Effects of function on rat femora

Oliver Grundnes and Olav Reikerås

We evaluated the mechanical effects of different degrees of function and weight bearing on intact rat femora. Thirty rats were allocated to either an exercising, a nonweight-bearing, or a control group. The exercising rats were trained on a treadmill with a 6 weeks' running program of progressive intensity and duration. In the nonweight-bearing group, the left achilles tendon was severed at its insertion. The control rats were allowed normal activity. After 6 weeks, the animals were killed. The maximum bending moment and bending rigidity were increased in the exercised rats, whereas no differences were found between the control and nonweight-bearing rats. Bone weight and bone dimensions did not differ in the three groups. In the nonweight-bearing group, the maximum bending moment and bending rigidity were higher in the nonoperated on limb, as well as the outer and inner bone dimensions and the area moment of inertia. Our results indicate that exercise improves the mechanical characteristics of bone.

Institute of Clinical Medicine, Department of Orthopedics, University Hospital, N-9012 Tromsø, Norway
Tel +47–83 26000. Fax +47–83 26042
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Long bones are responsive to physiologic changes that occur during exercise. In animal experiments, it has been shown that collagen density, bone volume, bone weight, calcium content, and cortical thickness are increased by exercise (Woo et al. 1981, Beyer et al. 1985, Pohlman et al. 1985, Rubin and Lanyon 1985); and the beneficial effect of modest exercise on osteoporosis in postmenopausal women is well documented from clinical studies (Chow et al. 1987, Simkin et al. 1987). On the other hand, inactivity osteoporosis is a well-known clinical entity, and immobilization of animals in plaster casts and sciatic-nerve resection have proved to cause bone atrophy (Burkhart and Jowsey 1967, Pennock et al. 1972). Rubin and Lanyon (1984) demonstrated decreased bone mass after removal of load bearing in an isolated avian-bone preparation.

Our study was designed to evaluate the biomechanical effects of exercise and nonweight bearing on rat femora.

Materials and methods

The study was performed in 30 male Wistar rats (Møllegård Avlslaboratorium, Eiby, Denmark) weighing 304 (279–318) g. Initially, the rats were given two short sessions of exercise on the treadmill on 3 consecutive days. The rats were then assigned to either an exercise, a nonweight-bearing, or a control group.

Training was performed on a treadmill set at 10-percent inclination in a dark room. The exercising rats followed a 6 weeks' running program. The intensity and duration were progressive the first 2 weeks until the rats were running 2 h/day at a speed of 20 m/min. To prevent weight bearing in one of the groups, the left achilles tendon was severed just proximal to its insertion site. Thus, the rats in this group were permitted full mobility of the limb, whereas weight bearing was impossible. These rats were observed once a day to be sure of the effect of the tenotomy. The control rats were allowed unrestricted activity.

At the end of the experimental period, the animals were killed in a CO_2 chamber. In the exercised and control animals, the left femur was used for evaluations, whereas both femora were evaluated in the nonweight-bearing group. The bones were carefully dissected free from all the soft tissue and weighed. Their lengths were determined with a sliding caliper (accuracy of 0.01 mm) as the distance from the top of the femoral head to the distal end of the medial condyle. The strength of the bones was then tested in three-point bending by dorsal deflection of the distal half of the bone according to the method described by Engesæter et al. (1978). A standard hydraulic testing machine was run at a constant rate of 0.04 rads. The load values were transferred to a chart recorder displaying the load-deformation curve. The strength was calculated as the maximum bending moment. The bending rigidity was determined from the slope of the linear elastic part of the curve. Energy absorption was

Table 1. Maximum bending moment (Nm × 10^{-1}), bending rigidity (Nm/rad), and energy absorption (Nm × rad × 10^{-2}) of intact femora in exercised, control, and nonweight-bearing rats. Medians and 25–75 percentiles

	Exercised	Control	Nonweight-bearing	*P*-value		
				Exercised vs control	Nonweight bearing vs control	Nonweight bearing vs exercised
Maximum bending moment	6.33 5.60–6.82	5.47 4.78–5.83	4.73 4.36–5.52	< 0.01	NS	< 0.005
Bending rigidity	2.22 2.07–2.39	1.67 1.50–1.80	1.84 1.67–2.07	< 0.005	NS	< 0.005
Energy absorption	4.85 4.50–5.30	5.15 4.15–5.50	4.10 3.14–4.90	NS	NS	NS

NS Not significant.

defined as the energy absorbed until maximum bending moment. The outer and inner anteroposterior and transverse diameters at the fracture site were measured with a sliding caliper (accuracy of 0.01 mm). The area moment of inertia (I) was calculated assuming an elliptical cross-section using the formula:

$$I = [ab^3 - (a-t)\,(b-t)^3]/4$$

where a and b are the major and minor external radii of the hollow ellipse, and t is the thickness of the hollow ellipse.

Data are presented with medians and 25 and 75 percentiles. For statistical evaluations, we used the nonparametric Kruskall-Wallis test comparing several groups. When significant differences were found, differences between each group were calculated using the Wilcoxon's rank-sum test for two groups. To evaluate differences between the two limbs in the nonweight-bearing group, Wilcoxon's signed rank test for paired data was used. $P < 0.05$ was considered significant. To examine the correlation between the area moment of inertia and the mechanical properties, we used the Sperman's rank correlation method, and the correlation coefficient is given as r.

Results

The tenotomized rats moved their limb with ease, but function and weight bearing were insufficient throughout the experimental period as intended. The weight gain was 59 (37–75) g in exercised rats, 70 (52–89) g in tenotomized rats, and 82 (61–94) g in control animals, i.e., lower in exercised animals.

The maximum bending moment and rigidity were both increased in the exercised rats (Table 1). No differences in energy absorption were seen between the three groups. When the right femur in the nonweight-bearing group was compared with the control femurs, differences were found in bending rigidity, whereas maximum bending moment and energy absorption were not different.

In the nonweight-bearing rats, we found differences in maximum bending moment and bending rigidity between the weight-bearing and tenotomized limb ($P < 0.01$), but no differences in energy absorption were found (Table 2). In this group the outer bone dimension in the weight-bearing limb was 13.6 (12.9–14.4) mm^2, and 12.2 (11.5–13.3) mm^2 in the nonweight-bearing limb ($P < 0.01$). The inner bone dimensions in the two limbs were 4.10 (3.43–4.62) mm^2 and 3.22 (3.12–4.07) mm^2 ($P < 0.05$), respectively; and a calculation of the area moment of inertia showed differences between the two limbs (Table 2). This tendency of increases in outer and inner bone dimensions as a response to increasing load was also observed when the three groups were compared. The outer bone dimension in the exercising group was 13.1

Table 2. Maximum bending moment (Nm × 10^{-1}), bending rigidity (Nm/rad), energy absorption (Nm × rad × 10^{-2}) and area moment of inertia (mm^4) of intact femora in nonweight-bearing and weight-bearing limbs in tenotomized rats. Medians and 25–75 percentiles

	Weight-bearing side	*P*-value	Nonweight-bearing side
Maximum bending moment	5.67 5.36–5.94	< 0.01	4.73 4.36–5.52
Maximum bending rigidity	2.19 1.89–2.55	< 0.01	1.84 1.67–2.07
Energy absorption	4.39 3.42–5.25	NS	4.10 3.14–4.90
Area moment of inertia	5.74 5.52–6.27	< 0.01	4.84 4.11–5.57

Table 3. Area moment of inertia (mm^4), bone weight (g), and bone weight/body weight (10^{-2}) of intact femora in exercised, nonweight-bearing, and control rats. Medians and 25–75 percentiles. None significant differences were found

	Exercised	Control	Nonweight-bearing
Area moment of inertia	5.66 4.76–6.05	5.21 4.89–5.72	4.84 4.11–5.57
Bone weight	1.14 1.02–1.21	1.21 1.07–1.33	1.14 1.00–1.24
Bone weight/ body weight	2.37 2.07–2.51	2.26 2.01–2.40	2.32 2.11–2.50

(12.3–13.4) mm^2 and 12.6 (12.3–13.4) mm^2 in the control rats. The inner dimensions were 3.91 (3.72–4.09) mm^2 and 3.40 (3.14–3.86) mm^2, respectively. However, no differences between the three groups were found. Bone weight, relative bone weight, and area moment of inertia were not found to be different in the three groups (Table 3).

Regression analysis showed close adjustments between the area moment of inertia and energy absorption (r = 0.7573), and a slightly lower correlation between area moment of inertia and maximum bending moment (r = 0.6927). The lowest correlation was found between the area moment of inertia and bending rigidity (r = 0.4151).

Discussion

Our results confirm that systematic training leads to a better mechanical quality of bone. The results are parallel to those of other studies in man (Scoutens et al. 1989a) and in the rat (Forwood and Parker 1987, Myburgh et al. 1989).

Our results are in opposition to those of Saville and Whyte (1969) and Kiiskinen (1977), who failed to observe such effects in growing rats and mice. However, these authors used rather inaccurate methods for mechanical testing. Further, Woo et al. (1981) exercised pigs for 8 months, and found no changes in mechanical properties of cortical bones. In their study, however, only bone strips of 4 mm from the anterior, posterior, lateral, and medial parts of the diaphysis were tested, and not the entire bone as in our study.

In growing rats, increases in bone volume and bone mineral content in response to a running program have been demonstrated (Saville and Whyte 1969, Beyer et al. 1985). Intensive training may inhibit bone growth, especially in younger animals. Kiiskinen (1977) ran young mice on a 5° inclined treadmill at 30 cm/sec for 30 min/day (moderate training) and found an increase in femoral bone weights. However, when he intensified the training to 180 min/day, the femoral bone volume and length decreased. The rats in our study underwent the same program as was used by Saville and Whyte (1969), who reported an increase in calcium content due to an increase in bone volume without a change in density and unchanged mechanical properties. Such diverging results may indicate that bone may respond to physical loads in different ways, depending on both the maturity of the bone and the degree of exercise.

Hypoplasia of bone induced by immobilization has previously been studied either by means of nerve resection (Pennock et al. 1972) or plaster casts (Burckhart and Jowsey 1967), with the focus being on the structural changes. Pennock et al. (1972), using sciatic-nerve resection, demonstrated that bone loss resulting from disuse is due to diminished subperiosteal appositional growth of bone. This osteoporosis results from decreased osteoblastic activity in contrast to the osteoporosis described by Burckhart and Jowsey (1967) following immobilization in a plaster cast, which is dependent on increased bone resorption. Rubin and Lanyon (1985) demonstrated a graded dose response relationship between the peak strain magnitude and change in the mass of bone. The bone loss was achieved by increased remodeling activity, endosteal resorption, and increased intracortical porosis. We used tenotomy of the achilles tendon to obtain nonweight bearing of a limb. The rats moved their limb freely, and the musculature encircling the femur was not affected. Our results concerning the outer bone dimensions (cortical area) indicate a relation between appositional growth and degree of load. However, this increase was counteracted by increased endosteal bone resorption (inner bone dimension). The effects in this respect were small, as there were no differences when the three groups were compared. The close correlation between the area moment of inertia and energy absorption is inductive for the contention that increased appositional growth has a positive effect on the structural mechanical properties in bones. We are, however, aware that our method for determining bone resorption/apposition is not very sensitive. However, because only the inner and outer zones of cortical bone participate in remodeling (Ream et al. 1983, Danielsen et al. 1986), we consider our method to be accurate for evaluation of significant changes in this respect. Changes in bone resulting from disuse may depend on species, age, and the method by which immobilization is produced. The term *disuse osteoporosis* may be too imprecise to be applied to all the circumstances in which partial or complete immobilization of a limb occurs.

Our model, then, is comparable to the clinical situation in which a person uses crutches to prevent weight bearing. The differences in maximum bending moment, bending rigidity, outer and inner bone dimensions, and area moment of inertia between the weight-bearing and tenotomized limb document the effect of the method and reflect the effects of reduced load on one limb and increased load on the other.

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