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Effect of Body Weight on Osteopenia in Ovariectomized Rats

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Summary. Bilateral ovariectomies or sham surgeries were performed in female Sprague Dawley rats that were 78 days of age and weighed an average of 210 g. Food was available ad libitum to the control rats and to a group of ovariectomized rats (obese OVX). The food consumption of a second group of ovariectomized rats (weight-matched OVX) was restricted to match their body weights to those of the control rats. All rats were sacrificed at 14 weeks postovariectomy. Radioimmunoassay of terminal serum estradiol confirmed the success of ovariectomy. The estradiol concentration in control rats was 24.9 ± 20.2 pg/ml, whereas the hormone was undetectable (<10 pg/ml) in both groups of OVX rats. The final body weights of control and weight-matched OVX rats were nearly identical (~260 g). In contrast, obese OVX rats weighed significantly more than both of the above groups (~320 g, $P < 0.001$). The proximal tibia and lumbar vertebra were processed undecalcified for quantitative bone histomorphometry. Tibial trabecular bone volume (TBV) was determined to be 17.6 ± 4.5%, 7.9 ± 5.3%, and 3.6 ± 3.1% for the control, obese OVX, and weight-matched OVX groups, respectively. Tibial TBV for both OVX groups was significantly less than the control value ($P < 0.001$). The difference in tibial TBV between obese OVX and weight-matched OVX rats was also statistically significant ($P < 0.02$). Histologic indices of bone resorption and formation were indicative of increased bone turnover in the proximal tibia of both OVX groups. In comparison to control rats, both groups of OVX rats exhibited a strong trend for a reduction in vertebral TBV, but no significant differences were observed among the three groups. Our results suggest that increased body weight provides partial protection against osteopenia in the long bones of OVX rats. However, it is important to note that this protective effect is only partial and that marked osteopenia develops in the long bones of OVX rats regardless of body weight.

Key words: Quantitative bone histomorphometry — Estrogen deficiency — Body weight — Osteopenia — Bone turnover.

Numerous studies have shown that ovariectomy induces osteopenia and increased body weight in rats [1–7]. A common criticism of such studies is that the increased body weight of ovariectomized rats may affect the development of osteopenia. This potential complicating factor is especially meaningful in view of reports that obesity reduces bone loss and fracture incidence in postmenopausal women [8–10]. The purpose of the current study is to evaluate the effect of body weight on the development of osteopenia in ovariectomized rats.

Materials and Methods

The experimental animals were 30 female Sprague Dawley rats that were 78 days of age and weighed an average of 210 g at the beginning of the experiment. All rats were anesthetized with an i.p. injection of ketamine hydrochloride and xylazine at doses of 50 mg/kg body weight and 10 mg/kg body weight, respectively. Bilateral ovariectomies were performed in 20 rats from a dorsal approach [11]. Ten control rats were subjected to sham surgeries. Food (Purina Rat Laboratory Chow, St. Louis, MO) was available ad libitum to the sham-operated control rats and to 10 ovariectomized rats (obese OVX). The mean food consumption of the control and obese OVX rats was ~18 g/day and 19.5 g/day,

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respectively. The food consumption of the remaining 10 ovariectomized rats (weight-matched OVX) was restricted to ~15 g/day to match their body weights to those of the control rats. It was determined during the early stages of the experiment that pair-feeding OVX rats according to the food intake of control rats did not prevent the increase in body weight associated with ovariectomy. All rats were housed individually at 25°C with a 13 h/11 h light/dark cycle. Calcein (Sigma Co., St. Louis, MO) was administered to each rat by i.p. injection (10 mg/kg body weight) on the ninth and third days before sacrifice. This regimen results in deposition of a double calcein label at bone surfaces that were actively mineralizing throughout the injection period.

All rats were sacrificed at 14 weeks postovariectomy by cervical dislocation under ketamine/xylazine anesthesia. Success of ovariectomy was confirmed at necropsy by failure to detect ovarian tissue and by observation of marked atrophy of the uterine horns. In addition, terminal blood samples were collected from the abdominal aorta for radioimmunoassay of serum estradiol with a commercially available kit (Diagnostic Products Corp., Los Angeles, CA). Serum samples from experimental animals and estradiol calibrators from processed human serum were placed in estradiol antibody-coated tubes in duplicate. Buffered [^{125}I] estradiol was then added to each tube followed by incubation at room temperature for 3 hours. After thorough decanting, each sample was counted for 1 minute with a gamma counter (1282 Compugamma, LKB-Wallac, Finland). Estradiol concentration was calculated by interpolation from a logit-log representation of a calibration curve with correction for nonspecific binding.

The proximal tibiae and first lumbar vertebrae were defleshed and placed in 10% phosphate-buffered formalin for 24 hours. The bone specimens were then dehydrated in ethanol and embedded undecalcified in methyl methacrylate [12]. Longitudinal sections (4 μm thick) were cut with an AO Autocut/Jung 1150 microtome and stained with Masson-Goldner trichrome. The following bone parameters were measured manually at a magnification of 400× with the aid of a Merz eyepiece reticle [13]: trabecular bone volume (%), osteoclast surface (%), osteoblast surface (%), and numbers of osteoclasts and osteoblasts per mm trabecular bone perimeter. Bone parameters were quantified in trabecular bone tissue at distances greater than 1 mm from the growth plate-metaphyseal junction to exclude the primary spongiosa. Details of data collection and calculations have been published elsewhere [6, 7].

Calcein-based parameters were measured in unstained, 10 μm-thick sections of the proximal tibial metaphysis and first lumbar vertebra. The rate of longitudinal bone growth, percentage of trabecular bone surface with a double calcein label (active formation suface), and calcification rate were measured with a Merz eyepiece reticle and calibrated eyepiece micrometer as previously described [6, 7]. Values for calcification rate were not corrected for obliquity of the plane of section in trabecular bone [14].

Data are expressed as the mean ± SD of the control, obese OVX, and weight-matched OVX groups. Statistical differences among the three groups were evaluated initially by analysis of variance. Significant F values ($P < 0.05$) for a given bone parameter were followed by multiple comparisons between two groups with Student's t test. In accordance with Bonferroni's procedure [15], a more stringent level of significance ($P < 0.02$) was used to compensate for the multiple comparisons ($P < 0.05 \div 3 \simeq P < 0.02$).

Results

One of the obese OVX rats contracted a respiratory infection with accompanying weight loss. Data from this animal has been excluded from all results presented below. The final body weights of control and weight-matched OVX rats were nearly identical (261.4 ± 11.6 g vs. 259.7 ± 11.7 g, respectively). Obese OVX rats weighed ~60 g more than both of the above groups (322.4 ± 15.2 g, $P < 0.001$).

Radioimmunoassay of terminal serum estradiol confirmed the success of ovariectomy. The estradiol concentration in control rats was 24.8 ± 20.2 pg/ml. The large standard deviation is probably due to random progression of control rats through the estrous cycle, during which serum estradiol has been shown to vary from 15–90 pg/ml [16]. Serum estradiol was undetectable (<10 pg/ml) in both groups of OVX rats.

Table 1 lists values for static histomorphometric parameters in trabecular bone of the proximal tibial metaphysis and lumbar vertebral body. Obese OVX and weight-matched OVX rats both exhibited a marked reduction in tibial trabecular bone volume relative to control rats ($P < 0.001$). In addition, trabecular bone volume in the proximal tibia of the weight-matched OVX group was significantly less than that of the obese OVX group ($P < 0.02$). The differences in tibial trabecular bone volume among the three groups are seen in Figure 1. A strong trend for increased osteoclast surface was observed in the proximal tibia of both OVX groups, but this trend was not statistically significant ($P < 0.05$). On the other hand, tibial osteoclast number was significantly elevated relative to control values in the obese OVX rats ($P < 0.01$) as well as the weight-matched OVX rats ($P < 0.02$). Histologic indices of bone formation (osteoblast surface and number) were also significantly increased in the proximal tibia of both OVX groups with the exception of osteoblast number in the weight-matched OVX rats ($P < 0.05$). Tibial osteoblastic parameters tended to be elevated to a greater extent in obese OVX rats relative to weight-matched OVX rats.

Bone changes in the lumbar vertebra of OVX rats were not as marked as those observed in the proximal tibia (Table 1). The 12–15% decline in vertebral trabecular bone volume in both groups of OVX rats was not statistically significant, although it approached significance in weight-matched OVX rats ($P < 0.05$). Osteoclastic parameters were not significantly increased in obese OVX rats relative to control rats. However, statistically significant incre-

Table 1. Static histomorphometric parameters in the proximal tibial metaphysis and lumbar vertebral body

	Group 1	Group 2	Group 3	*P* values[a]		
	Control (N = 10)	Obese OVX (N = 9)	Weight-matched OVX (N = 10)	1–2	1–3	2–3
Tibia						
Trabecular bone vol (%)	17.6 ± 4.5	7.9 ± 5.3	3.6 ± 3.1	0.001	0.001	0.02
Osteoclast surface (%)	3.2 ± 2.2	5.3 ± 2.0	6.6 ± 3.9	NS	NS	NS
Osteoblast surface (%)	3.3 ± 1.8	7.9 ± 3.2	6.4 ± 2.9	0.001	0.02	NS
Osteoclasts/mm	0.7 ± 0.5	1.4 ± 0.6	1.5 ± 0.8	0.01	0.02	NS
Osteoblasts/mm	1.2 ± 0.7	2.9 ± 1.3	2.4 ± 1.3	0.01	NS	NS
Vertebra						
Trabecular bone vol (%)	33.0 ± 5.1	28.9 ± 8.1	28.2 ± 4.6	NS	NS	NS
Osteoclast surface (%)	1.5 ± 0.9	2.2 ± 1.6	3.2 ± 1.8	NS	0.02	NS
Osteoblast surface (%)	3.1 ± 2.1	4.5 ± 2.9	4.9 ± 3.3	NS	NS	NS
Osteoclasts/mm	0.3 ± 0.2	0.4 ± 0.3	0.6 ± 0.3	NS	0.01	NS
Osteoblasts/mm	1.1 ± 0.8	1.8 ± 1.3	1.5 ± 0.9	NS	NS	NS

All values are the mean ± SD

[a] Determined by Student's *t* test with $P < 0.02$ as the minimum level of significance to compensate for multiple comparisons

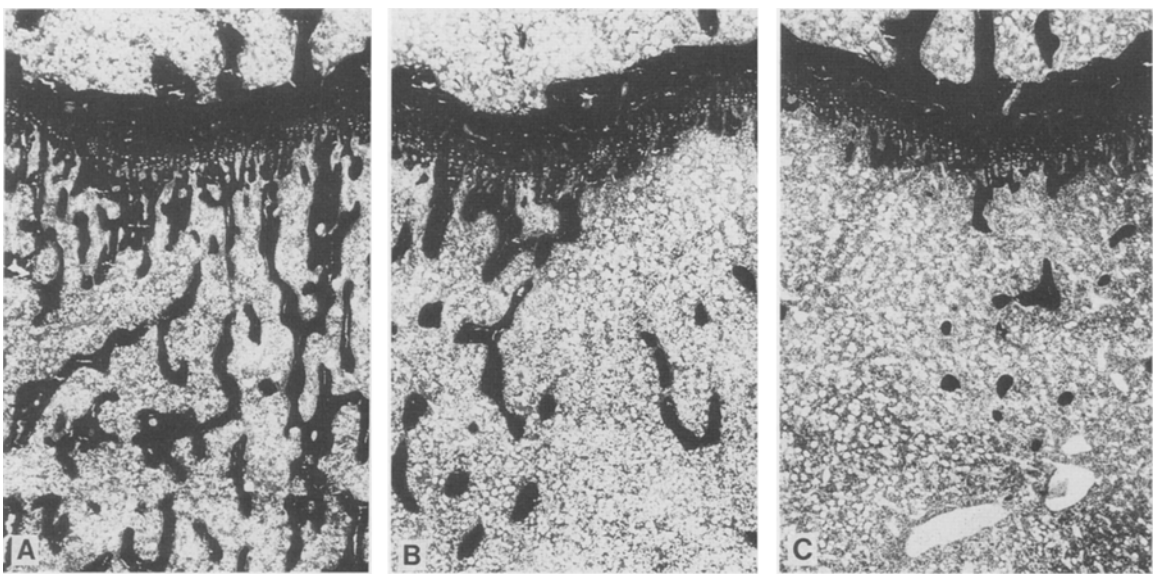

Fig. 1. Proximal tibial metaphysis of control (**A**), obese OVX (**B**), and weight-matched OVX (**C**) rats. Note the reduced mass of darkly stained trabecular bone indicative of osteopenia in both OVX animals. Note also that osteopenia is more pronounced in the weight-matched OVX rat relative to the obese OVX rat. Von Kossa stain, ×16.

ments in osteoclastic parameters were noted in the lumbar vertebrae of weight-matched OVX rats. Vertebral osteoblastic parameters tended to be elevated in both OVX groups relative to the control group, but this trend was not statistically significant.

Values for dynamic calcein-based parameters in the proximal tibial metaphysis and lumbar vertebral body are shown in Table 2. Tibial longitudinal bone growth was nearly identical in the three groups. Active formation surface was significantly increased in the proximal tibia of obese OVX rats relative to control rats ($P < 0.02$), but weight-matched OVX rats exhibited only a nonsignificant trend for increased tibial active formation surface. No significant differences among the three groups were noted for vertebral active formation surface and for tibial and vertebral calcification rates.

Table 2. Dynamic calcein-based parameters in the proximal tibial metaphysis and lumbar vertebral body

	Group 1	Group 2	Group 3	*P* values[a]		
	Control (N = 7)	Obese OVX (N = 5)	Weight-matched OVX (N = 8)	1–2	1–3	2–3
Tibia						
Longitudinal bone growth (μm/day)	12.8 ± 2.5	11.4 ± 1.9	11.1 ± 2.8	NS	NS	NS
Active formation surface (%)[b]	11.2 ± 4.9	18.4 ± 3.5	15.3 ± 11.8	0.02	NS	NS
Calcification rate (μm/day)	1.3 ± 0.3	1.4 ± 0.2	1.4 ± 0.5	NS	NS	NS
Vertebra[c]						
Active formation surface (%)	6.1 ± 3.5	6.0 ± 2.7	9.3 ± 6.8	NS	NS	NS
Calcification rate (μm/day)	1.3 ± 0.3	1.2 ± 0.2	1.4 ± 0.1	NS	NS	NS

All values are the mean ± SD

[a] Determined by Student's *t* test with $P < 0.02$ as the minimum level of significance to compensate for multiple comparisons

[b] Defined as trabecular bone surface with a double calcein label

[c] Longitudinal bone growth in the lumbar vertebra is too slow to be measured with the calcein regimen used in this study

Discussion

This study demonstrates that increased body weight provides partial protection against the development of osteopenia in the long bones of ovariectomized rats. Although tibial trabecular bone volume was markedly reduced in both the obese OVX and weight-matched OVX rats, the observed bone loss was more pronounced in the latter group. Since the weight-matched OVX rats weighed ~60 g less than the obese OVX rats, body weight is implicated as a factor affecting the development of osteopenia in the estrogen-deficient state. This finding is consistent with reports of decreased bone loss and fracture incidence in obese postmenopausal women [8–10]. Nevertheless, it is important to note that the protective effect of obesity against osteopenia in OVX rats is only partial and that marked osteopenia develops in the long bones of OVX rats regardless of body weight.

The mechanism by which increased body weight slows the bone loss associated with ovariectomy is unclear. For the most part, histologic indices of bone turnover in the proximal tibia were found to be elevated in obese OVX rats as well as weight-matched OVX rats. However, tibial bone formation parameters such as osteoblast surface, osteoblast number, and active formation surface tended to be elevated to a greater extent in obese OVX rats relative to weight-matched OVX rats. If real, this trend suggests that the increased body weight of obese OVX rats may have provided an additional stimulus for bone formation in the weight-bearing long bones. Diminished bone loss in obese OVX rats may be explained on this basis. The tendency for increased bone resorption in weight-matched OVX rats relative to obese OVX rats may also contribute to greater bone loss in the former animals. Another factor to consider is the reduced food intake of weight-matched OVX rats that was essential to maintain normal body weights. Shires et al. [17] reported that semistarvation induces osteopenia and decreased bone turnover in rats. Although the food consumption of these semistarved rats was not specified, the dietary restrictions were presumably greater than those of the current study.

The partial protective effect of increased body weight against osteopenia in OVX rats was apparently confined to the long bones. Although the data are inconclusive, both groups of OVX rats exhibited strong trends for reduced trabecular bone volume in their lumbar vertebrae relative to control vertebrae. However, vertebral trabecular bone volume was nearly identical in obese OVX and weight-matched OVX rats. Therefore, the tendency for osteopenia in the vertebral column of OVX rats did not appear to be affected by body weight. Since the tibia is undoubtedly subjected to greater mechanical forces than the vertebrae in quadruped animals such as the rat, it seems logical to assume that a protective effect associated with increased weight-bearing would be more pronounced in the long bones. This appears to be the case regarding the development of osteopenia in OVX rats. In contrast, obesity may be expected to diminish bone loss in the vertebral column of postmenopausal women due to their biped posture and increased vertebral weight bearing.

We previously described marked elevations in histologic indices of bone resorption and formation in the proximal tibia and lumbar vertebra of OVX rats at 5 and 10 weeks postovariectomy [6, 7]. In the current study at 14 weeks postovariectomy, bone turnover was still elevated in the proximal tibia of OVX rats, but not to the same extent as noted at earlier times postovariectomy. Further-

more, these same OVX rats exhibited trends for increased vertebral bone turnover, but these trends were, for the most part, statistically nonsignificant. For comparative purposes, it should be noted that the ages and body weights of the rats from the three studies (5, 10, and 14 weeks postovariectomy) were nearly identical at the time of surgery. We interpret these data as evidence that increased bone turnover in OVX rats is transient. This concept should not be surprising in view of the fact that other skeletal effects of estrogen deficiency in rats were found to be transient. For example, longitudinal bone growth was significantly increased in the proximal tibia of OVX rats at 5 weeks postovariectomy [6], but returned to control levels by 10 weeks postovariectomy [7]. Tibial calcification rate was found to be elevated in OVX rats at 5 and 10 weeks postovariectomy [6, 7], but was nearly identical in OVX and control rats in the current study at 14 weeks postovariectomy. Draper et al. [18] reported that ^{45}Ca excretion indicative of bone resorption was markedly increased in OVX rats during the first several months postovariectomy, then approached control levels by the 15th week postovariectomy. In view of these findings, the accelerated skeletal metabolism induced by ovariectomy in rats appears to be transient. Parfitt et al. [19] hypothesized that the increased rate of bone turnover observed in postmenopausal women during the early stages of estrogen deficiency [20] is also a transient phenomenon.

In summary, increased body weight was found to provide partial protection against the development of osteopenia in the long bones of ovariectomized rats. This finding is consistent with reports of diminished bone loss in obese postmenopausal women. Although osteopenia was less pronounced in obese OVX rats, it is important to note that the protective effect of increased body weight is only partial and that marked osteopenia develops in the long bones of ovariectomized rats regardless of body weight.

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