Influence of ladder climbing exercise on bone of rats induced to osteoporosis and immobilization

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Summary

Introduction: The oophorectomy surgery produces menopause, which in turn predisposes to many problems such as osteoporosis, with consequent deterioration of microarchitecture and decreasing mass bone, predisposing to the risk of fractures, which are often treated with immobilization, which negatively affects the muscle, cartilage and bone tissues. Despite the rich literature on exercise as a way of remobilization, both in humans and in animals, there is a gap with respect to some types of exercise, such as that performed with climb ladders. The aim of this study was to analyze the effects of ladder climbing exercise on rats bone histomorphometry induced osteoporosis and subjected to immobilization.
Methods: 36 female Wistar rats were separated into six groups: G1, G2 and G3 subjected to pseudo-oophorectomy; and G4, G5 and G6 to oophorectomy. After 60 days rest, G2, G3, G5 and G6 had immobilized the right hind limb for 15 days, followed by remobilization for the same period, being free in the box to G2 and G5, and ladder climbing exercise to G3 and G6. At the end of the experiment, the rats were euthanized, their tibias removed bilaterally and submitted to histological routine.
Results: There was a significant decrease in cortical bone (area and thickness) and osteocytes numbers, and increased medullary canal, in immobilized limbs of ovariectomized rats. However, the exercise of climbing a ladder was able to reverse these losses due to oophorectomy associated with immobilization. There was also a significant decrease in the area and trabecular thickness in members subjected to immobilization, being reversed with the free remobilization and in ladder.
Conclusions: the ladder climbing exercise was effective in the recovery process of bone tissue damaged by immobilization on osteoporosis model by ovariectomy in rats.

Key words:
Ovariectomy
Immobilization.
Exercise therapy.

Influencia del ejercicio en escalera sobre el hueso de ratas inducidas a la osteoporosis e inmovilización

Resumen

Introducción: La cirugía ooforectomía produce menopausia, que a su vez predispone a muchos problemas tales como la osteoporosis, con el consiguiente deterioro de la microarquitectura ósea, lo que aumenta el riesgo de fracturas, que a menudo son tratadas con la inmovilización, que afecta negativamente el tejido muscular, cartilaginoso y óseo. A pesar de la abundante literatura sobre el ejercicio físico como medio de recuperación, tanto en humanos como en animales, existe una brecha con respecto a algunos tipos de ejercicios, como los realizados con escaleras de ascenso. El objetivo de este estudio fue analizar los efectos del ejercicio de subir una escalera sobre el hueso de ratas con osteoporosis inducida y sometidos a inmovilización.
Métodos: Se dividieron 36 ratas Wistar en seis grupos: G1, G2 y G3 sometidos a pseudo-ooforectomía; y G4, G5 y G6 a ooforectomía. Después de 60 días de descanso, G2, G3, G5 y G6 habían inmovilizado la extremidad posterior derecha durante 15 días, seguido de removilización durante el mismo tiempo, realizando ejercicio libre en la jaula los grupos G2 y G5, o ejercicio subir escaleras para los grupos G3 y G6. Al final del experimento, las ratas fueron sacrificadas, sus tibias fueron retiradas bilateralmente y sometidas a un análisis histológico.
Resultados: Se observó una disminución significativa en el hueso cortical (área y espesor) y del número de osteocitos, y el aumento del canal medular, en las extremidades inmovilizadas de ratas ovariectomizadas. Sin embargo, el ejercicio de subir una escalera fue capaz de revertir estas pérdidas debidas a ooforectomía asociada con la inmovilización. También hubo una disminución significativa en el espesor de la área trabecular de los miembros sometidos a inmovilización, siendo revertido con la removilización libre y en escalera.
Conclusiones: El ejercicio de subida en una escalera fue eficaz en el proceso de recuperación del hueso dañado por inmovilización en el modelo de osteoporosis por la ovariectomía en ratas.

Palabras clave:
Ovariectomía.
Inmovilización.
Terapia por ejercicio.

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Introduction

Menopause in humans is characterized by last menstrual period and may occur spontaneously at about 51 years of age or by surgical induction in cases of oophorectomy and hysterectomy[1]. This alteration in the female body causes a slow and gradual decrease in estrogen and progesterone hormones, predisposing the onset and aggravation of some disorders and diseases such as osteoporosis. Consequently, this metabolic bone disease causes deterioration of the tissue microarchitecture and decrease of bone mass, leading to bone fragility and predisposing to fractures, even for minor traumas[1-3]. Around a third of postmenopausal women are affected by osteoporosis, with higher prevalence between 65 and 75 years of age and it may reach 70% in women over 80 years old. It is related to high rates of morbimortality, mainly due to fractures caused by bone fragility[2,4-7]. In the United States, from the total fractures treatment expenditure, about 50-67% are specifically of osteoporotic fractures, which increases the health care costs[8].

The studies involving the effects of immobilization in osteoporotic bones, especially when it comes to women's life situation, such as postmenopausal are still few. Immobilization is a conservative therapeutic resource used in post-operative, post-fractures, sprains and muscle injuries, in order to maintain a body segment at rest; aims to reduce the pain symptoms and protect the affected musculoskeletal structures affected by injuries[9]. However, the immobilization also leads to problems for tissues, affects negatively the muscle, bone and cartilaginous tissues, causing mass and joint range of motion decreasing, besides functional deficits[10-12]. In addition to these effects, is still observed decrease in strength and in the cross sectional area of the muscle; loss and reduction of the synthesis of proteoglycans of the cartilage matrix; necrosis and ulceration of cartilage; mass reduction and total cartilage volume; significant loss of cortical and cancellous bone[9,13-19].

Several studies emphasize that remobilization exercise has been shown to be effective in the recovery of patients and animals that were subjected to a body segment immobilization, promoting muscle hypertrophy, improving and maintaining bone mass[20-23]. Kemmler *et al.*[24] reinforces that even a single session of exercise can positively influence the hormones that affect bone metabolism, improving the remodeling process. However, up to the present, were not found in literature studies about morphological changes that the exercise of stair climbing can cause on the bone tissue of animals, osteoporotic females due to hormonal deprivation, in mimetization of postmenopausal period. The increase of life expectancy of the population, with consequent increase in the arising of chronic diseases, osteoporosis as a major public health problem nowadays, the disabilities and deleterious effects on tissues resulting from a restraint, and also from the need of making scientific evidence therapeutic resources used in clinical practice justify this study, which aimed to analyze the effects of ladder climbing exercise on bone histomorphometry in female rats induced to osteoporosis and submitted to immobilization.

Materials and methods

Experimental Groups

It were used 36 adult female Wistar rats, (10 ± 2 weeks), nulliparous, with a mean weight of 317.2 ± 22.1 g, kept in polypropylene cages, with free access to water and food, photoperiod light/dark of 12 hours, controlled room temperature (25 ± 1° C). The study was conducted according to the international ethical standards of animal experimentation and was approved by the Ethics Committee on Animal Experimentation of the State University of West of Paraná (Unioeste), under the number 4112.

The rats were divided randomly into six groups:

- G1 (n=6): submitted to simulated oophorectomy surgery (pseudo-oophorectomy) and remained 60 days without intervention;
- G2 (n=6): submitted to pseudo-oophorectomy and remained 60 days without intervention. After that, an immobilization of the right hind limb was performed (RHL) for two weeks. Posteriorly, they remained in free remobilization for the same period, being just put in contact with a ladder, in the last 10 centimeters (cm);
- G3 (n=6): submitted to pseudo-oophorectomy and remained 60 days without intervention. After that, an immobilization of the RHL was performed for two weeks and subsequently subjected to the exercise of stairs climbing for 10 days, with an interval of two days after the fifth session;
- G4 (n=6): bilateral oophorectomy surgery and remained 60 days without intervention;
- G5 (n=6): bilateral oophorectomy and remained 60 days without intervention. After that, it was performed immobilization and remobilization similar to G2;
- G6 (n=6): bilateral oophorectomy and remained 60 days without intervention. After that, it was performed immobilization procedure and remobilization similar to G3.

Pseudo and Oophorectomy Protocol

To carry out oophorectomy, pseudo-oophorectomy, immobilization and euthanasia, the rats were weighed and subjected to a protocol of anesthesia, which consisted of intraperitoneal injection of xylazine (12 mg/kg) and ketamine (80 mg/kg). To do the oophorectomy a trichotomy and antisepsis with iodine alcohol was performed in the region of the lower abdomen, followed by a longitudinal incision with a scalpel blade nº 11. After accessing the peritoneal cavity, the adipose tissue was removed until the oviduct and ovaries could be identified. Then, a suture with simple wire catgut 4.0 was done on the area of the uterine horns, promoting ovarian resection bilaterally. At the end of the procedure, internal sutures with resorbable simple wire catgut 4.0, and external with nylon 4.0 were performed. The pseudo-oophorectomy consisted of all surgical steps similar to ovariectomy, except for the ovaries removal. Subsequent to surgery, the rats remained for 60 days without any intervention, free in the cage[25].

Immobilization Protocol

Prior to immobilization, the anesthetic procedure was performed, and then the RHL, from the hip to the ankle, wrapped in a tubular

mesh with cotton bandages. Then the static orthosis was shaped, of approximately 50 grams, using quick drying plaster bandages, with the RHL in full extension of the knee and ankle in maximum plantar flexion, in rats of G2, G3, G5 and G6 as model proposed by Booth and Kelso[26], being reconfigured to only one of the members according to a study by Matheus *et al*.[27].

Ladder Climbing Protocol

After immobilization removal, the rats of G3 and G6 were submitted to the exercise of ladder climbing, with 10 repetitions per day in the first week (five days) and 20 repetitions per day in the second week (five days), with an interval of one minute between the climbs and two days between the weeks. The rats of G2 and G5 performed remobilization free of the cage, being placed in contact with a ladder at 10 cm from the end of it, only once, in the same period in which the exercise was performed on the ladder to G3 and G6. The equipment used to perform the exercise consisted of a ladder with 67 steps, spaced 1.5 cm between the steps of the grid, 20.5 cm wide, 118 cm height and vertical with 80° inclination angle. At the upper area there was a 28.5 cm long, 18.5 cm high and 15 cm wide darkroom, used for the rats to rest between the climbing sets, as well as to make them feel attracted to a shelter and encouraged to perform the exercise[20,22,28,29].

Histomorphometric Analysis

At the end of the experiment, all the rats were weighed and underwent anesthetic protocol being subsequently euthanized by decapitation in guillotine. After these procedures, the right and left tibia (left hind limb – LHL) were dissected and placed in flasks containing formalin at 10%. Then, after the fixation, the tibiae were washed with distilled water and immersed in trichloroacetic acid (TCA) at 5%, during five days, for decalcification. The next steps were dehydration, diafanization, measurement of the parts in its length with the help of a digital pachymeter to perform in the medial region of the bone a cut in the transversal plane.

In the proximal part, a cut in the frontal plane was performed, being reserved the anterior portions. After that, the bone pieces were impregnated and embedded in paraffin, being arranged in the blocks for subsequent microtomization as follows: the proximal portion, in frontal cut; and the distal portion, with transversal cut, positioned with the upper region for visualization. Cuts of 7 µm were performed in microtome, and confectioned blades with hematoxylin and eosin (HE) and photomicrographed with Olympus DP71® microscope.

The photomicrographs were submitted to analysis using the Image-Pro Plus 6.0® program, in the transversal plane performed with magnification of 40 times, for measurements of medullary canal area and cortical bone area, being the cortical thickness measured at three points (M1 , M2 and M3)[30] (Figure 1A); and magnification 400 times for counting the osteocytes, measured in three regions (between the points M1 and M2, M1 and M3, and M2 and M3) by means of a rectangle of 200 µm base by 100 µm height, overlaid on the image. There were excluded from the count, the cells that were in contact with the upper edge and right side (Figure 1B). In the frontal plane, photomicrographs were taken in three regions, designated P1, P2, and P3, which correspond respectively to the lateral, intermediate and medial portions of the proximal region of the tibia, with a magnification of 40 times to measure the length (vertical tracing) and area (circular tracing) trabecular bone (region between epiphyseal plate the and upper articular cartilage)[31,32] (Figure 1C). After collecting the data, the means were made and the statistical analyzes were performed.

Statistical Analysis

The survey data were evaluated by comparing the results obtained on the left hind limb (control) and right (immobilized), between the rats of the same group and between the experimental groups. For this, the BioEstat 5.0® program was used, with values presented as mean and standard deviation. The Student t paired test was applied for comparison between the right and left side, within the same group, and one-way ANOVA with Bonferroni post hoc test, for comparison between experimental groups. The level of significance was $p \leq 0.05$.

Figure 1. Photomicrographs with statements of histomorphometric analyzes performed on the ovariectomized rat right tibia subjected to immobilization and remobilization ladder. In A, measuring the thickness of cortical bone in three points (M1, M2 and M3) and medullar canal area, cross-section and 40X magnification; B overlaid base of 200 µm by 100 µm high for counting osteocytes in the area between the points M1 and M2, 400X magnification (obs .: rectangle asterisks exclusion demarcate the edges (top and right side); and C, measurement of area (circular route) and thickness (vertical layout) of trabecular bone (upper region of the plate and articular cartilage) in medial (P3), 40X magnification. HE staining.

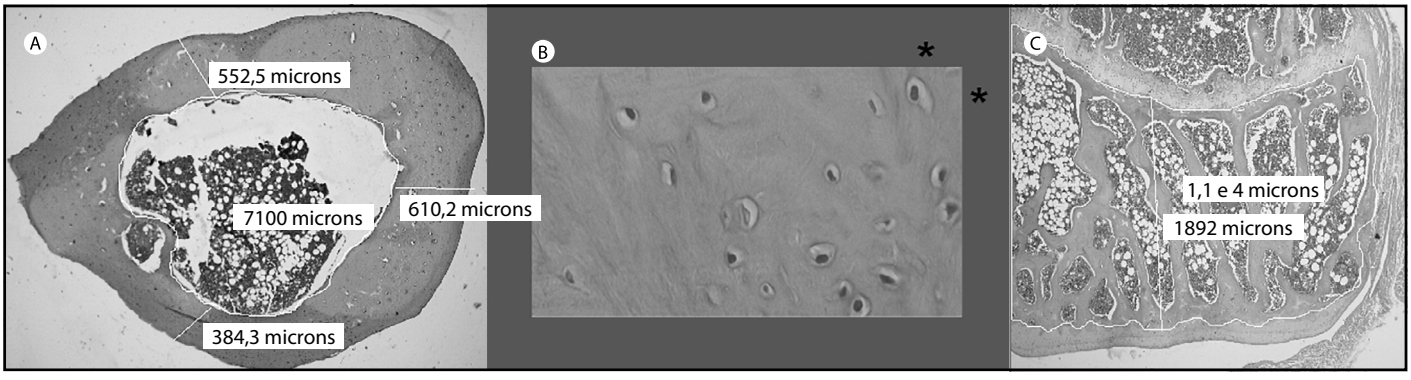

Table 1. Area and cortical thickness data, comparing the rats distributed between the study groups (G1 - G6), right (RHL - target) and left hind limbs (LHL - control).

Groups	Cortical Area (µm²) Note.: values elevated to the 6th Power RHL (mean ± sd)	LHL (mean ± sd)
G1	4.20 ± 5.47 (a)	4.90 ± 8.91 (cd)
G2	4.65 ± 11.71 (ab)	4.78 ± 9.97 (c)
G3	5.60 ± 18.36 (ab)	5.80 ± 12.58 (cd)
G4	3.80 ± 6.78 (a)	3.73 ± 4.77 (d)
G5*	4.00 ± 5.57 (a)	5.40 ± 7.91 (cd)
G6	6.66 ± 9.22 (b)	6.22 ± 12.32 (c)

Groups	Cortical Thickness (µm) RHL (mean ± sd)	LHL (mean ± sd)
G1	600.38 ± 64.44 (ab)	661.46 ± 122.49 (c)
G2	617.68 ± 69.30 (ab)	690.43 ± 89.07 (c)
G3	683.07 ± 69.83 (ab)	702.05 ± 39.53 (c)
G4	394.68 ± 35.83 (a)	427.55 ± 103.78 (d)
G5*	374.96 ± 28.40 (a)	624.19 ± 90.31 (c)
G6	646.12 ± 112.36 (b)	711.63 ± 78.82 (c)

Obs.: same letters mean similarities, and the different letters mean significant differences between the experimental groups to the same side. * Significant difference between the sides of the same group.

Results

Cortical Bone Area and Thickness

In the comparison between rats of the same group, it could be noted a significant decrease in cortical bone of the RHL regarding the LHL in G5, as evidenced by data from both the area (p=0.0178) and thickness (p=0.0024). The other groups (G1, G2, G3, G4 and G6) showed no significant differences between their hind limbs so that remobilization (G6) was able to reverse the loss of cortical bone in the immobilized limb when compared to the contralateral non-immobilized (Table 1).

When the comparison was made between the groups, with mean values of RHL, it was observed a significant increase in G6 compared to G1, G4 and G5 (p=0.0007), to area, but also to G4 and G5 (p=0.0062), for thickness. Thus, it is evident that the exercise was able to reverse the cortical bone loss caused by ovariectomy and immobilization, with values that exceed even those found in rats only pseudo-oophorectomized (G1). It is noteworthy that the free remobilization was not able to recover the loss of cortical bone in the limb of ovariectomized rats (Table 1).

In the LHL analysis between groups, there was a significant decrease in area of G4 in relation to G2 and G6 (p=0.0031), and in thickness of G4 in relation to the other groups (p=0.0001). This finding shows that ovariectomy was able to promote cortical bone loss and that both, free remobilization, and in ladder were able to recover from this loss (Table 1).

Table 2. Data of the medullary canal area, comparing the rats distributed between the study groups (G1 - G6), right (RHL - target) and left hind limbs (LHL - control).

Groups	Medullary Canal Area (µm²) Note.: values elevated to the 6th Power RHL (mean ± sd)	LHL (mean ± sd)
G1	1.90 ± 2.53 (a)	2.13 ± 4.89 (c)
G2	2.48 ± 6.61 (ab)	2.00 ± 4.29 (c)
G3	1.77 ± 4.32 (a)	2.08 ± 7.57 (c)
G4	2.00 ± 5.21 (a)	2.25 ± 4.93 (c)
G5*	3.42 ± 1.07 (b)	2.22 ± 3.34 (c)
G6	2.00 ± 8.74 (a)	2.40 ± 5.73 (c)

Obs.: same letters mean similarities, and the different letters mean significant differences between the experimental groups to the same side. * Significant difference between the sides of the same group.

Table 3. Data of osteocytes number, comparing the rats distributed between the study groups (G1 - G6), right (RHL - target) and left hind limbs (LHL - control).

Groups	Osteocytes Number (un) RHL (mean ± sd)	LHL (mean ± sd)
G1	22.67 ± 5.32 (a)	20.06 ± 3.32 (c)
G2	18.83 ± 3.48 (a)	23.33 ± 6.36 (c)
G3	20.44 ± 1.76 (a)	21.33 ± 4.73 (c)
G4	20.33 ± 1.63 (a)	19.67 ± 1.71 (c)
G5*	10.07 ± 0.83 (b)	20.07 ± 1.93 (c)
G6	23.94 ± 1.97 (a)	24.94 ± 2.89 (c)

Obs.: same letters mean similarities, and the different letters mean significant differences between the experimental groups to the same side. * Significant difference between the sides of the same group.

Medullary Canal Area

As to the medullar canal area, G5 rats showed a significant increase of RHL in relation to LHL (p=0.0384), also for the G5 RHL showed higher values than G1, G3, G4 and G6 (p=0.0043). In LHL there was no significant difference between the groups, ie, the area of the medullary canal was maintained even after the interventions (Table 2).

Thus, it was found that oophorectomy associated with immobilization and free remobilization (G5), produced a significant increase in the medullary canal area of the immobilized limb, when compared to the RHL from the other groups and that the ladder climbing exercise was able to reverse this increase. This was evident in G3 and G6, which had similar means between themselves and to the non-immobilized groups (G1 and G4) (Table 2).

Osteocytes Number

Regarding the osteocytes number, it was observed a statistically significant difference between RHL and LHL of G5 (p<0.0001), revealing that the free remobilization is not able to reverse the cell loss caused by immobilization in ovariectomized rats. However, as there was no

Table 4. Data referring to the trabecular bone area and thickness, comparing the rats distributed between the study groups (G1 - G6), right (target) and left hind limbs (control).

Groups	Trabecular Area (μm²) Note: values elevated to the 6th power RHL (mean ± sd)	LHL (mean ± sd)
G1	2.67 ± 9.22 (a)	2.15 ± 6.74 (b)
G2	3.55 ± 7.73 (a)	3.55 ± 5.56 (b)
G3	3.34 ± 5.07 (a)	3.45 ± 7.85 (b)
G4	3.52 ± 1.24 (a)	3.84 ± 1.02 (b)
G5	3.03 ± 8.43 (a)	3.02 ± 3.72 (b)
G6	3.70 ± 6.83 (a)	4.69 ± 2.40 (c)
Groups	**Trabecular Thickness (μm) RHL (mean ± sd)**	**LHL (mean ± sd)**
G1	1587.6 ± 255.82 (a)	1353.87 ± 118.44 (b)
G2	1774.83 ± 215.35 (a)	1795.06 ± 81.48 (c)
G3	1927 ± 156.10 (a)	1928.89 ± 257.36 (c)
G4	1655.11 ± 175.77 (a)	1716.03 ± 206.04 (bc)
G5	1728.07 ± 227.24 (a)	1645.6 ± 232.20 (bc)
G6	1880.72 ± 307.6 (a)	1928.5 ± 354.04 (c)

Obs.: same letters mean similarities, and the different letters mean significant differences between the experimental groups to the same side.

difference between the hind limbs of rats belonging to the G6; it is believed that the ladder exercise was able to reverse this loss (Table 3).

In the comparison between the groups, it was observed lower values only in RHL at G5 comparing to the other groups (p=<0.0001). Thus, immobilization with free remobilization caused severe loss of osteocytes in ovariectomized rats and the ladder climbing exercise proved to be effective in recovering the number of osteocytes (Table 3).

Trabecular Bone Area and Thickness

In the trabecular bone area and thickness analysis of the same group rats, there was no significant difference between RHL and LHL. In the intergroup analysis there were significant differences in comparison of G1 LHL with G6 (p=0.0265) for the area and thickness, and G1 to G2 and G3, only for thickness (p=0.0015) (Table 4).

Discussion

According to the results, it was observed a significant loss of cortical bone and the osteocytes number in immobilized limbs in ovariectomized rats subjected to the free remobilization, which was reversed by remobilization climbing ladder exercise. It was also observed that the osteoporotic rats, not immobilized, had significant loss of cortical bone, however, there was reversibility with the free remobilization and ladder climbing exercise. It was also observed an increase in trabecular bone in the not immobilized limbs of rats submitted to exercise ladder. Thus, the results obtained are consistent and affirm the hypothesis of this study that the ladder climbing exercise is effective in reduction or reversibility of bone loss caused by osteoporosis and immobilization.

In the present study, it was opted for the use of ovariectomized rats that mimetize some osteoporotic features found in postmenopausal women and for immobilization in plaster static orthoses, using the histomorphometry method for evaluation. These were chosen for been effective, and for best representing the daily clinic routine and with lower operational costs. There are several experimental models for inducing osteoporosis, either by hormonal interventions (surgical or pharmacological), dietary, genetic alterations, or immobilization (conservative or surgical)[30,31]. The oophorectomy consists on the removal of the ovaries, the main source of estrogens, thus inducing the restriction of this hormone circulating in the body. After surgery already begins the process of bone remodeling, with reabsorption exceeding neoformation, causing bone loss[29,30].

In the evaluation by means of histomorphometry is possible to analyze bone mass and architecture with accuracy as well as indexes of bone fragility, number of osteoblasts, osteoclasts, osteocytes and active osteoblasts in relation to the bone perimeter. Some authors have emphasized that the histological analysis technique is considered to be more effective, however it shows some limitations, such as difficulty in assessing various areas in different structures and at the same time. It is usually possible to analyze only a small area of the tissue, at a particular bone, which does not represent changes throughout the skeleton. However, it presents a great reliability when the sampling area is comparable in all groups[30,32]. Thus, it was opted for the bone histomorphometry in the proximal and medial region of the tibia, this bone was chosen due to its anatomical and biomechanical importance.

In some studies on oophorectomy significant bone losses were observed after 14, 72 and 74 days at the proximal tibia metaphysis, after 60 and 73 days in the lumbar vertebral body and after 30 days in the femoral neck[30,33]. Other authors report that the first changes in the width of the cortical bone and the medullar cavity of the femur and tibia, are noted in periods around 90 and 120 days after ovariectomy[34-36]. In the present study, was observed significant bone loss at 60 days after ovariectomy and 90 days after the association of oophorectomy, immobilization and free remobilization in the proximal region (trabecular) and tibia medial (cortical). However, the loss was more pronounced in 90-day period in the oophorectomized group, immobilized and free remobilization, and may be associated with longer duration of hormone estrogen deprivation, but also by association with immobilization, as it accentuated the decrease in bone mass.

Strong evidences show that the combination of oophorectomy and immobilization can reduce the time of significant bone loss, especially the cortical, when compared to the techniques isolated[30,34-36]. In view of the findings of this study, such association may also accentuate the effects of the significant loss of bone mass. These findings corroborate with previous studies showing the accuracy of measurements taken in the medial inferior areas of the cortical axis, which are very reliable, because most bone losses occur in this location[30,33,37].

The reduction in bone mass was clearly demonstrated in this study, because it is well seen in the cortical bone by the enlargement of the medullar cavity. This occurs due to the increasing in endosteal reabsorption and periosteal bone apposition[30,33,37]. With area measurements and

cortical thickness as well as the medullar canal area, only G5 RHL showed significant decrease compared to the LHL, which may be related to the effects of estrogen hormone deprivation associated with immobilisation of the RHL. The free remobilization was not effective to recover this loss, as in G2, G3 and G6 there was no difference. Thus, it is believed that both the free exercise and in stairs were effective to recover the bone loss associated with immobilisation in the pseudo-ovariectomized groups (G2 and G3) and that only the ladder exercise was effective in this reversibility in immobilized osteoporotic rats.

In intergroup comparison to the RHL, it was found that the exercise on ladder was effective in the recovery of cortical bone loss caused by ovariectomy alone or in combination with immobilization and free remobilization. It was also found that G6 was significantly higher than G1 regarding the cortical area, ie, showing that the exercise on ladder produces increasing in the upper bone mass even when compared with animals that did not undergo significant interventions, only the surgical procedure stress without removal of the ovaries.

In intergroup comparison for LHL, there was a significant decrease in cortical bone area and cortical bone thickness of G4 compared to G2 and G6. Regarding G2 can be linked to the fact that there was an overload on the LHL in relation to the immobilized limb (RHL), leading to a growth of cortical bone in higher values than those from the group which was subjected only to hormone deprivation (G4) with significant loss of cortical bone. However, in G6, it is evident the outcomes of therapeutic intervention, ie, that the ladder exercise was a clinical resource very well employed in remobilization for the recovery of bone loss caused due to hormonal estrogen deprivation and immobilization. As there was no difference compared to G5, it is believed that osteoporosis may have been determinative in the reduction of bone mass, as in G4, as well as in the free activity in the box was not enough to reverse the decrease in cortical bone mass in osteoporotic rats.

Regarding the trabecular bone, there was a significant LHL loss of G1 compared to G2, G3 and G6. This may be related to overload exerted on LHL due to immobilization of RHL associated with the effectiveness of exercise of climbing stairs, in ovariectomized and pseudo-oophorectomized rats being even able to promote an increase in this type of tissue. For rats not subjected to hormone deprivation, even the free exercises associated to the overload in LHL were able to promote growth of the trabecular bone. It was not observed diminution in consequence of ovariectomy and immobilization, which may be related to post-surgical period and immobilization. Maeda *et al.*[18] found a significant loss of cancellous bone in rats subjected to six weeks of immobilization.

Even with therapeutic interventions applied correctly, the complete reversibility of the damage caused by immobilization will not always occur[38,39]. Nevertheless, the exercise has been a therapeutic resource widely used for both the treatment of osteoporosis and in remobilization periods. However, the benefits of physical exercise on the skeleton depend on the intensity and bone characteristics, such as age, type and region involved[40,41]. In this study it was found that both the free exercise and the stairs climbing were effective in recovering the bone loss in rats that have undergone hormonal deprivation, through the protocol used. However, when these ovariectomized rats underwent immobilization, it was found that the free exercise was not able to recover bone loss, unlike the ladder exercise.

Several authors studied the therapeutic action of various resources and remobilization techniques in animal models, such as muscle stretching[42], free remobilization1[5,21,42], swimming[15], jump[21] and treadmill running[40,43]. It were found improvements in gait, cartilage conditions, subchondral bone, biomechanical and articular capsule[15]; restoration of the trabecular architectural integrity[21]; increase in bone mass and mineral density[21,40,43]; decrease in the number of osteocytes, even after remobilization[42]. This study verified that the osteocytes number of immobilized limbs was reestablished in both free remobilization and climbing stairs, in pseudo-ovariectomized rats. In osteoporotic rats, only the exercise of stairs climbing was able to recover the number of osteocytes, with values that even resemble the groups not exposed to hormone deprivation.

Study performed with rats subjected to tail suspension for 21 days and climbing stairs exercise for the same period, doing eight sets, with overload equivalent to 80% of their maximum strength, five times a week, observed that this type of exercise was able to restore the BMD and bone stiffness values[44]. In this study, the exercise of climbing stairs was held without load. Nevertheless, the protocol used for this exercise was effective in the recovery of bone mass and cell losses due to ovariectomy and immobilisation verified by the histomorphometry method. Cassilhas *et al.*[20] add that the ladder exercise promotes hypertrophy of the gastrocnemius muscle, the flexor digitorum longus and plantaris, which can help on the protection of the ankle joint and the bone matrix. Nascimento *et al.*[22] showed that this climbing exercise also promotes hypertrophy of the brachial triceps muscle, ie, achieving, with positive results with inferences even for the forelimbs.

Thus, it is apparent that the stair climbing exercise promotes the adaptation of muscle tissue, cartilage and bone, with an improvement in BMD, bone stiffness, hypertrophy of gastrocnemius muscles, flexor digitorum longus, plantaris and triceps proprioceptive stimulation and resistance, thus proving to be an important and effective kinesiotherapeutic feature, mainly because of its global action throughout the body, not only in a focus or member[20,22,45]. It is believed that exercise stimulates ladder climbing action osteoprotegerin, neutralizing the effects of osteoclastic bone resorption by the interaction of receptor activator kB ligand (RANKL) and receptor activator kB (RANK) due to hypoestrogenism. This possibly leads to an increased formation of vitamin D and serum calcium uptake in the bone matrix, providing increased mass and bone strength, which was evidenced by area and cortical thickness, and number of osteocytes, in the groups submitted to exercise climb staircase[46].

Thus, it might be noted that the ladder climbing exercise appears to be a good resource for clinical practice, taking in account many benefits that it provides to the treatment of osteoporosis in periods of remobilization and the combination of both situations. This causes it to expand the clinical reasoning of professionals and to increase the possibilities of therapeutic methods to be applied with greater accuracy and resolution in the proposed treatments. One limitation of the present study was the absence of a control group subjected only to immobilization, without remobilization and hormonal intervention. It is reiterated regarding the difficulty of other comparisons with the exercise protocol used, using times, series and different intensities. However, following the 3Rs ethical rule (replacement, reduction and refinement) for the use of animals in research, it was opted to follow this model due to the number of rats to be used.

In summary, upon the climbing stairs exercise protocol used in this study and with its limitations, it is concluded that this therapeutic approach was effective in the recovery of bone loss in rats subjected to a post-menopausal osteoporosis experimental model and immobilization, with recovery of thickness, and cortical and trabecular area, as well as the number of osteocytes. It is emphasized that future studies could be conducted with greater times of hormonal deprivation and immobilization, implementing other exercise protocols, in association with other therapeutic resources, as well as evaluating the effects on other systems and potential impact on functional activities.

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