THE EFFECTS OF A PROGRESSIVE LOADING EXERCISE PROGRAM ON FEMORAL PHYSICAL PROPERTIES AND STRENGTH OF OSTEOPENIC RATS

*Ana Claudia Muniz Renno[1], Flavia Roberta Faganello[1], Fernanda Mendes de Moura[2],
Nádia Slemer Andrade dos Santos [2], Renata Passarelli Tirico[2], Paulo Sérgio Bossini[3], Jose Antonio Zuanon[4],
Carlos Benatti Neto[4], Nivaldo Antonio Parizotto[5]*

SUMMARY

Background: Many studies have shown that physical exercises are able to stimulate bone formation and increase bone mass, constituting a therapeutic modality to treat bone loss due to osteoporosis. However, some points about the intensity, duration and frequency of the exercises remain confusing and contradictory. Thus, the aim of this study was to determine the effects of a progressive loading exercise program on femur of osteopenic rats. To induce osteopenia we used the animal model of ovariectomy (OVX). Forty animals was studied and divided into 4 groups: sham-operated sedentary (SS); ovariectomy-sedentary (OS); sham-operated training (ST) and ovariectomy training (OT). The trained groups performed jumps into water: 4 series of 10 jumps each, with an overload of 50% to 80% of the animal's body weight, during 8 weeks. Femora were submitted to a physical properties evaluation, a biomechanical test, calcium and phosphorus content measurement and a morphometric histological evaluation. Results: osteopenic animals showed a decrease of bone strength and lower values of bone weights, bone density and calcium content. The exercised osteopenic rats showed higher values of geometrical, physical properties, bone strength and calcium content compared to controls. The results of the present study indicate that the progressive loading exercise program had stimulatory effects on femora of osteopenic rats. It seems that the intensity and duration of the protocol used produced bone structural adaptations, which contributed to reverse bone loss due to ovariectomy.

Keywords: *Osteoporosis; Rats; Ovariectomy; Physical exercise.*

Citation: *Renno ACM, Faganello FR, Moura FM, Santos NSA, Tirico RP, Bossini PS et al. The effects of a progressive loading exercise program on femoral physical properties and strength of osteopenic rats. Acta Ortop Bras. [serial on the Internet]. 2007; 15(5): 276-279. Available from URL: http://www.scielo.br/aob.*

INTRODUCTION

Osteoporosis is a disease with an immense social-economic significance and it has been recognized as a major public health problem. It is a bone disorder, characterized by loss of bone mass, resulting in bone weakness and in an increased of susceptibility to fractures(1,2).

Many treatments have been developed with the aim of preventing bone loss and increasing bone mass, including estrogen replacement, bisphosphonate compounds and physical activity programs(3). Physical activity has been advocated as offering a potential means to increase and to maintain bone mineral density (BMD), constituting an efficient treatment to manage bone loss in osteoporotic patients.

Animal studies investigating osteogenic responses to exercise have examined many kinds of training including running and walking(4,5) and swimming exercises(8,9) in young and osteopenic rats.

Recent studies have showed that dynamic exercises are more effective to stimulate bone tissue than static exercises(10,11). It is thought that bone sensitivity wanes quickly after exercise initiation, reaching a mechanosensorial saturation, after which bone mass just increase if the magnitude of loading increases(8,11). Moreover, many authors state that short periods of exercise, with rest periods between them are more effective to produce an osteogenic response than a single longer session recovery period(10,11).

Although, many studies have demonstrated a stimulatory effect of exercises on bone tissue, the mechanism by which exercise acts on bones is not fully understood. Moreover, some points about the intensity, duration and frequency of the exercises remain confusing and contradictory. Therefore, it is important to study all aspects of bone response to physical exercises thus it can be used with confidence as a treatment within the clinical setting.

Little is known about the effects of a progressive loading exercise programs performed in aquatic environments on bone tissue of osteopenic rats. We hypothesized that muscle contractions performed during jump exercises could be efficient to induce an osteogenic effect, promoting an increase in femoral bone strength and mass, even into the water, where the gravitational force are decreased. This type of exercise could be useful in osteoporotic patients, constituting a safer therapeutic modality instead of traditional exercises, as walking, once the risk of falls and consequently fractures are decreased in the aquatic environment. In this context, the aim of this study was to determine the response of femora of osteopenic rats to a progressive exercise training. Femora were examined anthropometrically, evaluated using a morphometric analysis and a biomechanical test to determine their mechanical strength was performed.

Study conducted at the Federal University of São Carlos (UFSCar).
Correspondences to: Rua Jose Paulino 1800 apt. 93, Centro, Campinas - São Paulo, Brasil, CEP 13013002.E-mail: acmr_ft@yahoo.com.br

1. PhD, Department of Physiotherapy, Federal University of São Carlos;
2. Physiotherapy Student, Federal University of São Carlos,
3. Master Degree Student, Department of Physiotherapy, Federal University of São Carlos;
4. PhD, Department of Physiology and Pathology, São Paulo State University- UNESP;
5. PhD, Department of Physiology and Pathology, São Paulo State University- UNESP;
6. PhD, Department of Physiotherapy, Federal University of São Carlos.

Received in: 12/12/06; approved in: 07/05/07

MATERIALS AND METHODS

Forty female Wistar rats (12 weeks, ± 250 g) were used in this experiment. Rats were randomized into 4 groups, with 10 animals each: sham-operated sedentary (SS); ovariectomy-sedentary (OS); sham-operated training (ST) and ovariectomy training (OT).

The remaining animals were kept in a temperature-controlled room (22 ± 2° C) at the Animal Experimentation Laboratory of Federal University of Sao Carlos, with a light-dark cycle of 12/12 hours. The animals were given standard laboratory rat food and were provided with water ***ad libitum***.

Surgical Procedure of Ovariectomy

Ovariectomy (OVX) was performed via bilateral translumbar incisions, under Ketamine/ Xylazine anesthesia (80/10 mg/ Kg). The uterine tubes were ligated (Catgut 4.0) and after removal of the ovaries, the incisions were closed (Catgut 3.0). Sham operated groups were submitted to a skin incision and sutures. After the surgery, all animals were conditioned during 8 weeks with the purpose of inducing osteopenia(3).

Progressive loading exercise program

The rats were trained at the same time of the day. The training program was consisted of a progressive loading exercise program. Training occurred in a container (Height: 1m; Diameter: 80 cm), 60% filled with warm water (33° C). The training started on the day 61 post-OVX. Exercised rats were trained 3 times/ week, for 8 weeks. The training program was carried out fixing an additional load on the animal body trough an appropriated vest that allowed the jump execution without the vest slipping off from the animal's body(12). The load progressed during the experiment and was adjusted daily (Table 1). To reduce animal stress, the animals were adapted to water in the first week (pre-training). This adaptation consisted of sessions of weight lifting (40% body weight load), once a day for 5 days. After the pre-training week, animals initiate the experimental training protocol which consisted of jumps into the water[12] (Figure 1).

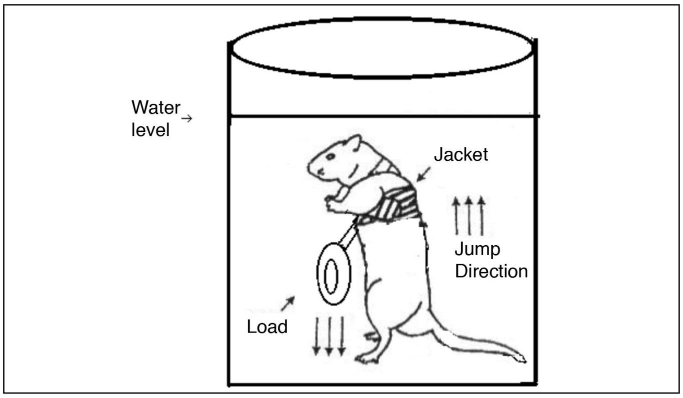

***Figure 1**- Schematic view of the container*

Weeks	Weight
Day 1	20% of body mass
Day 2	30% of body mass
Day 3	40% of body mass
Days 4 -7	50% of body mass
2	50% of body mass
3 and 4	60% of body mass
5 and 6	70% of body mass
7 and 8	80% of body mass

***Table 1** - Physical exercise program*

Briefly, the training protocol was as follows. First and second training weeks: 4 series × 10 jumps, overload at 50% of body weight. Second week: 4 series × 10 jumps, overload at 60% of the rat body weight. Third and fourth: 4 series × 10 jumps, at 60%. Fifth and sixth weeks: 4 series × 10 jumps, at 70% and seventh and eighty weeks: 4 series × 10 jumps, at 80%. A rest of 30 s was allowed among the series. An observer was present during all the training sessions(12).

This study was approved by the Ethic Committee of animal studies of the Federal University of Sao Carlos.

After 8 experimental weeks, rats were sacrificed by an overdose of general anesthetic. The success of the operation was confirmed at necropsy by failure to detect ovary tissue and by observation on marked atrophy of uterine tube. Both femora were collected and cleaned from soft tissues for analysis.

Femoral Length: this measure was taken in the left femora, from the femoral major throcanter until the tip of lateral condyle, using a digital caliper (Bron-Sharp, error measure = 0,1%). In sequence, the left femora were submitted to the biomechanical test.

Mechanical testing: this test was performed in the laboratory of Human Physiology of Federal University of São Carlos. Biomechanical properties of femora were determined by a three-point bending test in an Instron Universal Testing Machine (Instron cop, Cantn, MA), 4444 model). Before the test, the bones were thawed at room temperature. Each bone was placed on a special holding device with 2 supports located at a distance of 13 mm. Femora were loaded to failure in the three-point bending test at the middle point of femora, with a perpendicular cross head speed of 5 mm/min. From de load-deformation curve, the maximal load (N), structural stiffness (N/mm) and energy absorption (mJ) were obtained.

Physical Properties: After the mechanical test, both parts of right femora were burned at 800°C for 24 h, to obtain the ash weight (AW), using a digital electronic scale of high precision (Chyo, error measure = 0,1%). The bone density (BD) and volume (BV) were calculated using the Archimedes' principle(13).

Geometry properties: After sacrifice, left femora were fixed in formalin and decalcified (using the Morse solution- Sodium Citrate, 20% and Formic Acid, 50%). For geometry properties analysis, femora were cut in the middle and both parts were embedded in paraffin and processed for histological analyses. Transversal 5 mc sections were cut and stained with Masson stain. Pictures of each section were taken, using a Microscope (OLYMPUS), with a 40 X objective, and a camera coupled to the microscope. A specific software system analyzer Imagelab was used to calculate the cross-sectional area, medullar area and the inner and outer diameters.

Calcium and phosphorus content: Calcium and phosphorus content: Spectrophotometric analysis indicating overall calcium and phosphorus content was determined using at an absorbance wavelength at 490nm.

STATISTICAL ANALYSIS

The results are given in mean and standard deviation. The ANOVA test was used to compare changes among the groups and the Duncan´s test to identify the differences. A p level of ≤ 0.05 was considered as being statically significant.

RESULTS

It can be observed in table 2 that the initial body mass of the animals did not show any statistical difference among them.

Ovariectomy produced a statistically significant increase in final body mass and in the variation of body mass during the experiment (Table 2).

Groups (n)	Initial body mass (g)	Final body mass (g)	Increase of body mass (g)
Basal	251,66 ± 9,42		
OS	248 ± 6,6	305,5 ± 8,5	57,5 ± 2
SS	246,5 ±14,11	285,5 ± 17,9 ^{i}	38,5 ± 3,7 ^{i}
ST	255,5 ± 13,54	288,8 ±14,65 ^{i}	33,3 ± 0,2 ^{i}
OT	249,2 ± 5,7	302,4± 11,84 $^{* a}$	53,2 ± 6,1* ^{a}

i vs OS; * vs SS; a vs ST, p ≤ 0.05

***Table 2** - Body mass of rats*

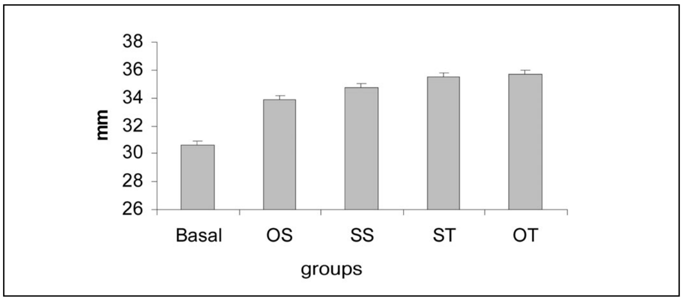

Figure 2** -Femoral Length; $^{@}$**vs basal; i vs OS; p ≤ 0.05

OVX led to a significant decrease in cross-sectional area, medullar area and outer diameter when compared to sham-operated and OT groups. Inner-diameter of Sham-operated sedentary animals was higher than the other groups. The medullar area and outer diameter of the osteopenic trained group showed statistically significant higher values compared to the animals of the osteopenic sedentary animals (p = 0,00021 and p = 0,0038, respectively) and showed no difference comparing to the animals of SS and ST groups (Table 3).

Groups	Cross-section area (mm^2)	Medullar area (mm^2)	Outer Diameter (mm)	Inner Diameter (mm)
Basal	3,5 ± 0,4	1,8 ± 0,12	3 ± 0,2	2,1± 0,13
OS	3,7± 0,26	2,0 ±0,14	3,1± 0,26	2,3 ± 0,26
SS	4,7±0,7 $^{@i}$	2,5±0,25 $^{@ i}$	3,7±0,4 $^{@ i}$	2,6±0,12 $^{@ i}$
ST	4,9± 0,35 $^{@ i}$	2,4± 0,39 $^{@ i}$	4± 0,15 $^{@ i}$	2,3± 0,14*
OT	4,7 ±0,32 $^{@ i}$	2,02± 0,15 $^{@* a}$	3,9 ±0,16 $^{@ i}$	2,3±0,1*

$^{@}$ vs basal; i vs OS; * vs SS; a vs ST. p ≤ 0.05

***Table 3** - Geometrical properties of femora*

OVX produced a significant decrease of femoral length compared to SS group. Moreover, we can observe in table 3 that the exercised animals demonstrated higher values of femoral lengths, suggesting that that the training program had a stimulatory effect on the bone growing.

Femoral physical properties (ash weight, bone volume, bone density and mineral density) were significantly lower in osteopenic sedentary animals compared to other groups, excepting the bone density and mineral density of the sham operated sedentary animals (SS). Exercised animals showed higher values of ash weight, bone density and mineral density than SS groups, indicating the positive effect of exercise. No difference was observed between the animals of the ST and OT groups (Table 4).

The mean maximal load of the animals of OS group was significantly lower than SS, ST and OT groups (p = 0.0091, p = 0.004 and p = 0.0027, respectively). Maximal load of ST and OT groups were higher than SS group. No statistically significant difference was observed between sham operated exercised and osteopenic exercised rats. Structural stiffness was higher in the animals of SS, ST and OT groups compared to OS. The energy absorption of the sham-operated sedentary animals was higher than the other groups. No other difference was found for this variable (Table 5).

Groups	Ash Weight (g)	Bone Volume (cm3)	Bone Density (g/cm^3)	Mineral Density (g/cm^3)
Basal	0,28 ± 0,02	0,5± 0,05	1,46 ± 0,05	0,59 ± 0,04
OS	0,3 ± 0,003 $^{@}$	0,52 ± 0,05 $^{@}$	1,5 ± 0,02 $^{@}$	0,59 ± 0,02
SS	0,34 ± 0,004 $^{@ i}$	0,57 ± 0,04 $^{@ i}$	1,50 ± 0,03 $^{@}$	0,60 ± 0,04 $^{@}$
ST	0,41± 0,004 $^{@ i*}$	0,60 ± 0,04 $^{@ i}$	1,56 ± 0,02 $^{@ i*}$	0,68± 0,02 $^{@ i *}$
OT	0,39 ± 0,02 $^{@ i*}$	0,59 ±0,04 $^{@ i}$	1,57 ± 0,02 $^{@ i*}$	0,68±0,02 $^{@ i *}$

$^{@}$ vs basal; i vs OS; * vs SS; p ≤ 0.05

***Table 4 -** Physical properties of femora*

Groups	Maximal load (N)	Energy absorption (mJ)	Structural stiffness (N/mm)
Basal	82,11 ± 14,89	52,2 ± 4,52	152,26 ± 4,89
OS	89,16 ± 14,89	57,12 ±6,65	186,59 ± 26,62
SS	109,67 ± 13,6 $^{@i}$	61 ± 14	232,1 ± 35,14 $^{@i}$
ST	129,78 ± 20 $^{@i*}$	77,8 ± 18 $^{i *}$	253,31 ± 35,2 $^{@i}$
OT	124,97 ± 13,23 $^{@ i *}$	65,33 ± 13,4	231,89 ± 54,8 $^{@ i}$

$^{@}$ vs basal; i vs OS; * vs SS; p ≤ 0.05

***Table 5 -** Biomechanical properties*

Table 6 demonstrated the values found in the mineral content evaluation. The osteopenic sedentary animals showed lower values of calcium content. No other differences were found among the other groups.

Groups	Calcium (mg/ Kg)	Phosphorus (mg/ kg))
Basal	343,49 ±21,85	165,04 ± 7,76
OS	332,50 ± 16,59	159,96 ±13,65
SS	367 ± 25,80 $^{@i}$	160,39 ± 13,08
ST	375,71 ± 20,70 $^{@i}$	160,55 ± 8,64
OT	368,66 ± 17,78 $^{@i}$	153,59 ± 13,71

$^{@}$ vs basal; i vs OS; p ≤ 0.05

Table 6 - Calcium and Phosphorus content

DISCUSSION

In this study, we observed that the ovariectomy-rat model was efficient to induce osteopenia in the animals studied. The animals of the group OS presented a significant decrease in cross-sectional areas, outer diameters, medullar areas, bone weights and bone strength compared to other groups. Osteopenic rats were then examined to determine the effect of aquatic jump exercise on their femora. Jump exercise performed into the water was found to have a stimulatory effect on osteopenic bones, improving femoral strength, femoral physical and geometric properties and increasing calcium content in osteopenic exercised rats.

As we presented in the introduction, exercises physical programs have been widely used as a part of osteoporosis treatment and prevention(14). Physical activity induces an increase of mechanical load that act on bone tissue, due to external forces and muscles contractions. The increased mechanical load generates a strain force, which suppress bone remodeling and conserve/increase bone mass(4,5). Turner and Robling(15) stated that mechanical load on bones creates a gradient within bone's fluid filled lacunar-canalicular network, which promotes a cascade of cellular events, including elevation of intracellular calcium, expression of growth factors and increase of bone matrix production.

Our results corroborate with other studies which found that a program of exercises can stimulate osteogenesis, increasing the values of bone weights, bone strength and cross-sectional area after OVX(4-8,16,17). Moreover, Peng et al.(5) and Hart et al.(6) found that the OVX leads to a significant weakening of bone strength and a program of exercises was able to reverse the decrease.

Some of the main concerns about the role of physical activities on stimulating bone tissue are the modality and the intensity of the exercise(5 ,18). Many studies have shown that weight bearing exercise can increase bone mineral density (BMD)(19-21). Although weight bearing is one of the most important factors influencing cancellous bone formation and re-absorption, there is evidence that non-weight-bearing exercises could also benefit bone osteogenesis(6,7). For example, in humans, Yung et al(22) found that swimmers had higher calcaneous bone mass than the sedentary controls. Snyder et al(23) who compare the effects of a running program and a swimming training, observed that the swimmer rats showed higher bone mineral content than runner rats.

It is suggested that muscle contractions performed during swimming exercise may have osteogenic effects, been responsible by positive bone mass adaptation(6) . It seems that more than 70% of the bending moment on a bone is transmitted by muscle force rather than body weight, supporting the idea that muscle strength places greater loads on bones than do gravitational forces associated with weight(24) .

We can hypothesize that the stimulatory effects of the present exercise program on femora of the osteopenic rats could be related to the lower limb muscle contractions required to perform jumps. Probably, the forces generated by muscles during the exercise, overloaded sufficiently bone tissue, producing bone adaptation, with an increase of bone metabolism(25).

Moreover, the exercise regime used in the present study overloaded progressively the animals during the 8 week-exercise training and it was consisted of low repetitions. It corroborates with recent theories that suggest that protocols consisting of fewer numbers, several sessions rather than repetitive single bout(11), or a longer interval (30 s) rather than shorter interval between each loading(26) are more effective to increase bone mass and strength(8). In addition, the exercise protocol was performed in an aquatic environment which could be safer for elderly people in improving muscle force and bone quality and body coordination than high impact loading of step aerobic.

Despite the positive effects of the present exercise program on bone tissue, we should consider some limitations of this study. First, our study failed in comparing the effects of the present training program with other type of exercise, as walking or running. Second, we could not directly measure the magnitude of loading neither the force generated by animals during the jumping exercise. Third, we have suggested that the positive effects of the exercise protocol on bone mass were due to the increase in muscle hypertrophy. However, the muscle mass and its correlation with increase in bone mass were not measured.

In conclusion, the progressive load exercise performed in the aquatic environment was able to enhance the osteogenic response in the osteopenic tissue. Although the gravitational force and impact loading are decreased into the water, probably the muscle hypertrophy due to exercise overloaded bone tissue, increasing femoral mass and femoral strength in the osteopenic rats. Thus, the exercise protocol used in this study might constitute a safer alternative therapeutic modality to be used in the treatment and prevention of bone loss, instead of traditional weight-bearing exercises. However more studies are needed to clarify the influences of muscle contractions on bone mass and to compare the effects of the present exercise protocol with other types of exercise.

REFERENCES

1. Culhan EG, Jimenez HI, King CBK. Thoracic kyphosis, rib mobility, and lung volumes in normal women and women with osteoporosis. Spine. 1994; 19:1250-55.
2. Klein RF, Mitchell SR, Phillips TJ, Belknap JK, Orwoll ES. Quantitative trait loci affecting peak bone mineral density in mice. J Bone Min Res. 1998; 13:1648-56.
3. Kalu DN. The ovariectomized rat model of postmenopausal bone loss. Bone Miner. 1991; 15: 175-191.
4. Barengolts EI, Lathon PV, Curry, DJ, Kukreja SC. Effects of endurance exercise on bone histomorphometric parameters in intact and ovariectomized rats. Bone Miner. 1994; 26:133-40.
5. Peng Z, Tuukkanen H, Zhang H, Jamsa T. The mechanical strength of bone in different rat models of experimental osteoporosis. Bone. 1994; 15:523-32.
6. Hart KJ, Shaw JM., Vajda E, Hegsted M, Miller SC. Swim-trained rats have greater bone mass, density, strength, and dynamics. J Appl Physiol. 2001; 91:1663-8.
7. Bourrin S, Palle S, Genty C, Alexandre C. Physical exercise during remobilization restores a normal bone trabecular network after tail suspension-induced osteopenia in young rats. J Bone Miner Res. 1995;10:820-8.
8. Honda A, Sogo S, Nagasawa S, Shimizu T, Umemura Y. High-impact exercise strengthens bone in rats ovariectomized with the same outcome as Sham rats. J Appl Physiol. 2003; 95:1032-7.
9. Notomi T, Lee SJ, Okimoto N, Okazaki Y, Takamoto T, Nakamura T, Suzuki M. Effects of resistance exercise training on mass, strength, and turnover of bone in growing rats. Eur J Appl Physiol. 2000; 82:268-74.
10. Turner CH., Robling AG. Exercises for improving bone strength. Br J Sports Med. 2005; 39:188-9.
11. Robling AG, Hinant FM, Burr DB, Turner CH. Shorter, more frequent mechanical loading sessions enhance bone mass. Med Sci Sports Exerc. 2002; 34:196-202.
12. Marqueti RC, Parizotto NA, Chriguer RS, Perez SE, Selistre-de-Araujo HS. Androgenic-anabolic steroids associated with mechanical loading inhibit matrix metallopeptidase activity and affect the remodeling of the achilles tendon in rats. Am J Sports Med. 2006; 34:1274-80.
13. Martin RB. Effects of simulated weightlessness on bone properties in rats. J Biomech. 1990; 23:1021-29.
14. Bayramoglu M, Sozay S, Karatas M, Kilinc S. Relationships between muscle strength and bone mineral density of three body regions in sedentary postmenopausal women. Rheumatol Int. 2005; 25:513-7.
15. Turner CH. Three rules for bone adaptation to mechanical stimuli. Bone. 1998; 23: 399–407.
16. Donahue HJ, Mazzeo RS, Horvath S.M. Endurance training and bone loss in calcium-deficient and ovariectomized rats. Metabolism. 1988; 37:741-4.
17. Myburgh KH, Noakes TD, Roodt M., Hough FS. Effect of exercise on the development of osteoporosis in adult rats. J Appl Physiol. 1989; 66:14-9.
18. Nordsletten L, Kaastad TS, Madsen JE, Reikeras O, Ovstebo R, Stromme JH, et al. The development of femoral osteopenia in ovariectomized rats is not reduced by high intensity treadmill training: a mechanical and densitometric study. Calcif Tissue Int. 1994; 55:436-42.
19. Grimston SK. An application of mechanostat theory to research design: a theoretical model. Med Sci Sports Exerc. 1993; 25:1293-7.
20. Kung AW, Ng, F. A rat model of thyroid hormone-induced bone loss: effect of antiresorptive agents on regional bone density and osteocalcin gene expression. Thyroid. 1994; 4:93-8.
21. Heinonen A, Sievanen H, Kannus P, Oja P, Pasanen M, Vuori I. High-impact exercise and bones of growing girls: a 9-month controlled trial. Osteoporos Int. 2000; 11:1010-7.
22. Yung P, Lai YM, Tung PY, Tsui HT, Wong CK, Hung VW, Oin L. Effects of weight bearing and non-weight bearing exercises on bone properties using calcaneal quantitative ultrasound. Br J Sports Med, 2005; 39:547-51
23. Snyder A, Zierath JR, Hawley JA, Sleeper MD, Craig BW. The effects of exercise mode, swimming vs. running, upon bone growth in the rapidly growing female rat. Mech Ageing Dev. 1992; 66:59-69.
24. Iwamoto J, Yeh JK, Aloia JF. Differential effect of treadmill exercise on three cancellous bone sites in the young growing rat. Bone. 1999; 24:163-9.
25. Mullender M, El Haj AJ, Yang Y, van Duin MA, Burger EH, Klein-Nulend J. Mechanotransduction of bone cells in vitro: mechanobiology of bone tissue. Med Biol Eng Comput. 2004; 42:14-21
26. Umemura Y, Sogo N, Honda A. Effects of intervals between jumps or bouts on osteogenic response to loading. J Appl Physiol. 2002; 93:1345-8.