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Original Article

Hylonome

Physical activity in osteopenia treatment improved the mass of bones directly and indirectly submitted to mechanical impact

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Abstract

The effect of physical activity in the treatment of osteopenia induced by ovariectomy was studied in 34 two-month-old Wistar female rats. Animals were divided into three groups in which two were formed by ovariectomized (OVX) animals and the other one had sham-operated animals. Group 1, active OVX'd rats; group 2, sedentary OVX'd rats and group 3, sham-operated ones (control). After three months of daily physical activity in a motor-driven treadmill all rats were sacrificed. In order to perform a histomorphometric analysis, long bones, vertebrae, and nasal bone were selected at necropsy. Ovariectomized rats which exercised showed an increased trabecular bone volume, cortical thickness in the long bones and vertebrae and also an increased nasal bone thickness. Physical activity also increased the connection of osteocytes. It was concluded that physical activity in osteopenia treatment increases and restores the mass of bones directly and indirectly submitted to physical impact.

Keywords: Bone, Exercise, Osteopenia, Ovariectomy, Rat

Introduction

As life expectancy has increased, osteoporosis has been increasingly diagnosed in women and men worldwide[1]. In spite of constant researchers' efforts, further studies are important to elucidate its etiopathogenesis and treatment. Sex steroids are assumed to play a role in the genesis of human osteoporosis, mainly menopause[2]. But the lack of physical activity constitutes a risk factor[3].

Most researchers agree that, either directly or indirectly, physical activity presents a complex and powerful effect on the bones, but results are contradictory, probably due to the heterogeneity of some factors such as the animal's age, type and length of exercise and also the bone site analyzed[4-6]. In addition, the effect of exercise on the human skeleton has been assessed by densitometry[7] or bone biopsy[8], which may not be enough to conclude about the whole skeleton response to physical stimuli.

Although, molecular response of bone tissue to physical activity impact has been studied, there is still a lot to be explained. It is known that the osteocyte is the main cell type responsible for mechanotransduction translation of mechanical forces into biochemical signs which account for turnover[9,10]. Nevertheless, changes in osteocytes' morphology and activity as a consequence of physical force imposed on them deserve more attention[11] and these studies will be helpful in further understanding of these numerous cell functions in the bones.

Most researchers have worried about the effect of physical activity on osteoporosis prevention[12,13]. Throughout the literature studied, only one report was found of a recent study with rats that verified the effect of low impact physical activity on the treatment of osteopenia after osteopenic

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changes had occurred. However, this study does not mention the effect of exercise on the whole skeleton and on bones, which were not affected by physical impact[14].

To date, there are no studies on the assessment of physical activity effect on bone, which did not receive any physical impact, focusing mainly on the indirect effect of exercise on bone tissue. No studies have evaluated the effect of physical activity on the whole skeleton, which is one of our goals in this study.

Thus, the goal of this study was to verify, through histomorphometry, the effect of controlled physical activity on rat bones directly and indirectly submitted to mechanical impact.

Materials and methods

Animal Care and Study Protocol

Thirty-four Wistar female rats were used in this study. Two-month-old animals were randomly divided into two groups of animals, one ovariectomized and the other sham-operated (control). They were submitted to bilateral ovariectomy as a standard procedure approved by the Ethical Committee of Universidade Federal de Minas Gerais. Three months after ovariectomy, five rats from the sham-operated group and eight rats from the ovariectomized group were sacrificed. This procedure was carried out to check the reduction of bone mass induced by ovariectomy and to prove bone mass of OVX'd rats before beginning physical activity. Three months after ovariectomy, more than enough time to induce osteopenia[15], ovariectomized groups were submitted to different treatments in the following three months. One group had regular and controlled physical activity (active OVX'd group) and the other one was kept sedentary (sedentary OVX'd group). Thus, the rats were divided into three experimental groups: Group 1, active OVX'd rats (n=8); Group 2, sedentary OVX'd rats (n=8) and Group 3, sham-operated ones (control) (n=5). Animals of the same experimental group were housed five per cage under 12-hour-light/dark cycle. They were fed with commercial rat chow containing 22% of crude protein, 1.4% of calcium and 0.6% of phosphorus. Food and water were provided *ad libitum* to all animals.

The active OVX'd group performed physical activity on a motor-driven treadmill at 15m/min speed and 0° inclination once a day, five days a week for three months. The exercise lasted 15 min/day on the first week. From the 2nd week to the completion of this study, animals exercised for 30 min/day. This activity was considered of moderate degree[16]. Electric shocks or other means of artificial stimulation were not used at any stage. Animals in the sham-operated and sedentary OVX'd groups were handled weekly until the end of the experiment. Six months after surgery, three experimental groups were killed with an overdose of anesthesia (Tionembutal 2,5%). At necropsy, the success of ovariectomy was determined by uterus atrophy.

Histomorphometry

Long bones (humerus, radius, femur and tibia), nasal bone, cervical vertebrae, thoracic vertebrae (1-7 and 8-13) and lumbar vertebrae (1-3 and 4-6) after being fixed in 10% buffered formalin, were demineralized in 10% formic acid solution at pH 4.5 under moderate vacuum for 14 days.

Length of long bones was determined from epiphysis to epiphysis with a ruler. Length of vertebrae was measured in histological sections with a millimetric ruler and 4.68× magnification. Ultimately, a correction factor obtained by the scale of a micrometric slide was applied.

After the complete demineralization (controlled by X-ray) and 24 hours' washing in tap water, bones were sectioned and processed for routine paraffin embedding technique. All vertebral segments were longitudinally sectioned. Long bones were sectioned in the middle diaphysis and jaws were sectioned longitudinally next to the molars in order to assess nasal bone. Paraffin sections were stained with hematoxylin and eosin and analyzed by optical light microscopy.

Sections were also impregnated by silver in order to have a better view of osteocyte connection. Silver impregnation was performed with histological, deparaffinated and hydrated 4 μm-sections. In order to carry out this impregnation, water solution of silver nitrate was used with formic acid solution as well as microbiological jelly (data not shown).

Nasal bone thickness was determined in 50 points by ocular with a millimetric ruler and 40× magnification. Measurement number was determined by the technique to study the instability variation of mean values in relation to the original sample.

Trabecular bone volume (BV/TV%) was determined by morphometry in histological sections of vertebrae and long bones (proximal epiphysis and metaphysis of humerus, tibia, head and also distal epiphysis and femur metaphysis). These variables were determined by using an ocular containing a 25-point grid (Zeiss KPL 10x) and 40× magnification. In the long bones, the grid was superimposed across 10 fields in epiphysis and 10 fields in metaphysis completing 250 points per field. Fields were chosen at 1 mm under the epiphyseal plate and articular cartilage. In the vertebral segments, the same variables were determined in a total of nine fields/vertebra. Three fields were 1 mm under each epiphyseal area and the other three, in the middle region of vertebral body, totalized 225 points per vertebra.

Evaluation of cortical thickness of the mid-diaphysis of long bones, as well as the middle vertebrae. In the mid-diaphysis of long bones, an ocular with a millimetric ruler and 5× magnification was used and eight measurements, at the same distance, were taken in the transversal section. Vertebral cortical thickness was determined by an ocular 10× magnification and a millimetric ruler through six equidistant measurements of the vertebral body with three measurements on the superior cortical and three on the inferior one. Ultimately, a correction factor obtained by the scale of a micrometric slide was applied to cortical thickness means.

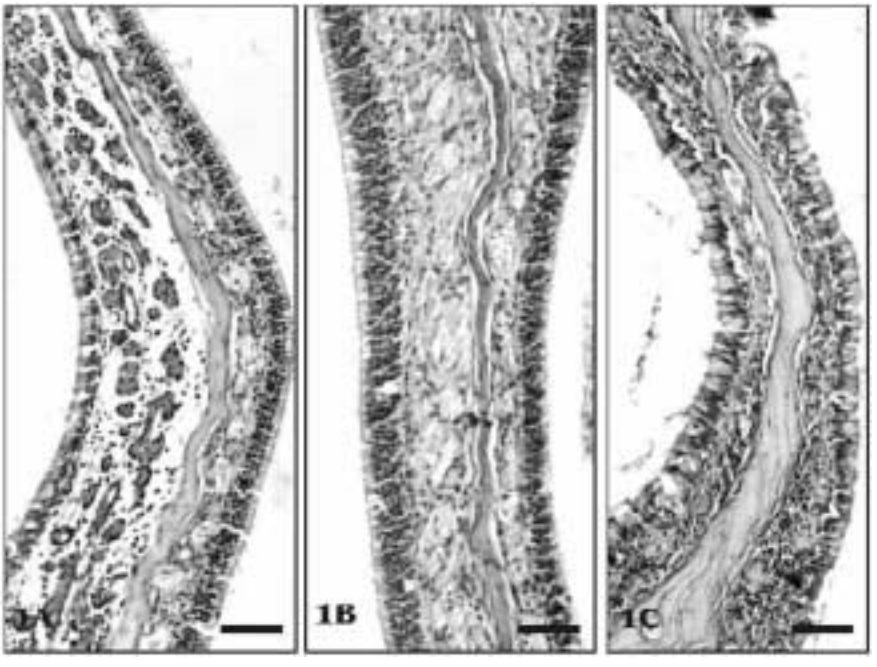

Figure 1. Rat, nasal bone, HE. A) Sham-operated group. Continuous nasal bone. B) Sedentary OVX'd group. Thin nasal bone. C) Active OVX'd group. Thicker nasal bone than that from sham-operated and sedentary OVX'd groups. Bar: 75 μm.

Statistics

A completely randomized design was employed. Means and standard deviations were calculated for each variable within each group. The following numeric variables were analyzed: nasal bone thickness, trabecular bone volume (BV/TV%), cortical thickness and length of long bones and vertebrae. Data were submitted to analysis of variance, and means were compared by SNK test.

Variable	Group		
	Sham-operated	**Sedentary OVX'd**	**Active OVX'd**
Thickness of nasal bone	16.19 ± 1.72^{B}	18.37 ± 3.58^{B}	23.38 ± 2.76^{A}
Different letters in the same row indicate a statistically significant difference ($P<0.05$).			

Table 1. The mean and standard deviations of nasal bone thickness (μm) in sham-operated rats, sedentary OVX'd rats and active OVX'd rats.

Results

Morphology and Morphometry

Three months after ovariectomy, a significant reduction was observed in trabecular bone volume of the whole skeleton. This result confirmed the induction of osteopenia before beginning physical activity (data not shown).

Sham-operated group (control)

Animals from the sham-operated group showed normal bone morphology. Nasal bone was continuous (Figure 1A) and covered by active osteoblasts. A large amount of epiphyseal and metaphyseal trabeculae of long bones and vertebrae was observed to be thick and confluent with cartilage matrix retention (cartilaginous core) (Figures 2A, 3A, 4A, 5A and 6A). Osteoblastic cover was oscillating from active cuboidal cells with large nuclei to thin ones with fusiform nuclei. Osteocytes were sometimes active with large nuclei located in wide lacunas; sometimes they were a little basophilic and inactive with small nuclei located in narrow lacunas.

Sedentary ovariectomized group

Rats from the sedentary OVX'd group presented marked osteopenia both in the trabecular bone tissue, and in the cortical bone tissue. Nasal bone was thin (Figure 1B) and not continuous. But no difference was observed regarding the sham-operated group (Table 1). Almost no osteoblastic cover was observed and cells were inactive with fusiform nuclei.

Epiphyseal and metaphyseal trabeculae of long bones and vertebrae were significantly reduced, when compared to the

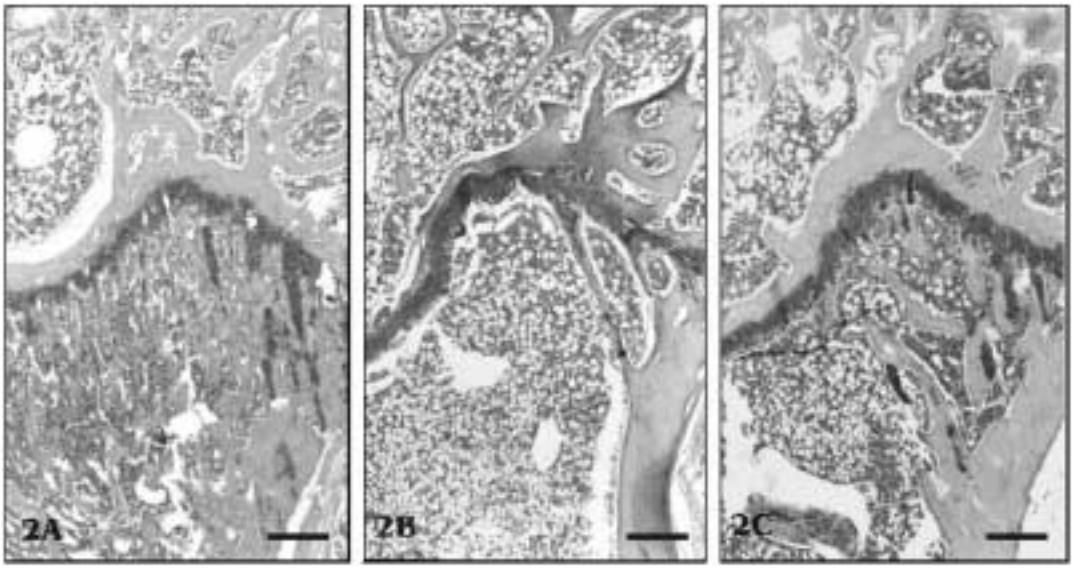

Figure 2. Rat, proximal metaphysis humerus, HE. A) Sham-operated group. Epiphyseal and metaphyseal trabeculae in a large quantity and confluent. B) Sedentary OVX'd group. Epiphyseal and metaphyseal trabeculae reduced and thin. C) Active OVX'd group. Epiphysis and metaphysis with larger amount of trabeculae than in the sedentary OVX'd group. Bar: 187 μm.

Variable	Group		
	Sham-operated	Sedentary OVX'd	Active OVX'd
Proximal humerus (epiphysis)	42.27 ± 5.83^{A}	10.10 ± 3.43^{C}	28.80 ± 11.71^{B}
Proximal humerus (metaphysis)	36.34 ± 5.95^{A}	5.07 ± 2.78^{C}	24.35 ± 7.28^{B}
Proximal tibia (epiphysis)	44.22 ± 9.21^{A}	14.58 ± 5.99^{C}	32.42 ± 8.27^{B}
Proximal tibia (metaphysis)	43.29 ± 6.71^{A}	7.25 ± 5.52^{C}	32.87 ± 6.66^{B}
Distal femur (epiphysis)	41.02 ± 7.84^{A}	15.61 ± 6.46^{C}	33.09 ± 4.90^{B}
Distal femur (metaphysis)	41.93 ± 7.51^{A}	8.18 ± 6.87^{C}	28.52 ± 6.62^{B}
Head of femur	53.59 ± 9.90^{A}	19.14 ± 5.82^{C}	40.19 ± 7.95^{B}
Cervical vertebrae	37.39 ± 5.41^{A}	19.37 ± 6.08^{B}	33.21 ± 6.12^{A}
Thoracic vertebrae 1-7	34.67 ± 5.37^{A}	17.51 ± 4.73^{B}	34.84 ± 4.10^{A}
Thoracic vertebrae 8-13	36.58 ± 7.51^{A}	19.75 ± 4.45^{B}	38.11 ± 5.30^{A}
Lumbar vertebrae 1-3	38.15 ± 6.73^{A}	16.84 ± 8.85^{B}	32.72 ± 10.24^{A}
Lumbar vertebrae 4-6	33.15 ± 3.09^{A}	14.87 ± 4.44^{B}	29.68 ± 5.16^{A}
Different letters in the same row indicate a statistically significant difference ($P<0.05$).			

Table 2. The mean and standard deviations of trabecular bone volume (BV/TV%) of long bones and vertebrae in sham-operated rats, sedentary OVX'd rats and active OVX'd rats.

sham-operated group (Table 2). Trabeculae were slightly thin, reduced in number and sometimes fragmented (Figures 2B, 3B, 4B, 5B and 6B).

Deep osteocytes were active with large nuclei located in wide lacunas and with lacunar ring and intense basophilic adjacent matrix. Osteocyte connections were less numerous, short, intensively rambling and little connected. This finding was different from that one observed in the sham-operated group, once the processes were well oriented and connected (Figure 8). Extensive bone loss was not observed in the cortex of long bones and vertebrae (Table 3).

Active ovariectomized group

Physical activity did not only stop bone loss caused by ovariectomy, but it also increased trabecular bone volume and cortical thickness. Thus, the amount of bone in all sites of these animals' skeletons was higher than in the rats three months after ovariectomy (before beginning physical activity) and the same or higher than that in the sham-operated group (Tables 2-5). Surprisingly, their nasal bone was significantly thicker than those in the sham-operated and seden-

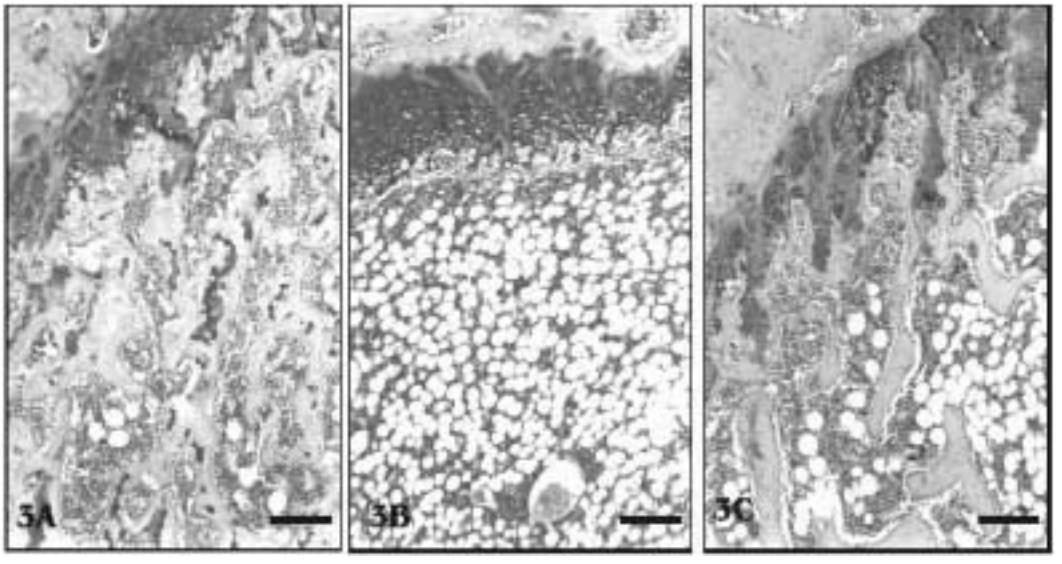

Figure 3. Rat, distal metaphysis femur, HE. A) Sham-operated group. Epiphyseal and metaphyseal trabeculae in a large quantity, thick and confluent. B) Sedentary OVX'd group. Absent metaphyseal trabeculae. C) Active OVX'd group. Metaphyseal trabeculae in a larger amount than in the sedentary OVX'd group. Bar: 184 μm.

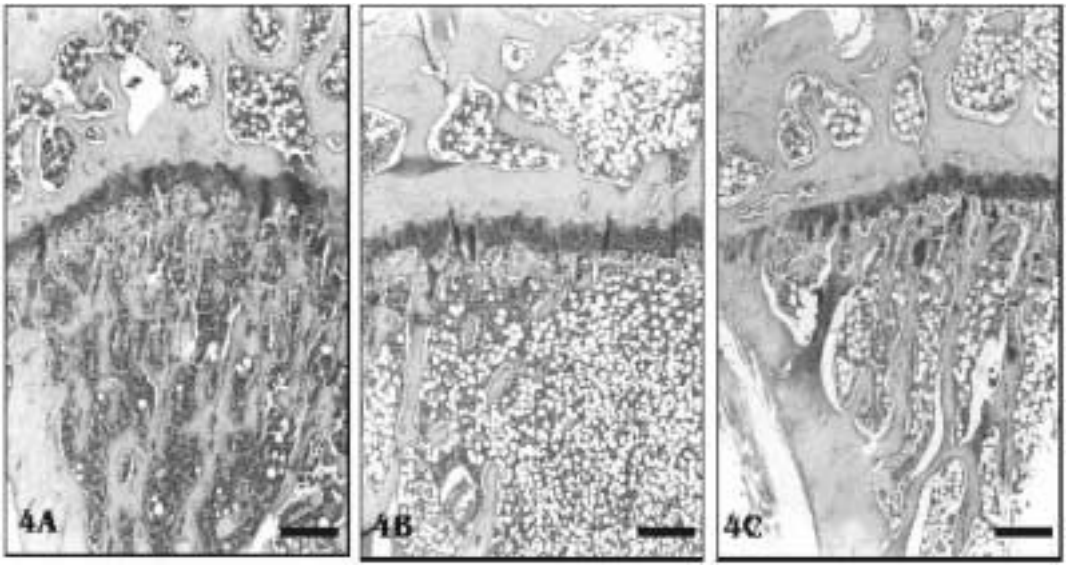

Figure 4. Rat, proximal metaphysis tibia, HE. A) Sham-operated group. Epiphyseal and metaphyseal trabeculae in a large amount and confluent. B) Sedentary OVX'd group. Metaphyseal trabeculae reduced. C) Active OVX'd group. Metaphyseal trabeculae in a larger amount than in the sedentary OVX'd group. Bar: 369 μm.

tary OVX'd groups (Figure 1C) (Table 1).

Trabecular bone volume in long bones and vertebrae was significantly higher in the active OVX'd rats (Figures 2C, 3C, 4C, 5C and 6C) (Table 2). At the end of the experiment, rats submitted to physical activity did not present any sign of osteopenia. They presented thick and confluent trabeculae with evident osteoblastic cover, formed by more than one row of apparently immature cells. This response was not observed in the sedentary OVX'd group where osteoblastic cover was almost non-existent and osteoblasts were inactive when it was present (Figures 7A and 7B). Active OVX'd animals showed an increase in trabecular bone volume and cortical thickness in long bones and vertebrae, as shown in Tables 2 and 3. In addition, osteocytes were active with large nuclei located in

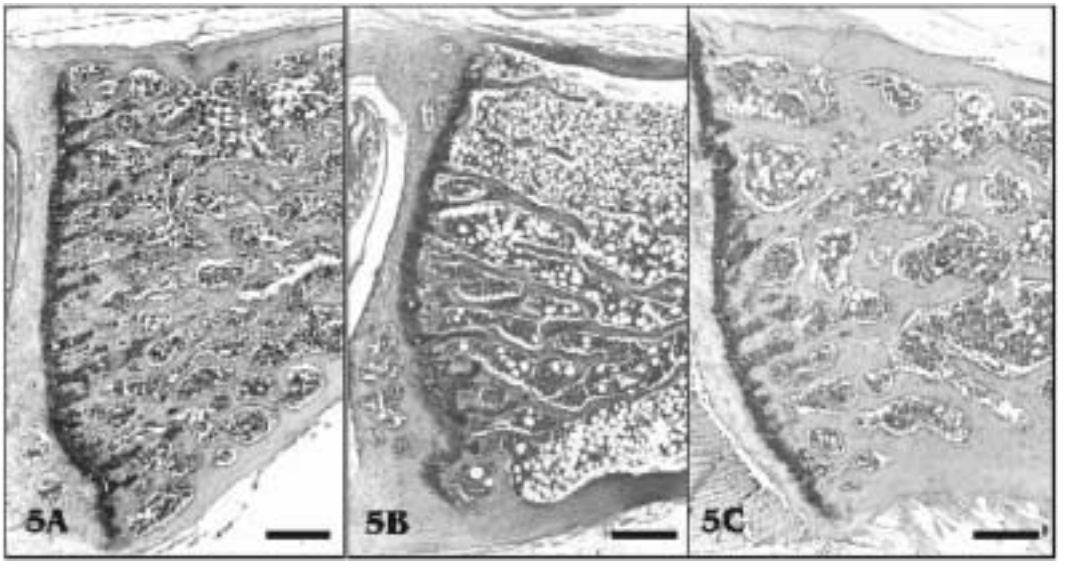

Figure 5. Rat, lumbar vertebra, HE. A) Sham-operated group. Trabeculae in a large amount, thick and confluent. B) Sedentary OVX'd group. Reduced, thinner and sometimes fragmented trabeculae and thin cortex. C) Active OVX'd group. Trabeculae in a larger amount, thick and confluent and thicker cortex than in the sham-operated and sedentary OVX'd groups. Bar: 613 μm.

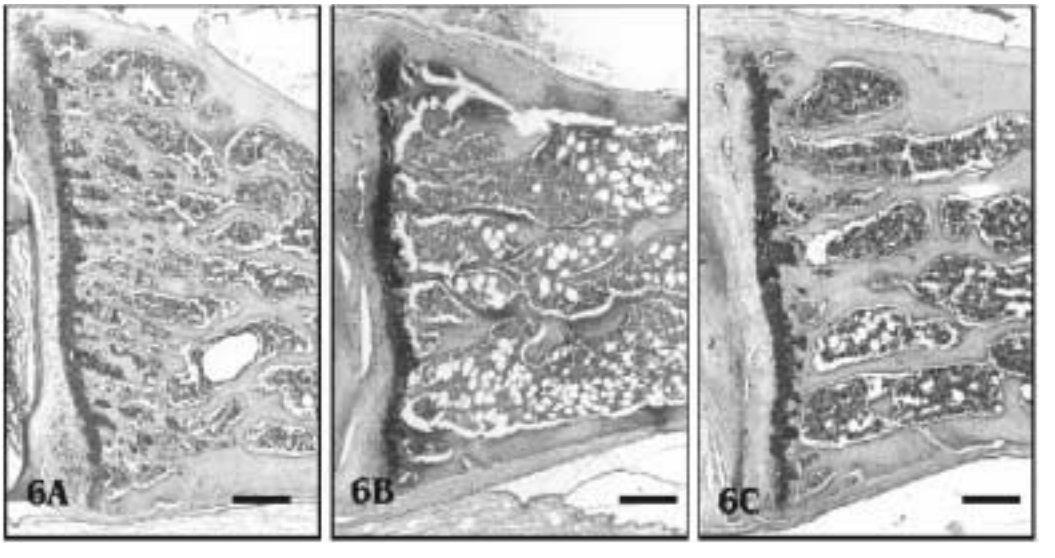

Figure 6. Rat, thoracic vertebra, HE. A) Sham-operated group. Trabeculae in a large amount, thick and confluent. B) Sedentary OVX'd group. Reduced and thin trabeculae and thin cortex. C) Active OVX'd group. Trabeculae in a larger amount, thick and confluent and thicker cortex than in the sedentary OVX'd group. Bar: 493 μm.

wide lacunae in active OVX'd rats. Osteocyte connections were more numerous, expanded, and well oriented than in the sham-operated group. They communicated with each other forming a larger connection net whereas sedentary OVX'd rat bones presented osteocytes with short fragmented connections with no communication (Figures 8A, 8B and 8C).

The length and width of long bones and vertebrae did not differ significantly among all groups studied (data not shown).

Discussion

The present study demonstrated that physical exercise in ovariectomized female rats increased the trabecular bone volume in vertebrae and in long bones and vertebral cortical thickness. Therefore, our results provide evidence through bone histomorphometry that exercise has important protective and therapeutic effects on osteopenia. To our knowledge,

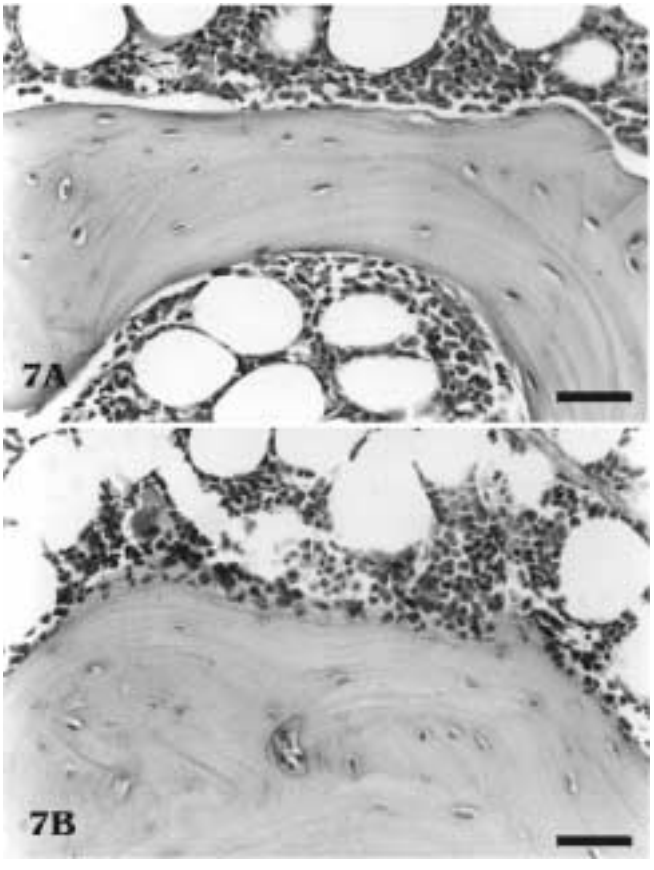

Figure 7. Rat, distal metaphysis femur, HE. A) Sedentary OVX'd group. Trabeculae with almost no osteoblastic cover and inactive osteoblasts with fusiform nucleus. B) Active OVX'd group. Trabeculae with evident osteoblastic cover, formed by more than one row of cells apparently immature. Bar: 26 μm.

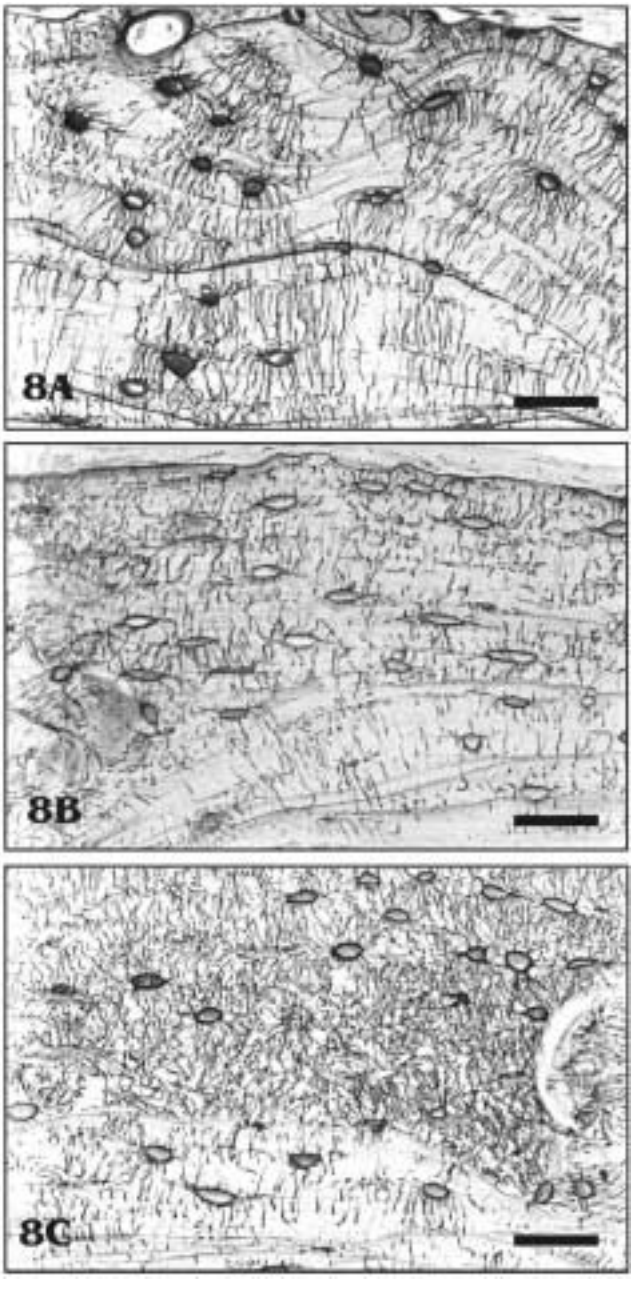

Figure 8. Rat, lumbar vertebra, silver impregnation. A) Sham-operated group. Connection of well oriented and connected osteocytes. B) Sedentary OVX'd group. Connection of less numerous, short, intensively rambling and little connected osteocytes. C) Active OVX'd group. Connection of more numerous, expanded, and well oriented osteocytes than those in the sham-operated and sedentary OVX'd groups. Bar: 31 μm.

this is the first study to describe the effect of physical activity on rat bones, which did not suffer direct mechanical impact.

Osteopenia was expected in ovariectomized animals and hypoplasia and hypotrophy were observed in the osteoblastic cover with a consequent reduction of bone matrix production. However, physical activity not only prevented bone loss caused by ovariectomy but also increased trabecular bone volume and cortical thickness in long bones and vertebrae. No significant difference was observed in the length of long bones and vertebrae among groups. Therefore, it is important to notice that the increase of bone promoted by physical activity did not suffer influence from growth.

Induction of osteopenia was evident after three months of ovariectomy. Thus, the effect of physical activity was observed during the treatment, and not only in the prevention, as is observed in most studies[12,13].

Surprisingly, nasal bone was significantly thicker in active OVX'd animals than in the sham-operated or sedentary OVX'd groups. However, there was no difference in nasal bone thickness between the sham-operated group and the sedentary OVX'd group. This fact was probably due to the effect of age in the reduction of this amount of bone (data not shown).

It is postulated that the action of mechanical forces mediates the direct effect of physical activity on bone mass improvement[17,18]. Although nasal bone does not suffer any kind of impact during exercise, thus suggesting that the ben-

Variable	Group		
	Sham-operated	Sedentary OVX'd	Active OVX'd
Humerus	398.65±56.80^{A}	408.78±50.43^{A}	432.43±62.97^{A}
Radius	416.21±24.17^{A}	407.09±63.56^{A}	420.60±41.77^{A}
Femur	481.08±42.30^{A}	459.46±45.51^{A}	506.75±68.14^{A}
Tibia	343.24±38.93^{B}	366.55±37.14^{AB}	413.85±64.58^{A}
Cervical vertebrae	141.22±25.74^{A}	120.52±10,50^{A}	144.22±22.91^{A}
Thoracic vertebrae 1-7	121.07±13.30^{A}	110.68±14.37^{A}	131.79±17.37^{A}
Thoracic vertebrae 8-13	141.05±15.26^{A}	115.03±17.05^{A}	143.67±28.75^{A}
Lumbar vertebrae 1-3	145.72±18.33^{B}	131.83±17.06^{B}	183.59±25.48^{A}
Lumbar vertebrae 4-6	140.08±18.85^{B}	126.53±14.45^{B}	172.35±32.42^{A}
Different letters in the same row indicate a statistically significant difference (P<0.05).			

Table 3. The mean and standard deviations of cortical thickness (μm) of long bones and vertebrae in sham-operated rats, sedentary OVX'd rats and active OVX'd rats.

Variable	Group	
	OVX'd before exercise	OVX'd after exercise (active OVX'd)
Proximal humerus (epiphysis)	22.62±7.07^{A}	28.80±11.71^{A}
Proximal humerus (metaphysis)	11.18±6.95^{B}	24.35±7.28^{A}
Proximal tibia (epiphysis)	21.87±9.99^{B}	32.42±8.27^{A}
Proximal tibia (metaphysis)	15.69±6.19^{B}	32.87±6.66^{A}
Distal femur (epiphysis)	23.87±3.54^{B}	33.09±4.90^{A}
Distal femur (metaphysis)	19.51±6.12^{B}	28.52±6.62^{A}
Head of femur	28.46±5.4^{B}	40.19±7.95^{A}
Cervical vertebrae	24.38±3.6^{B}	33.21±6.12^{A}
Thoracic vertebrae 1-7	22.23±5.82^{B}	34.84±4.10^{A}
Thoracic vertebrae 8-13	25.35±5.77^{B}	38.11±5.30^{A}
Lumbar vertebrae 1-3	21.42±4.49^{B}	32.72±10.24^{A}
Lumbar vertebrae 4-6	21.07±3.81^{B}	29.68±5.16^{A}
Different letters in the same row indicate a statistically significant difference (P<0.05).		

Table 4. The mean and standard deviations of trabecular bone volume (BV/TV%) of long bones and vertebrae in OVX'd rats before and after beginning physical activity.

Variable	Group	
	OVX'd before exercise	OVX'd after exercise (active OVX'd)
Humerus	403.71±36.4^{A}	432.43±62.97^{A}
Radius	403.71±51.80^{A}	420.60±41.77^{A}
Femur	484.78±44.19^{A}	506.75±68.14^{A}
Tíbia	374.52±51.58^{A}	413.85±64.58^{A}
Cervical vertebrae	131.21±23.56^{A}	144.22±22.91^{A}
Thoracic vertebrae 1-7	113.38±18.24^{A}	131.79±17.37^{A}
Thoracic vertebrae 8-13	116.64±26.18^{A}	143.67±28.75^{A}
Lumbar vertebrae 1-3	142.92±35.08^{B}	183.59±25.48^{A}
Lumbar vertebrae 4-6	121.7±27.71^{B}	172.35±32.42^{A}
Different letters in the same row indicate a statistically significant difference (P<0.05).		

Table 5. The mean and standard deviations of cortical thickness (μm) of long bones and vertebrae in OVX'd rats before and after beginning physical activity.

eficial effect of physical activity is not only mediated by mechanical force, but it is also indirectly mediated by hormones and growth factors. The vibrations due to exercise may be another hypothesis to explain the increase in bone mass caused by physical activity in bones with no direct mechanical impact. It is postulated that high frequency vibration on bone mass of OVX rats induced an increase in periosteal bone formation rate and inhibited the endocortical resorption seen in OVX rats[19]. In addition, exercise may increase the supply of oxygen that may contribute to systemic bone effects[16].

To our knowledge, there is no report concerning the effect of physical activity on bones that did not suffer direct mechanical impact during physical exercise. Indirect effect of physical activity on bones mediated by hormonal factors involves cytokine production and the release of growth factors by bone cells as a consequence of an increased osteoblastic activity[18]. It is known that physical exercise triggers a series of physiological responses involving hypothalamus-hypophysis-adrenal and hypothalamus-hypophysis-gonads axes[19] stimulating the release of growth hormone-GH[2,17,21-23], which has direct or indirect anabolic effect, mediated by insulin-like growth factor 1 (IGF-1)[17]. IGF-1 is a cytokine produced by bone cells and it is the most abundant growth factor in the bone matrix[24]. This cytokine stimulates DNA synthesis and collagen production by osteogenic cells increasing bone matrix synthesis *in vivo*[9]. Another function of IGF-1 is to induce an osteoblastic differentiation providing bone formation, either by its systemic or local effect[24]. However, our experimental design did not allow us to postulate if IGF-I would be the mediator of physical activity effects on nasal bone. Furthermore, there are several studies showing an increased plasma concentration of thyroid hormones during physical activity[25-28], though these hormones have not been observed to play a role in physical activity effect on bone metabolism[23].

Rats, submitted to physical activity, presented no signs of osteopenia at the end of the experiment. Physical activity promoted a more significant increase of bone mass on vertebrae than on long bones. This result is interesting because vertebrae suffer less mechanical impact than long bones. Effects on the bone vary depending on the type and intensity of exercise, which can be even harmful[22]. Then, it is important to point out that exercises imposed on these animals were considered of a moderate level and enough to observe their effect on the whole skeleton, without causing any harm.

Changes among osteocyte processes in response to physical activity were observed in active OVX'd rats. However, changes in morphology and activity of osteocytes, as a consequence of physical force imposed over them, deserve more attention. To our knowledge, this is the first study to describe changes among osteocyte processes in demineralized bone by optical microscopy.

Cherian et al.[10], showed that these canaliculi are important to the nutrition and maintenance of bone viability. Then, would they be involved in the beneficial response of bone to physical activity? There are evidences that one of the mechanisms by which physical activity improves bone mass is through direct effects mediated by mechanical forces[17,18]. When a mechanical force is applied on bone tissue, it forms endogenous signals that interfere with bone remodeling. These signs are caught by a mechanosensory system in which osteocytes are the main cells responsible for translating this mechanical force into biochemical signs that regulate bone turnover[9,10]. It is believed that cell deformation caused by direct force applied to it, increased intracanicular pressure caused by dynamic force and increased velocity of interstitial fluid flow are the factors that directly affect osteocytes[29]. The last factor represents the major stimulus to the osteocyte in response to mechanical strain. Interstitial fluid flow through canaliculi around osteocytes seems to be in charge of deformation of extracellular matrix and changes in cell membrane[31]. Autocrine function of prostaglandin (PGE2) is also discussed in the regulation of gap junctions and connexin (Cx43) expression in the osteocyte membrane. As a result of mechanical force application, arachidonic acid conversion into prostaglandin E2 in the osteocyte[31] would act as an autocrine factor, stimulating Cx43 protein formation and new gap junctions[10]. These gap junctions play an important role in signaling between osteoblasts and osteocytes in response to mechanical strain[32].

Conclusion

This study showed that daily physical activity in a motor-driven treadmill without inclination presented the following findings: 1) it reversed osteopenia signs in the whole skeleton; 2) it increased the quantity of trabecular and cortical bone in vertebrae, the trabecular bone tissue in the long bones and it also increased the thickness of nasal bones; 3) it increased the osteocyte connection and, 4) restored bone mass to sham level. Thus, physical activity in osteopenia treatment increases and restores the amount of bone mass in bones directly and indirectly submitted to physical impact.

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