
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<tr>
<td>Whole body weight, g</td>
<td>Initial</td>
</tr>
<tr>
<td>Final</td>
<td>275.83 ± 7.58b</td>
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<tr>
<td>Uterus weight, mg/g body weight</td>
<td>1.92 ± 0.15a</td>
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<tr>
<td>Body composition, g</td>
<td>Fat mass</td>
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<tr>
<td></td>
<td>Lean mass</td>
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<td>Bone mass</td>
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<td>Whole femur</td>
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<td>Width, mm</td>
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<td></td>
<td>BMD, mg/cm²</td>
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<tr>
<td>Femur midpoint</td>
<td>Yield load, n</td>
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<tr>
<td></td>
<td>Resilience, mJ</td>
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<td>Stiffness, n/mm</td>
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<td>LV4</td>
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<td>Height, mm</td>
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<td>Depth, mm</td>
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<td>Width, mm</td>
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<tr>
<td></td>
<td>Peak load, n</td>
</tr>
<tr>
<td>LV1–3</td>
<td>BMD, mg/cm²</td>
</tr>
</tbody>
</table>

1 Values are means ± SEM. Means in a row with superscripts without a common differ, P < 0.05.
2 Depth refers to the anteroposterior width at the midpoint of the femur and the LV4.
3 Width refers to the mediolateral width at the midpoint of the femur and the LV4.

*Different from SHAM and OVX-PF, P < 0.05. †Different from OVX-PF, P < 0.05.
Eventually stabilized to the levels of the other groups. Therefore, food intake appears to be the primary means through which accelerated weight gain is achieved postovariectomy, which is consistent with previous findings (7, 10, 39). The maintenance of the excess weight following transient hyperphagia, however, may depend on other mechanisms. Reduced energy expenditure and decreased metabolic rate, for example, have been observed in OVX rats, but the pathways of estrogenic action are not well elucidated (41).

In addition, fat mass was significantly greater in OVX-AL rats than in SHAM and OVX-PF rats, whereas the SHAM and the OVX-PF groups did not differ. Therefore, ovariectomy-induced hyperphagia not only resulted in significantly higher weight gain but also in the excess accumulation of fat mass. It was also shown that pair-feeding suppressed the increase in adipose tissue deposition. The increase in adiposity following the cessation of endogenous estrogen production is reported in both postmenopausal women and OVX rats (18–19). Ovariectomy stimulates, while estradiol replacement inhibits, adipose tissue lipoprotein lipase, a regulatory enzyme responsible for the hydrolysis of circulating triglycerides and their uptake and storage into adipocytes, thereby promoting the growth of fat mass (10). Lean mass, however, did not differ among any of the 3 groups. Therefore, the significantly greater body weights in OVX-AL rats can be mainly attributed to the excess accumulation of fat mass.

Despite having significantly greater body weight and fat mass, OVX-AL rats did not have added protection against the loss of bone mineral at either the femur or the LV compared with the OVX-PF rats. Similarly, vertebral strength, as measured by the peak load of LV4, was significantly lower among the OVX-AL and OVX-PF rats compared with the SHAM rats, indicating an increase in the risk of fracture among all OVX rats. To our knowledge, we are the first to report that increased weight gain associated with ovariectomy-induced hyperphagia does not attenuate loss of biomechanical bone strength at the LV. Moreover, the duration of our study, 14 wk, is a realistic duration for dietary studies investigating how foods or food components modulate bone metabolism postovariectomy.

The biomechanical strength properties at the femur midpoint did not differ significantly among groups. This finding is consistent with previously published studies that have shown that ovariectomy-induced estrogen deficiency manifests in a time- and site-specific fashion on the skeleton (42, 43) due in part to differences in the proportion and distribution of trabecular and cortical bone at different skeletal sites. Wronska et al. (44) reported that OVX-AL rats had significantly greater trabecular bone volume compared with weight-matched OVX rats; however, other histomorphometric measures did not differ. The authors concluded that “marked osteopenia develops in the long bones of OVX rats regardless of body weight,” which is consistent with our findings (44).

Numerous studies have linked obesity to increased BMD, leading to protective effects against osteoporosis and associated fracture risks (17–19). Body weight is thought to affect bone metabolism primarily through the effect of mechanical loading at weight-bearing bone sites (17–19). Additionally, fat mass, an important indicator of obesity and one of the most metabolically active tissues in the body, may modulate bone metabolism via aromatase and/or leptin (19). The fact that increased weight gain and fat mass did not protect against bone loss may be explained by the intricate interplay between the metabolic pathways of bone regulation.

In conclusion, consuming diet ad libitum or pair-feeding will not confound the effects of dietary interventions in rats of a similar age postovariectomy and in a study of similar duration. Studies of longer duration and thus in old OVX rats are indicated, because the effects of low-dose estrogen due to synthesis in adipose tissue and increased weight bearing may not be manifested in shorter-term interventions such as used in this study.

### Literature Cited

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