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Effects of Two Non-Endurance Exercise Protocols on Established Bone Loss in Ovariectomized Adult Rats

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Summary. The effects of non-endurance exercise on bone properties were evaluated in 9-month-old sham-operated (SH) and ovariectomized (OVX) rats. The studies were started 3 months postsurgery, after bone mass was decreased in OVX rats. The sham and OVX rats were either kept sedentary (SED) or were trained to run with one of two protocols: 12 m/minute, 50 minutes/day, 4 days/week (low intensity, frequent, EX-1); or 21 m/minute, 40 minutes/day, 1 day/week (moderate intensity, infrequent, EX-2). A group of seven rats evaluated at the beginning of the study served as baseline control. The bone mineral was assessed by the ash weight of the left femur, tibia, and 4th lumbar vertebra. Biomechanical (strength, deformation, stress, strain, and stiffness) and morphometric (length, cortical and medullary area, moment of inertia) properties were evaluated for the right femur. There was a significantly lower bone mineral and mechanical properties in OVX-SED ($n = 7$) than in SH-SED ($n = 10$) rats. The OVX-EX-1 ($n = 6$) rats had higher ash content of femur and tibia than OVX-SED rats, but the change was significant only for tibia. The EX-2 had no effect on the ash content, but femur stress was higher in OVX-EX-2 ($n = 8$) than in OVX-SED rats. The femur yield force and deformation were improved in OVX rats with both exercise protocols, whereas the vertebra ash weight, femur strain, modulus of elasticity, length, cortical area, and moment of inertia were not changed. Non-endurance exercise did not affect bone properties in either SH-EX-1 ($n = 7$) or SH-EX-2 ($n = 8$) groups. We conclude that non-endurance exercise has beneficial effects on established osteopenia in ovariectomized rats.

Key words: Osteoporosis – Exercise – Ovariectomy – Bone strength.

Oophorectomized rats have been used as a model for studies of postmenopausal osteoporosis [1, 2]. Ovariectomy (OVX) in adult rats induces osteopenia due to high bone turnover, with bone resorption exceeding bone formation [1–3]. Exercise has been used for bone protection from OVX-induced changes with variable effects. Tuukkanen et al. [4] observed decreased trabecular bone resorption with 8 weeks of exercise in young OVX rats. Exercise, however, was ineffective for prevention of OVX-induced osteopenia in calcium-deficient adult rats [5]. Case-control studies in humans have demonstrated the positive effect of exercise, but a few randomized trials failed to show protective effect of training on postmenopausal osteoporosis [6, 7]. Exercise protocols most frequently used for postmenopausal women either include 1–3 sessions of supervised moderate exercise per week or 4–6 days/week of unsupervised physical activity [7, 8].

We have used the OVX rat model and have shown that endurance exercise started immediately post-OVX resulted in an improvement of bone mass and femur strength [9]. The present study evaluated effects of two non-endurance exercise protocols started 3 months post-OVX, after osteopenia had occurred. One exercise protocol was designed to provide a low level of exercise 4 days/week and the other exercise protocol was designed to provide moderate exercise once a week. These exercise protocols were chosen to simulate the protocols frequently used in humans.

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Materials and Methods

Animals

Nine-month-old retired female breeders, weighing approximately 250 g, were obtained from Sprague-Dawley Breeding Laboratories (Indianapolis, IN). All animals received a standard diet containing 0.97% calcium, 0.85% phosphorus, and 1.0 IU/g vitamin D (AG-WAY, RNH 3000, Arlington Heights, IL). The rats were weight matched and randomly assigned to various groups. A group of rats ($n = 7$) was sacrificed at the beginning of the study and designated as the baseline control (control) group. The remaining animals were sham-operated (SH) or ovariectomized under ketamine hydrochloride/xylazine hydrochloride (88/10 mg/kg i.p.) anesthesia via an abdominal approach. Established osteoporosis was induced by OVX followed by no treatment for 3 months. The SH and OVX rats were subjected to the following protocols: (1) sedentary (SED), followed for 6 months (SH-SED and OVX-SED); (2) sedentary for 3 months and then trained with low intensity frequent exercise (EX) (described later) for 3 months (SH-EX-1 and OVX-EX-1); (3) sedentary for 3 months and then trained with moderate intensity infrequent exercise for 3 months (SH-EX-2 and OVX-EX-2). Eight animals were assigned to each group except for the SH-SED group which had 10 animals. A few animals died during the course of the studies and the number of animals available for analysis is indicated in Table 1. At the end of the experiment the rats were anesthetized with ketamine/xylazine (88/10 mg/kg i.p.) and sacrificed by exsanguination, as previously described [10]. At sacrifice, the left femur, left tibia, and fourth lumbar vertebra (L4) were removed and cleaned for ash analysis, and the right femur was obtained for biomechanical and morphometric properties measurement. Body weight as well as uterine weight were recorded for each rat.

Table 1. Body and uterine weight of adult female rats

Groups	No. rats	Body wt, g	Uterine wt, g
Control	7	248.0 ± 5.6	0.52 ± 0.02
SH-SED	10	258.2 ± 8.8	0.50 ± 0.07
SH-EX-1	7	275.0 ± 7.4	0.56 ± 0.05
SH-EX-2	8	255.4 ± 12.7	0.56 ± 0.07
OVX-SED	7	256.4 ± 10.9	0.10 ± 0.01[a]
OVX-EX-1	6	301.5 ± 4.6[b]	0.08 ± 0.02[a]
OVX-EX-2	8	287.8 ± 11.5	0.10 ± 0.01[a]

Values are mean ± SEM. SH = sham-operated, OVX = ovariectomized, SED = sedentary, EX = exercised, EX-1 = low intensity frequent EX, EX-2 = high intensity infrequent EX. Control animals were sacrificed at the beginning of the study at 9 months and were therefore 6 months younger than the animals in the other groups

[a] $P < 0.01$ vs SH-SED; [b] $P < 0.05$ vs OVX-SED

Animal Training

The exercising rats were trained to run on a motor-driven, six chamber Omni Pacer Treadmill with an electric grid at the rear of each compartment (Omnitech, Columbus, OH). During the conditioning period, rats started running at 6 m/minute, 0 grade, 10 minutes/day, and the speed and duration were then increased over 2 weeks until final protocol parameters were reached. The low intensity frequent exercise was defined as running at a speed of 12 m/minute, 0 grade, 50 minutes/day, 4 days/week (EX-1); the moderate intensity infrequent exercise consisted of running at 21 m/minute, 0 grade, 40 minutes/day, 1 day/week (EX-2). The experimental protocols were approved by the institutional Animal Care Committee.

Bone Analysis

Left femur, left tibia, and fourth lumbar vertebra were freed of soft tissue. The bones were ashed in porcelain crucibles in a muffle furnace at 800°C overnight and the ash weight was obtained. Mechanical properties of the right femur were determined by a three-point bending test in an Instron Universal Testing Machine (Model II25, Instron Corp, Canton, MA). The breaking force was applied perpendicular to the long axis of the femur with a crosshead speed of 1.0 mm/minute, as previously described [10]. The femur length was measured with calipers before breaking. The inner and outer diameters in anterior-posterior and medial-lateral axis were measured with a micrometer at the point of fracture and used to calculate cortical area, medullary area, and moment of inertia. Coefficient of variation for micrometer measurements was 1.5%. The moment of inertia provided the measure of bone distribution about the neutral axis. The three mechanical parameters of femur were directly derived from the force-deformation curves: (1) yield force, monitored by the point of deviation from the linear slope of the force-deformation curve; (2) maximum force, indicated by the maximum force on the force-deformation curve; (3) elastic deformation, measured as deflection at yield point. The yield stress (measure of tissue strength), the elastic strain (measure of the bone bending relative to its length), and the modulus of elasticity (indicator of bone stiffness) were calculated from the primary data, using previously described formulas [10, 11].

Statistical Analysis

Differences among groups were evaluated by analysis of variance followed by Dunnett squares method using an IBM PC II computer. A P value below 0.05 was considered significant [12].

Results

Body and Uterine Weights

Table 1 shows final body weights as well as uterine weights of rats from various groups. There were no significant differences in the final body weights among various groups, except for the rats in OVX-EX-1 group which had higher body weights than OVX-SED rats. The uterine weights were significantly decreased by OVX and were not affected by exercise.

Effects of Age

The ash weight of all three bones, as well as femur force, was higher in SH-SED than in control rats sacrificed at the beginning of the study at the age of 9 months (Figs. 1 and 2). The other femur biomechanical and morphometric properties were not modified by age (Table 2).

Effects of Ovariectomy on Bone Parameters

Ovariectomy resulted in a significantly lower ash weight of femur, tibia, and L4 in OVX-SED rats compared with the SH-SED group (Fig. 1). Femur biomechanical parameters—yield and maximum force, deformation, stress, strain, and modulus of elasticity—were significantly lower in OVX-SED rats than in SH-SED animals (Fig. 2 and Table 2). Femur geometry was also affected by OVX; the medullary area and moment of inertia were higher in OVX-SED rats than in SH-SED group (Table 2). Femur length and cortical area were not influenced by ovariectomy (Table 2).

Comparison of OVX-SED to baseline control rats showed that there was no net loss of bone mineral or femur force during the 6-month period of OVX (Figs. 1 and 2). However, the other biomechanical and morphometric properties were affected by OVX such that the differences observed between baseline control and OVX-SED rats were similar to those seen between SH-SED and OVX-SED rats (Table 2).

Effects of Exercise in Ovariectomized Rats

The femur and tibia ash weights were higher in OVX-EX-1, but not in OVX-EX-2 rats as compared with those in OVX-SED animals. The differences were statistically significant only for the tibia in the OVX-EX-1 group (Fig. 1). The vertebra ash weights were not significantly different between exercised and sedentary groups in either EX-1 or EX-2 OVX rats. The femur yield force and deformation were significantly higher in OVX rats on both exercise protocols compared with the OVX-SED group (Fig. 2 and Table 2). The

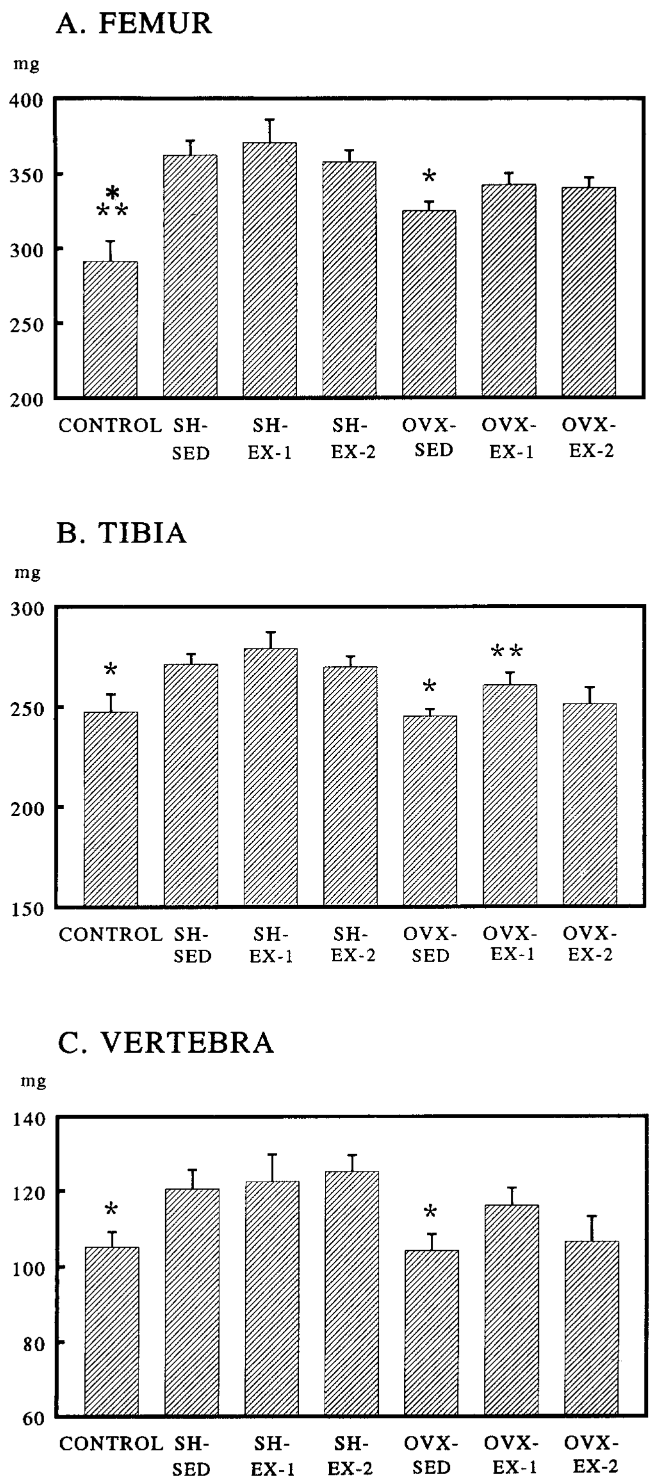

Fig. 1. Ash weights of various bones in baseline control, sham and ovariectomized, trained and untrained rats. Each bar represents mean ± SEM. The designations are indicated in Table 1. $*P < 0.05$ vs SH-SED; $**P < 0.05$ vs OVX-SED.

femur stress was significantly higher (Table 2) and the femur medullary area was significantly lower (Table 2) in the OVX-EX-2 training group compared with OVX-SED rats. The femur maximum force, strain, modulus of elasticity, length, cortical area, and moment of inertia were not changed by either exercise protocol in OVX rats (Table 2).

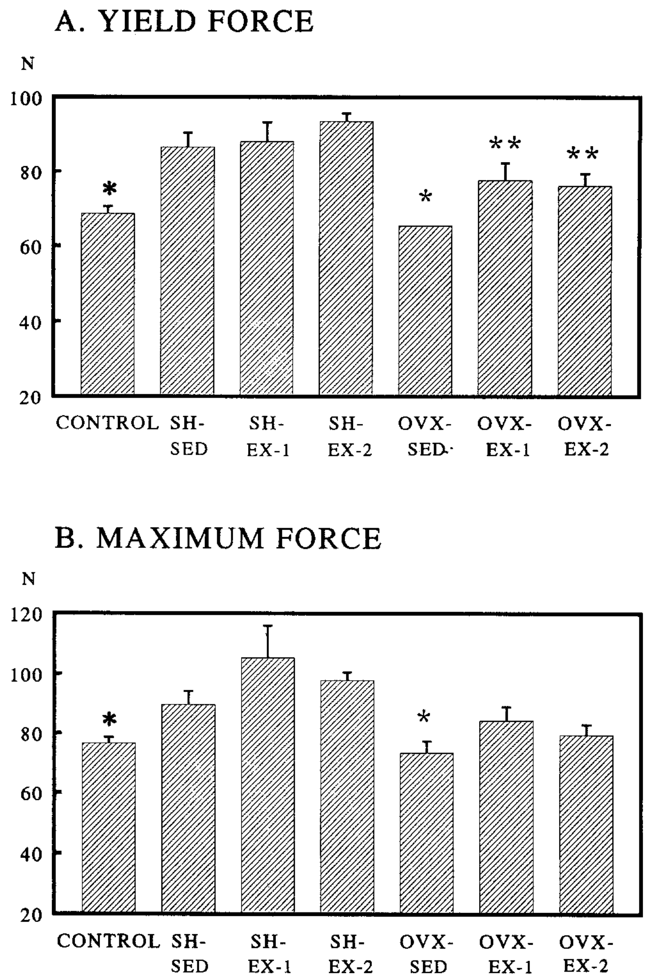

Fig. 2. Femur yield and maximum force in baseline control, sham and ovariectomized, trained and untrained rats. The data are presented as in Figure 1. The vertical axis represents yield and maximum force in Newtons (N).

Effects of Exercise in Sham-Operated Rats

The moment of inertia was higher in SH-EX-1 rats compared with SH-SED rats (Table 2). The other bone parameters, such as ash weight and biomechanical properties, were not affected by either exercise protocol in sham rats (Figs. 1 and 2, Table 2).

Discussion

We have previously demonstrated that ovariectomy is associated with lower bone mineral [9, 10]. Similarly, comparison of OVX-SED to SH-SED rats in the present studies has shown significant differences in the ash weights of various bones. In our earlier studies [10] the femur force, moment of inertia, stress, and stiffness were not changed 20 weeks post-OVX. This is in contrast to the findings in the present experiment where several of the biomechanical properties of the femur were modified by OVX, which is probably related to the lower bone mass due to longer duration of the studies (26 weeks).

The biomechanical properties of the bone are dependent not only on bone mineral but also on morphometry and/or

Table 2. Femur biomechanical and morphometric properties

Groups	Deformation, mm	Stress, MPa	Strain, $\times 10^3$	Elasticity, MPa
Biomechanical properties				
Control	0.499 ± 0.06[b]	154.2 ± 11.0[b]	18.9 ± 0.6[b]	8.27 ± 1.9
SH-SED	0.497 ± 0.02[b]	165.4 ± 9.3[b]	19.0 ± 0.9[b]	8.91 ± 0.7[b]
SH-EX-1	0.427 ± 0.02	150.3 ± 11.1	19.7 ± 0.8	7.66 ± 0.6
SH-EX-2	0.518 ± 0.01	173.6 ± 10.5	20.2 ± 0.6	8.11 ± 0.7
OVX-SED	0.374 ± 0.02[a]	108.7 ± 1.4[a]	15.9 ± 0.8[a]	6.9 ± 0.5[a]
OVX-EX-1	0.437 ± 0.02[b]	135.3 ± 14.2	17.8 ± 0.8	7.67 ± 0.9
OVX-EX-2	0.439 ± 0.02[b]	144.1 ± 7.1[b]	17.5 ± 1.1	8.44 ± 0.9

Morphometric properties

Groups	Length, mm	Cortical area, mm^2	Medullary area, mm^2	MI, cm^4 $\times 10^{-3}$
Control	36.2 ± 0.4	4.95 ± 0.29	3.36 ± 0.24[b]	0.383 ± 0.05[b]
SH-SED	36.1 ± 0.1	5.55 ± 0.14	3.36 ± 0.13[b]	0.418 ± 0.02[b]
SH-EX-1	35.6 ± 0.4	5.94 ± 0.26	3.54 ± 0.22	0.506 ± 0.05[a]
SH-EX-2	35.7 ± 0.2	5.59 ± 0.19	3.53 ± 0.17	0.451 ± 0.04
OVX-SED	36.3 ± 0.3	5.36 ± 0.27	4.6 ± 0.31[a]	0.541 ± 0.04[a]
OVX-EX-1	35.7 ± 0.4	5.63 ± 0.2	3.98 ± 0.3	0.503 ± 0.04
OVX-EX-2	36.3 ± 0.3	5.38 ± 0.22	3.58 ± 0.14[b]	0.444 ± 0.04

Values are mean ± SEM. MI = moment of inertia. Group designations are as in Table 1
[a] $P < 0.05$ vs SH-SED; [b]$P < 0.05$ vs OVX-SED

architecture. In the present studies, an increase in the femur medullary area observed in OVX-SED rats is consistent with increased endosteal resorption, which is one of the mechanisms of ovariectomy-induced osteopenia in cortical bone [13]. The medullary area was significantly smaller in the OVX-EX-2 than in the OVX-SED group, suggesting that resorption may be decreased by exercise. This observation is in agreement with previous findings that total resorptive surfaces are decreased by exercise in osteopenic rats [14].

Amount of bone mineral correlates well with bone resistance to fracture and is suggested to account for 70–80% of the mechanical strength, the remainder being explained on the basis of changes in bone organization and/or architecture [15, 16]. Moment of inertia is a measurement of bone distribution around the central axis and reflects changes in bone architecture. An increase in moment of inertia is proposed to be an adaptive response of bone to aging in female [17] and male [11] rats, reinforcing bone resistance to fracture. A recent study of age-related changes in human cortical bone found increased remodeling activity and increased endocortical porosity with aging [18]. In addition in these studies, an increase in periosteal bone formation was observed with aging. This may serve as a mechanism of preservation of mechanical integrity via expansion of outer cortical diameter [18]. In the present studies there was a trend to an increase in the moment of inertia in older sedentary rats compared with younger baseline control rats. Similarly, this parameter was increased in the OVX rats, suggesting that the bones in these animals may adapt to a decrease in bone mineral by redistribution of mass.

Femur and tibia ash weights were greater in OVX-EX-1 rats compared with OVX-SED rats. The difference was, however, significant only for tibia, suggesting that tibia is more sensitive than femur to exercise intervention, probably due to higher trabecular bone content. The beneficial effect of exercise was limited to weight-bearing bones, tibia, and femur, as there was no effect of exercise on vertebra ash weight.

The mechanism of improved bone resistance to fracture with exercise is not clearly understood. During normal activity, bone has to resist predominantly to compressive forces. Tensile force, however, is the main force acting on bone during fracture. Bending was used as a test of femur strength in the present studies as bending best reflects forces acting during fracture [19]. Bone resistance to failure in tension depends on collagen content, and resistance to compression depends on mineral content [19]. Femur resistance to load and femur tissue strength were significantly increased in OVX exercising compared with OVX sedentary rats. The corresponding increases in the femur ash were small and not statistically significant. These observations are consistent with the view that bone strength may depend not only on mineral, but also on other factors such as bone architecture and possibly matrix collagen content [15, 16, 19, 20].

In the present study, non-endurance exercise did not influence either bone mineral or femur biomechanical properties in sham-operated animals. This contrasts with the findings of our previous studies which showed a significant improvement of the femur force with endurance exercise [9]. The differences between the findings of the two studies may be explained by the different levels (non-endurance versus endurance) of exercise. In our present and previous studies, the effects of exercise were more pronounced in the OVX rats. Ovariectomy-induced decrease in bone mass is associated with a state of high bone turnover with resorption exceeding formation. This state of high bone turnover following OVX probably makes the bones more sensitive to various therapeutic manipulations.

In conclusion, the data from the present studies indicate that (1) at 26 weeks after ovariectomy, bone mineral as well as biomechanical and morphometric properties of bone are adversely affected; (2) exercise has a positive effect on bone ash in ovariectomized rats even when training is started 3 months post-OVX, after bone mass was decreased; (3) bone biomechanical properties including yield force, stress, and deformation, but not stiffness, are improved by non-endurance exercise in OVX rats with established osteopenia; (4) low- and moderate-intensity exercise do not affect bone properties of sham-operated rats.

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References

1. Saville PD (1969) Changes in skeletal mass and fragility with castration in the rat: a model of osteoporosis. J Am Geriatr Soc 17:155–166
2. Kalu DN, Liu C, Hardin RR, Holis BW (1989) The aged rat model of ovarian hormone deficiency bone loss. Endocrinology 124:7–16
3. Wronski TJ, Cintron M, Dann LM (1988) Temporal relationship between bone loss and increased bone turnover in ovariectomized rats. Calcif Tissue Int 43:179–183
4. Tuukkanen J, Peng Z, Vaananen HK (1991) Effect of exercise on osteoporosis induced by ovariectomy in rats. Calcif Tissue Int 49:S80
5. Donahue HJ, Mazzeo RS, Horvath SM (1988) Endurance training and bone loss in calcium-deficient and ovariectomized rats. Metabolism 37:741–744
6. Block JE, Smith R, Friedlander A, Genant HK (1989) Preventing osteoporosis with exercise: a review with emphasis on methodology. Med Hypotheses 30:9–19
7. Smith EL, Gilligan C (1991) Physical activity effects on bone metabolism. Calcif Tissue Int 49:S50–S54
8. Prince RL, Smith M, Dick IM, Price RI, Webb PG, Henderson NK, Harris MM (1991) Prevention of postmenopausal osteoporosis. A comparative study of exercise, calcium, and hormone-replacement therapy. N Engl J Med 325:1189–1204
9. Barengolts EB, Curry DJ, Bapna M, Kukreja SC (1991) Effects of exercise on bone loss in sham-operated or oophorectomized rats. J Bone Miner Res 6:S105
10. Barengolts EB, Gajardo HF, Rosol TJ, Kukreja SC (1990) Effects of progesterone on post-ovariectomy bone loss in aged rats. J Bone Miner Res 5:1143–1147
11. Kiebzak GM, Smith R, Gundberg CC, Howe JC, Sactor B (1988) Bone status of senescent male rats: chemical, morphometric, and mechanical analysis. J Bone Miner Res 3:37–45
12. Dunnett CW (1955) A multiple comparisons procedure for comparing several treatments with a control. J Am Stat Assoc 50: 1096
13. Turner RT, Vandersteenhoven JJ, Bell NH (1987) The effects of ovariectomy and 17β-estradiol on cortical bone histomorphometry in growing rats. J Bone Miner Res 2:115–122
14. Myburgh KH, Noaks TD, Roodt M, Hough FS (1989) Effect of exercise on the development of osteoporosis in adult rats. J Appl Physiol 66:14–19
15. Currey JD (1988) The effect of porosity and ash content on the Young's modulus of elasticity of compact bone. J Biomech 21: 131–139
16. Schaffler MB, Burr DB (1988) Stiffness of compact bone: effects of porosity and density. J Biomech 21:13–16
17. Kiebzak GM, Smith R, Howe JC, Gundberg CM, Sacktor B (1988) Bone status of senescent female rats: chemical, morphometric, and biomechanical analyses. J Bone Miner Res 3:439–446
18. Simmons ED, Pritzker KPH, Grynpas MD (1991) Age-related changes in the human femoral cortex. J Orthop Res 9:155–167
19. Albright JA (1987) Bone: physical properties. In: Albright JA, Brand RA (eds) The scientific basis of orthopaedics. New York, p 135
20. Beyer RE, Huang JC, Wilshire GB (1985) The effect of endurance exercise on bone dimensions, collagen, and calcium in the aged male rat. Experiment Geront 20:315–323