

# Training increases the in vivo fracture strength in osteoporotic bone

## Protection by muscle contraction examined in rat tibiae

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The effect of high-intensity training on the in vivo lower leg fracture strength during muscle contraction was investigated in osteoporotic rats. 20 Wistar rats were ovariectomized and given a low calcium (0.01%) diet. 7 weeks after ovariectomy they were randomized into training (T) and sedentary (S). The S group was kept cage-confined without any intervention. The T group ran on a treadmill with 10° inclination 5/7 days for 8 weeks. A maximum intensity of 27 m/min was reached after 4 weeks. After 8 weeks, the right lower legs of the anesthetized animals were loaded in three-point ventral bending until fracture occurred during electrically-induced muscle contraction. The left tibiae were excised and fractured at the same level as the right tibiae. Weight gain was equal in the two groups. Energy absorption and de-

flection at fracture were significantly higher in the T group than in the S group in vivo during muscle contraction. In vitro, there were no significant differences in mechanical results. The mediolateral outer diameter was larger in the T group, and the maximal stress that the tibia could withstand was lower than in the S group.

We conclude that 8 weeks of high-intensity training of osteoporotic rats increased the structural lower leg strength during muscle contraction. The reduced maximal stress in the training animals indicates a reduction in bone material quality. The increase of in vivo structural strength must reflect an increased protective effect of muscle contraction due to training.

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Several studies show the positive effects of physical activity in preventing hip fractures (Cooper et al. 1988, Paganini-Hill et al. 1991, Jaglal et al. 1993) and some exercise programs show an effect on bone density (Chow et al. 1987, Cheng et al. 1991, Nelson et al. 1994). Although bone mineral density is correlated to physical ability (Vico et al. 1995) and to moderate walking activities in postmenopausal women (Krall and Dawson-Hughes 1994), it seems difficult to increase bone mass by exercise after the menopause (Lau et al. 1992, Pruitt et al. 1992). Aniansson et al. (1984) found an impaired muscle function with aging that correlates with decreased physical activity. Quadriceps strength was strongly correlated with activities of daily living such as climbing steps and walking, and satisfactory quadriceps function may protect against falls. Even very old people can strengthen their muscles immensely (Fiatarone et al. 1990, Lexell et al. 1995, McCartney et al. 1995). However, the ideal training program for preventing hip fractures has yet to be devised.

In rats, the results of training vary and depend on training intensities or on the bones studied (Pohlman et al. 1985, Donahue et al. 1988, Raab et al. 1990, Forwood and Parker 1991, Li et al. 1991, Tuukkanen et al. 1991, Grundnes and Reikerås 1992, Bourrin et al. 1994, Chen et al. 1994, Kannus et al. 1994, Mosekilde et al. 1994, Peng et al. 1994, Sjøgaard et al. 1994, Tuukkanen et al. 1994). Less attention has been paid to effects on other structures in the musculoskeletal system (Saville and Whyte 1969). Nordsletten and Ekeland (1993b) found that muscle had a protective effect against lower leg fracture just by being there and that the structural strength of normal rat tibia was substantially greater during muscle contraction than when tested with relaxed muscles (Nordsletten and Ekeland 1993a). The same protective effect of muscle contraction has been found in osteoporotic and in normal female rats (Nordsletten et al. 1994). Treadmill training increased this effect in male rats (Nordsletten et al. 1993). Training of female rats during the first 8 weeks after ovariectomy had no effect

compared to sedentary controls (Kaastad et al. 1996). We investigated the effect of high intensity training on the in vivo lower leg fracture strength in rats with established osteoporosis.

## Animals and methods

20 female Wistar rats (Møllegaard, Copenhagen), aged 12 weeks, with a mean weight of 202 (8.2) g were ovariectomized via the dorsal route (Waynforth 1980) and given a low calcium diet (Ca 0.01%, P 1.2 %, Vitamin D<sub>3</sub> 1500 IU/kg). They had free access to food and tap water and 3 or 4 were housed in wire-top plastic cages (24 × 33 cm wide, 15 cm high) in a room with a light/dark cycle of 12/12 hours. Ovariectomy was confirmed at autopsy by failure to detect ovarian tissue. During operation and testing, the animals were anesthetized with a combination of Hypnorm® and Dormicum®. The dose was 0.2 mL/100g body weight subcutaneously. The experiment conformed to the Norwegian Council's of Animal Research Code for the Care and Use of Animals for Experimental Purposes. 7 weeks after ovariectomy the rats were randomized into 2 groups: training (T) and sedentary (S).

### Experimental groups

**Training.** 10 animals were conditioned to run on a treadmill having a 10% inclination with increasing speed for one hour 5 days a week. A maximum intensity of 27 m/min was reached after 4 weeks, and the original one hour daily was reduced to 45 min. because of increasing exhaustion among the animals. Each treadmill lane measured 52 × 6.5 cm and was equipped with a system that delivered compressed air at the rear to encourage running. The frontal half of the treadmill was covered to give a dark area in which the rats preferred to run.

**Sedentary.** 10 animals were kept cage-confined without any intervention.

### Test fracture

8 weeks after the start of training, the right lower legs of all animals were tested in vivo during muscle contraction. This test fracture has been described previously (Nordsletten and Ekeland 1993a). The ischiatic nerve was connected to a nerve stimulator (Pulsar 6i, Frederick Haer, Brunswick, ME, USA). A clamp was fixed to the distal lower leg with suspension under the foot, and the rat was placed in a modular test apparatus. The lower leg was deflected ventrally in three-point bending until tibial fracture occurred at a quasi-static rate of 0.095 radians/second (5.43°/s) during supramaximal stimulation (0.5 ms square pulses of 80

Hz with amplitude of 6V) of the ischiatic nerve. The cam engaged the soft tissues dorsally to the upper part of the tibia. A fulcrum positioned anteriorly to the leg was the third point of force application.

Both tibiae were resected and cleansed of all soft tissues. The in vitro tibiae were kept wet in Ringers® solution between dissection and testing. The tibial length from the malleolar plane to the intercondylar ridge was measured in the in vitro tibiae with sliding callipers (accuracy of ± 0.05 mm). The fracture position in the tibia tested in vivo was measured from the malleolar plane, and the corresponding position was marked on the contralateral tibia. The in vitro tibia was placed in the test apparatus and loaded in the same way as the in vivo tibia with the fulcrum positioned over the fracture mark.

The load in the test apparatus was measured with a load cell connected to a microcomputer via an amplifier. The load/deflection curve was recorded on-line in WorkBenchMac (Strawberry Tree Incorporated, Sunnyvale, CA, USA). Ultimate bending moment, ultimate energy absorption, bending stiffness and deflections were read directly or calculated from the computer recordings of the load/deflection curves (Nordsletten and Ekeland 1993a).

Before the rats were killed, blood was withdrawn from the aorta for determinations of the serum corticosterone.

### Geometry

The anteroposterior and mediolateral periosteal diameters of the left tibia were measured with sliding callipers just proximal to the fracture line. The diameter of the medullary canal was measured by inserting cannulae of increasing diameters varying by 0.05 mm. The diameter of the largest cannula that could be inserted was taken as the medullary diameter. The cross-section of the tibia was assumed to be elliptical in shape with a circular marrow canal, and the area moment of inertia (I) was calculated by the formula

$$I = \frac{1}{64} \pi (a^3b - d^4)$$

where a and b are the anteroposterior and mediolateral periosteal diameters and d is the diameter of the medullary canal. The ultimate stress ( $\sigma_{ult}$ ) at the surface of the tibia was calculated as

$$\sigma_{ult} = \frac{BM_{ult} \frac{a}{2}}{I}$$

where  $BM_{ult}$  is the ultimate bending moment and a is the anteroposterior periosteal diameter (Bak and Jensen 1992).

Table 1. Mechanical results in the right lower leg, tested during tetanic muscle contraction (in vivo), and in the left lower leg, tested excised (in vitro), in ovariectomized rats on a low calcium diet undergoing training (T) or kept sedentary (S). Values are mean (SD)

Group	n	Bending moment (Nm $\times 10^{-2}$ )		Energy absorption (J $\times 10^{-2}$ )		Bending stiffness (Nm $^{\circ}$ $\times 10^{-3}$ )		Deflection ( $^{\circ}$ )	
		In vivo	In vitro	In vivo	In vitro	In vivo	In vitro	In vivo	In vitro
T	8	57 (6.3)	40 (5.3)	20 (3.6)	9.1 (3.0)	18 (2.8)	26 (2.0)	40 (4.7)	21 (3.7)
	p	0.3	0.6	0.04	0.9	0.6	0.8	0.03	0.8
S	10	53 (9.7)	42 (5.1)	16 (4.5)	9.2 (3.2)	17 (3.6)	25 (5.0)	34 (4.9)	20 (4.2)

Table 2. Geometry, area moment of inertia (I), and ultimate stress in the left tibial shaft in ovariectomized rats on a low calcium diet undergoing training (T) or kept sedentary (S). Values are mean (SD)

Group	n	Mediolateral diameter (mm)	Anteroposterior diameter (mm)	Medullary diameter (mm)	I (mm $^4$ )	Maximum stress (N/mm $^2$ )
		T	8	2.61 (0.12)	2.67 (0.16)	1.24 (0.10)
	p	0.05	0.5	0.4	0.3	0.04
S	10	2.48 (0.13)	2.62 (0.16)	1.20 (0.11)	2.10 (0.44)	264 (36)

### Statistics

The results are given as mean (standard deviation). The Student's two sample t-test was used for evaluation of group differences.  $P < 0.05$  (two-sided) was considered significant.

### Results

In the training (T) group, 1 rat was excluded because she refused to run after 2 weeks. The others completed the training program, but failure in muscle stimulation at testing excluded yet another T animal.

Weight gain was equal in the 2 groups with final weights 329 (35) g in the T group and 322 (21) g in the S group. Corticosterone levels did not differ and were 2999 (439) nmoL/L in the T group and 2743 (855) nmoL/L in the S group. Tibial length was 40.0 (0.8) mm in the T and 40.0 (0.7) mm in the S animals, and the mean fracture site measured from the malleolar plane was 15.9 mm in vivo and 16.4 mm in vitro in the T rats and 16.6 and 16.5 mm, respectively, in the S rats.

The mechanical parameters at fracture in vivo during muscle stimulation were all higher in the T group than in the S group, with differences for energy absorption (25% higher,  $p 0.04$ ) and deflection (16% higher,  $p 0.03$ ) (Table 1). There were no differences between T and S animals in the mechanical parameters at in vitro fracture (Table 1).

The mediolateral diameter was larger in the T group ( $p 0.05$ ), while the anteroposterior and medul-

lary diameters were similar. This resulted in an insignificant increase in area moment of inertia, but the maximal stress the leg could withstand was significantly lower in the T rats ( $p 0.04$ ) (Table 2).

### Discussion

The increase in lower leg structural strength during muscle contraction in the trained rats occurred without a corresponding increase of strength in the excised nude tibiae. Therefore, the protective effect against fracture from high-intensity training must have been a function of the soft tissues. Saville and Whyte (1969) found that running exercise in the rat increased bone volume and mass as well as muscle mass, but breaking force in the femur and humerus in vitro were not affected. They did not test the fracture strength in vivo, but it has been shown more recently that active muscle contraction increased the in vivo structural strength in the lower legs of the rat (Nordsletten and Ekeland 1993a). The increased in vivo strength found in trained rats in the current study indicates that high-intensity training increased this muscle-protective effect. The same training effect has been found in male rats (Nordsletten et al. 1993), while female rats training at the same intensity in the first 8 weeks after ovariectomy, had no increase in the protective effect of muscle contraction (Kaastad et al. 1996). In the latter study the trained rats had a significantly increased serum corticosterone level three days after the last training session. This prolonged cortico-

sterone increase might have reduced the muscle training potential by reducing muscle protein synthesis (Southorn et al. 1990) and increasing myofibrillar protein breakdown (Hall-Angerås et al. 1990). In the present study, corticosterone values were similar in trained and sedentary rats, and it seems that the lower leg muscles were able to respond positively to training, in spite of the depletion of estrogen and calcium. Male rats training at the same intensity were hormone- and calcium-replete (Nordsletten et al. 1993).

Prospective human studies are not very helpful for evaluating postmenopausal bone changes directly by histomorphometric and mechanical measurements, and several authors have used rat models for this purpose (Kalu et al. 1989, Wronski et al. 1989, Frost and Jee 1992). The ovariectomized rat model causes changes in bone that resemble most of the characteristics of human postmenopausal bone loss, but the increased fracture risk associated with osteoporosis is missing. This causes some to refer to the bone changes seen as osteopenia rather than osteoporosis. On the other hand, Kalu (1991) found that the wide-ranging similarities made this model suitable for studying problems that are relevant to postmenopausal bone loss. The ideal age of the rat has been discussed. We chose 12-week-old, mature female Wistar rats in which ovariectomy alone produces osteoporosis in cancellous bone within a few weeks (Wronski et al. 1989). However intracortical remodeling usually occurs only in older rats (Jee et al. 1990, Kalu 1991) making it more difficult to achieve cortical osteoporosis. Hodgkinson et al. (1978) found that the combination of ovariectomy and calcium deprivation in female rats increased bone loss significantly compared to calcium deprivation or ovariectomy alone. Nordsletten et al. (1994) have confirmed that ovariectomy induces significant cortical and trabecular osteoporosis after seven weeks on a low-calcium diet. They also found that muscle contraction was not affected by the lower, yet physiological, serum calcium in the ovariectomized rats (Nordsletten et al. 1994).

In our study, high-intensity training, 27 m/min treadmill running, was chosen as the weight-bearing exercise (Forwood and Parker 1991, Li et al. 1991). This program has been shown to have an effect on lower leg structural strength, without affecting skeletal strength in male rats (Nordsletten et al. 1993). The ovariectomized rats started the training program after seven weeks when osteoporosis was established (Nordsletten et al. 1994). The exercise protocol started at 13 m/min with a 10% inclination, reaching 22 m/min for one hour by the second week. This intensity was well tolerated by all the training rats, but as the intensity increased half the group completed it with-

out problems and the other half suffered after about 30 min. The chosen intensity of 27 m/min was reached after four weeks of training, but due to increasing exhaustion among the bad runners, the duration was reduced to 45 min. 5/7 days. There were no differences in the test results between the good and the bad runners. Hou et al. (1990) trained younger, unoperated female Sprague-Dawley rats (160g) for 10 weeks at a similar intensity, without problems. The reduced training capacity is probably related to the estrogen depletion. Kendrick et al. (1987) found that estradiol influenced myocardial glycogen utilization and also spared tissue glycogen during submaximal exercise when ovariectomized rats ran to exhaustion on a treadmill. Estradiol-replaced rats ran significantly longer and completed more total work than did sham-injected controls.

Tibial lengths were equal in the two groups indicating that bone growth was not affected by the exercise. Geometrical measurements of the *in vitro* fractured left tibiae showed a significant increase in mediolateral diameter in the training animals. The same effect was found in male rats trained at the same intensity (Nordsletten et al. 1993). As in the male rats, there were no differences between the training and sedentary rats in structural strength of the excised tibiae. However, the maximal stress which the tibiae could withstand was significantly decreased in the training animals, indicating a reduction in the material qualities of the bone. Forwood and Parker (1991) looked for microdamage in tibiae and femora of male Wistar rats after repeated bouts of treadmill-running at the same intensity as ours. The histological analyses revealed no evidence of microdamage, and they suggested that the damage in cortical bone during loading involved a more subtle disruption of the tissue on a submicroscopic scale (Forwood and Parker 1991).

In our study, high-intensity training of ovariectomized rats increased the *in vivo* structural strength of the lower leg thanks to an increased protective effect of muscle contraction in spite of unchanged fracture strength and reduced bone material quality of the tibia.

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## References

- Aniansson A, Zetterberg C, Hedberg M, Henriksson K G. Impaired muscle function with aging. A background factor in the incidence of fractures of the proximal end of the femur. *Clin Orthop* 1984; 191: 193-201.
- Bak B, Jensen K S. Standardization of tibial fractures in the rat. *Bone* 1992; 13 (4): 289-95.
- Bourrin S, Genty C, Palle S, Gharib C, Alexandre C. Adverse effects of strenuous exercise: a densitometric and histomorphometric study in the rat. *J Appl Physiol* 1994; 76 (5): 1999-2005.
- Chen M M, Yeh J K, Aloia J F, Tierney J M, Sprintz S. Effect of treadmill exercise on tibial cortical bone in aged female rats—a histomorphometry and dual energy X-Ray absorptiometry study. *Bone* 1994; 15 (3): 313-9.
- Cheng S, Suominen H, Rantanen T, Parkatti T, Heikkinen E. Bone mineral density and physical activity in 50-60-year-old women. *Bone Miner* 1991; 12 (2): 123-32.
- Chow R, Harrison J E, Notarius C. Effect of two randomised exercise programmes on bone mass of healthy postmenopausal women. *Br Med J* 1987; 295 (6611): 1441-4.
- Cooper C, Barker D J, Wickham C. Physical activity, muscle strength, and calcium intake in fracture of the proximal femur in Britain. *Br Med J* 1988; 297: 1443-6.
- Donahue H J, Mazzeo R S, Horvath S M. Endurance training and bone loss in calcium-deficient and ovariectomized rats. *Metabolism* 1988; 37: 741-4.
- Fiatarone M A, Marks E C, Ryan N D, Meredith C N, Lipsitz L A, Evans W J. High-intensity strength training in nonagenarians. Effects on skeletal muscle. *JAMA* 1990; 263 (22): 3029-34.
- Forwood M R, Parker A W. Repetitive loading, in vivo, of the tibiae and femora of rats: Effects of repeated bouts of treadmill-running. *Bone Miner* 1991; 13 (1): 35-46.
- Frost H M, Jee W. On the rat model of human osteopenias and osteoporosis. *Bone Miner* 1992; 18 (3): 227-36.
- Grundnes O, Reikerås O. Effects of function on rat femora. *Acta Orthop Scand* 1992; 63 (1): 70-3.
- Hall-Angerås M, Angerås U, Zamir O, Hasselgren P-O, Fischer J. Interaction between corticosterone and tumor necrosis factor stimulated protein breakdown in rat skeletal muscle, similar to sepsis. *Surgery* 1990; 108 (2): 460-6.
- Hodgkinson A, Aaron J E, Horsman A, McLachlan M S F, Nordin B E C. Effect of oophorectomy and calcium deprivation on bone mass in the rat. *Clin Sci Mol Med* 1978; 54: 439-46.
- Hou J C, Salem G J, Zernicke R F, Barnard R J. Structural and mechanical adaptations of immature trabecular bone to strenuous exercise. *J Appl Physiol* 1990; 69 (4): 1309-14.
- Jaglal S B, Kreiger N, Darlington G. Past and recent physical activity and risk of hip fracture. *Am J Epidemiol* 1993; 138 (2): 107-18.
- Jee W, Mori S, Li X, Chan S. Prostaglandin E2 enhances cortical bone mass and activates intracortical bone remodeling in intact and ovariectomized female rats. *Bone* 1990; 11: 253-66.
- Kaastad T S, Reikerås O, Narum S, Madsen J E, Haug E, Obrant K J, Nordsletten L. Effect of intensive training on lower leg structural strength: an in vivo study in oophorectomized rats. 1996; Submitted.
- Kalu D N. The ovariectomized rat model of postmenopausal bone loss. *Bone Miner* 1991; 15 (3): 175-91.
- Kalu D N, Liu C C, Hardin R R, Hollis B W. The aged rat model of ovarian hormone deficiency bone loss. *Endocrinology* 1989; 124 (1): 7-16.
- Kannus P, Sievanen H, Jarvinen T L, Jarvinen M, Kvist M, Oja P, Vuori I, Jozsa L. Effects of free mobilization and low- to high-intensity treadmill running on the immobilization-induced bone loss in rats. *J Bone Miner Res* 1994; 9 (10): 1613-9.
- Kendrick Z V, Steffen C A, Rumsey W L, Goldberg D I. Effects of estradiol on tissue glycogen metabolism in exercised oophorectomized rats. *J Appl Physiol* 1987; 63: 492-6.
- Krall E, Dawson-Hughes B. Walking is related to bone density and rates of bone loss. *Am J Med* 1994; 96: 20-6.
- Lau E, Woo J, Leung P C, Swaminathan R, Leung D. The effects of calcium supplementation and exercise on bone density in elderly Chinese women. *Osteoporosis Int* 1992; 2 (4): 168-73.
- Lexell J, Downham D, Larsson Y, Bruhn E, Morsing B. Heavy-resistance training in older Scandinavian men and women: short- and long-term effects on arm and leg muscles. *Scand J Med Sci Sports* 1995; 5: 329-41.
- Li K C, Zernicke R F, Barnard R J, Li A F. Differential response of rat limb bones to strenuous exercise. *J Appl Physiol* 1991; 70 (2): 554-60.
- McCartney N, Hicks A, Martin J, Webber C. Long-term resistance training in the elderly: effects on dynamic strength, exercise capacity, muscle, and bone. *Biol Sci* 1995; 50A (2): B97-104.
- Mosekilde L, Danielsen C C, Sogaard C H, Thorling E. The effect of long-term exercise on vertebral and femoral bone mass, dimensions, and strength - assessed in a rat model. *Bone* 1994; 15 (3): 293-301.
- Nelson M E, Fiatarone M A, Morganti C M, Trice I, Greenberg R A, Evans W J. Effects of high-intensity strength training on multiple risk factors for osteoporotic fractures. A randomized controlled trial. *JAMA* 1994; 272 (24): 1909-14.
- Nordsletten L, Ekeland A. Muscle contraction increases the structural capacity of the lower leg: An in vivo study in the rat. *J Orthop Res* 1993a; 11 (2): 299-304.
- Nordsletten L, Ekeland A. Muscle contribution to tibial fracture strength in rats. *Acta Orthop Scand* 1993b; 64 (2): 157-60.
- Nordsletten L, Kaastad T S, Obrant K J, Skjeldal S, Kirkeby O J, Stokke O, Ekeland A. Muscle contraction increases the in vivo structural strength to the same degree in osteopenic and normal rat tibiae. *J Bone Miner Res* 1994; 9 (5): 679-85.
- Nordsletten L, Kaastad T S, Skjeldal S, Kirkeby O J, Reikerås O, Ekeland A. Training increases the in vivo strength of the lower leg: An experimental study in the rat. *J Bone Miner Res* 1993; 8 (9): 1089-95.
- Paganini-Hill A, Chao A, Ross R K, Henderson B E. Exercise and other factors in the prevention of hip fracture: The Leisure World study. *Epidemiology* 1991; 2 (1): 16-25.

- Peng Z, Tuukkanen J, Vaananen H K. Exercise can provide protection against bone loss and prevent the decrease in mechanical strength of femoral neck in ovariectomized rats. *J Bone Miner Res* 1994; 9 (10): 1559-64.
- Pohlman R L, Darby L A, Lechner A J. Morphometry and calcium contents in appendicular and axial bones of exercised ovariectomized rats. *Am J Physiol* 1985; 248: R12-7.
- Pruitt L A, Jackson R D, Bartels R L, Lehnhard H J. Weight-training effects on bone mineral density in early postmenopausal women. *J Bone Miner Res* 1992; 7 (2): 179-85.
- Raab D M, Smith E L, Crenshaw T D, Thomas D P. Bone mechanical properties after exercise training in young and old rats. *J Appl Physiol* 1990; 68 (1): 130-4.
- Saville P D, Whyte M P. Muscle and bone hypertrophy. Positive effect of running exercise in the rat. *Clin Orthop* 1969; 65: 81-8.
- Southorn B, Palmer R, Garlick P. Acute effects of corticosterone on tissue protein synthesis and insulin-sensitivity in rats in vivo. *Biochem J* 1990; 272 (1): 187-91.
- Søgaard C H, Danielsen C C, Thorling E B, Mosekilde L. Long-term exercise of young and adult female rats—effect on femoral neck biomechanical competence and bone structure. *J Bone Miner Res* 1994; 9 (3): 409-16.
- Tuukkanen J, Peng Z, Väänänen H K. Effect of exercise on osteoporosis induced by ovariectomy in rats. *Calcif Tissue Int* 1991; 49: S80.
- Tuukkanen J, Peng Z, Vaananen H K. Effect of running exercise on the bone loss induced by orchidectomy in the rat. *Calcif Tissue Int* 1994; 55 (1): 33-7.
- Vico L, Pouget J F, Calmels P, Chatard J C, Rehalia M, Minnaire P, Geysant A, Alexandre C. The relations between physical ability and bone mass in women aged over 65 years. *J Bone Miner Res* 1995; 10 (3): 374-83.
- Waynforth H B. Experimental and surgical technique in the rat. Academic Press Inc. Ltd, London 1980.
- Wronski T J, Dann L M, Scott K S, Cintron M. Long-term effects of ovariectomy and aging on the rat skeleton. *Calcif Tissue Int* 1989; 45 (6): 360-6.