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ORIGINAL ARTICLE

Jumping exercise preserves bone mineral density and mechanical properties in osteopenic ovariectomized rats even following established osteopenia

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Abstract
Summary The effects of jump training on bone structure before and after ovariectomy-induced osteopenia in rats were investigated. Jumping exercise induced favorable changes in bone mineral density, bone mechanical properties, and bone formation/resorption markers. This exercise is effective to prevent bone loss after ovariectomy even when osteopenia is already established.
Introduction The present study investigated the effects of jump training on bone structure before and after ovariectomy-induced osteopenia in 80 10-week-old Wistar rats.
Methods Forty rats (prevention program) were randomly allocated to one of four equal groups (n = 10): sham-operated sedentary (SHAM-SEDp), ovariectomized (OVX) sedentary (OVX-SEDp), sham-operated exercised (SHAM-EXp), and OVX exercised (OVX-EXp). SHAM-EXp and OVX-EXp animals began training 3 days after surgery. Another 40 rats (treatment program) were randomly allocated into another four groups (n = 10): sham-operated sedentary (SHAM-SEDt), OVX sedentary (OVX-SEDt), sham-operated exercised (SHAM-EXt), and OVX exercised (OVX-EXt). SHAM-EXt and OVX-EXt animals began training 60 days after surgery. The rats in the exercised groups jumped 20 times/day, 5 days/week, to a height of 40 cm for 12 weeks. At the end of the experimental period, serum osteocalcin, follicle-stimulating hormone (FSH) dosage, dual X-ray absorptiometry (DXA), histomorphometry, and biomechanical tests were analyzed.
Results The OVX groups showed higher values of FSH and body weight ($p < 0.05$). DXA showed that jump training significantly increased bone mineral density of the femur and fifth lumbar vertebra ($p < 0.05$). The stiffness of the left femur and fifth lumbar vertebra in the exercised groups was greater than that of the sedentary groups ($p < 0.05$). Ovariectomy induced significant difference in bone volume (BV/TV, percent), trabecular separation (Tb.Sp, micrometer), and trabecular number (Tb.N, per millimeter) ($p < 0.05$) compared to sham operation. Jump training in the OVX group induced significant differences in BV/TV, Tb.Sp, and Tb.N and decreased osteoblast number per bone perimeter ($p < 0.05$) compared with OVX nontraining, in the prevention groups. Osteocalcin dosage showed higher values in the exercised groups ($p < 0.05$).
Conclusions Jumping exercise induced favorable changes in bone mineral density, bone mechanical properties, and bone formation/resorption markers. Jump training is effective to

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prevent bone loss after ovariectomy even when osteopenia is already established.

Keywords Exercise therapy · Osteopenia · Ovariectomy · Rehabilitation medicine

Introduction

Osteopenia is characterized by low bone mass and microstructural deterioration of bone tissue, leading to bone fragility and increased susceptibility to fractures [1]. Osteopenia is reversible and precedes osteoporosis [2].

Physical activity and mechanical loading play important roles in the regulation of bone homeostasis, without the side effects of the use of drugs [3]. Exercise increases peak bone mass [4]. However, nonweight-bearing activities such as swimming and cycling exert controversial influence on bone mineral density [5, 6] and are not the best form of exercise for prevention and treatment of osteoporosis.

A most effective physical activity in eliciting an osteogenic response is jumping exercise, as it causes high tension and deformation in bones [7]. Thus, jumping appears to promote superior bone formation compared with low-impact aerobic exercises [8–12]. The mechanical stress induced by jumping training is crucial for the structural and functional integrities of the skeletal system, since it increases bone density and mechanical properties of the bones [7].

The ovariectomized rat bone loss model is suitable to study problems relevant to postmenopausal bone loss [13] and is most commonly used in research on postmenopausal osteoporosis. After ovariectomy, bone resorption initially exceeds bone formation, causing bone loss. Soon thereafter, bone remodeling reaches a steady state, where resorption and formation are balanced. Statistically significant bone loss is seen in the proximal tibial metaphysis after 14 days [14], in the lumbar vertebral body after 60 days [15], and in the femoral neck after 30 days [16, 17].

The present study investigated whether jumping exercise, before and after ovariectomy-induced osteopenia, might have a beneficial effect on bones in rats. Our hypothesis was that the chosen impact exercise regimen (prevention or treatment) would lead to increased levels of bone turnover in favor of bone formation in both situations.

Materials and methods

Female rats were used to evaluate the effects on bone in prevention (before installed osteopenia) and treatment (after installed osteopenia) programs of jumping exercise.

Animals

The protocols for animal experimentation were previously approved by the Institutional Animal Care and Use Committee of the university (protocol number CETEA 182/2008).

Eighty female Wistar rats, aged 10 weeks, were obtained from the University of São Paulo (Ribeirão Preto, São Paulo, Brazil). The rats were housed in standard cages (three to four animals per cage) at a constant temperature (25 °C) and a 12-h light-dark cycle. Food and water were provided ad libitum.

We used a system of sealed envelopes to allocate the rats within groups. An official of the central animal facility separated 80 animals all of the same age. We used a random numbers table to allocate blocks of ten rats. Starting with an arbitrary point in the table, we selected eight sequential random numbers. The first number was assigned to the single row block and the next was assigned to the double row block. These assignments were then arranged in an ascending order. This procedure produced a random sequence of consecutive surgery and treatment allocations. Sealed, opaque, and numbered envelopes containing the surgery (sham or ovariectomy) assignments were prepared, with care being taken to make sure that the order of the envelopes exactly matched the allocation schedule.

The first four numbers were allocated to the prevention program and the last four to the treatment program. On the day of the surgery, an experienced veterinary surgeon, who performed all the operations, opened the sealed envelope allocation for each numbered rat to have ovariectomy (OVX rats) or sham (Sham rats) procedure with each rat under anesthesia with intramuscular ketamine/xylazine (30 mg kg^{-1} ketamine and 3 mg kg^{-1} xylazine, i.m.).

Ovariectomy was confirmed with follicle-stimulating hormone (FSH) dosage.

On the day of the beginning of the training, the groups were randomized by sealed envelopes to establish whether a given block of animals would (or not) perform the exercises. On this day, a researcher opened one sealed envelope per rat, allocating them to the exercise or sedentary program.

All analyses were performed by a researcher who did not know to which groups the animals belonged.

Training program

Rats in the training groups jumped 20 times/day, 5 days per week, for 12 weeks. Each exercised rat jumped and grasped the top of a wooden box (40 cm in height) with their forelimbs and pulled themselves over the edge; the rats were then carefully returned to the bottom of the box by hand [18, 19]. Initially, the rats jumped with electrical stimulation, but, after a few days, they jumped without stimulation. Rats jumped at 3-s intervals and 2–3 min was required to complete a training session.

The peak ground reaction force of rats that jumped upwards to a height of 40 cm is about 6.3 times the body weight [20].

Experiment 1—prevention program

Forty rats were first randomly allocated to the prevention program and then randomly allocated to one of the following four groups: sham-operated sedentary (SHAM-SEDp; n = 10), ovariectomized (OVX) sedentary (OVX-SEDp; n = 10), sham-operated exercised (SHAM-EXp; n = 10), and OVX exercised (OVX-EXp; n = 10).

SHAM-EXp and OVX-EXp rats in the prevention protocols began training 3 days after surgery, for 12 weeks. The hypothesis of this experiment is that high-impact exercise can prevent bone from becoming osteopenic (Fig. 1).

Experiment 2—treatment program

In the treatment protocols, the rats began training 60 days after surgery, for 12 weeks. We hypothesized that high-impact exercise can arrest and/or reverse the osteopenia induced by ovariectomy.

Forty rats were randomly allocated to the treatment program and then randomly allocated into one of the following four groups: sham-operated sedentary (SHAM-SEDt; n = 10), OVX sedentary (OVX-SEDt; n = 10), sham-operated exercised (SHAM-EXt; n = 10), and OVX exercised (OVX-EXt; n = 10) (Fig. 1).

Data collection

All animals were weighed weekly. At the end of the experiments, the animals were euthanized with an intraperitoneal overdose of sodium pentobarbital. After confirmation of death, the sternum was sectioned to allow access to the thorax and to puncture the left ventricle. Blood samples were collected and centrifuged and the serum was stored at −70 °C for biochemical analysis.

The left femur and fifth lumbar (L5) vertebra were dissected from each rat and stored at −20 °C until bone mineral densitometry (BMD) and mechanical testing. Immediately after careful removal of all soft tissues, the right tibia was fixed with 70% ethanol and stored at 4 °C until histological assessment was performed.

Bone mineral densitometry

The BMD of the left femur and L5 vertebrae was measured by DXA using a Lunar DPX-L device (Lunar, Madison, WI, USA) adapted to measurement in small animals. A high-resolution mode (voltage of 76.0 kVp, current of 150 mA, and collimation of fine) was used, with scan width of 40 mm and scan length of 50 mm. The bones were submerged in a plastic vessel containing water at 2-cm depth to simulate the effect of soft tissue. The entire bones were scanned and the scans were analyzed.

Mechanical testing procedures

The left femur and L5 vertebrae were dissected and weighed and their length measured. The mechanical properties of the left femur and L5 vertebrae were assessed in all the samples collected. After thawing, the femoral neck was subjected to a bending-compression test and the L5 to a compression test. The L5 vertebrae were trimmed with removal of the spinous and transverse processes so that the vertebral body could be placed upright without support. A device having a small cylinder, compressing the central area of the vertebral body, had been developed for this purpose. We used a 50 kgf load cell, constant displacement speed of 0.1 mm/min, preload of 10.0 N, and 30-s time of accommodation. To achieve flexion-compression testing in the femora, the distal epiphysis was embedded in acrylic resin for fixing the base of the test machine. The load was applied in the center of the head, the

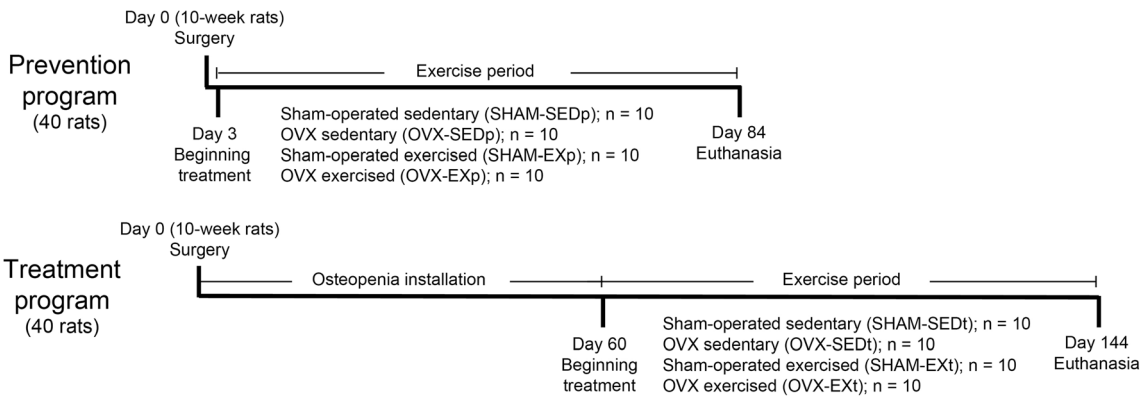

Fig. 1 Chart of the division of experimental groups. Note the initial division into two groups (*prevention* and *treatment*) and subsequent division in four groups (sham, ovariectomy, sham trained, and ovariectomy trained)

bone load cell used was 50 kgf, preload 10.0 N and 30-s settling time, and load application speed of 1 mm/min [21]. Maximum deformation and stiffness were calculated [22]. Ultimate load, deformation on ultimate load, and stiffness were determined from the load/deformation curves [21–23]. All tests were performed using a material testing machine (EMIC®, 10,000 N; EMIC, São José dos Pinhais, Paraná, Brazil) with ad hoc accessories.

Bone histomorphometry

Quantitative static bone histomorphometry was performed. The right tibia of all the animals was removed for histomorphometric analysis. At the end of the experimental period, the right tibia was carefully dissected out, immediately fixed in 70% ethanol at 4 °C for 7 days, dehydrated, and embedded in methyl methacrylate. Undecalcified 5- and 10-μm thick longitudinal sections of the proximal tibia were cut using a Polycut-S equipped with a tungsten carbide knife (Leica, Heidelberg, Germany) and mounted on gelatin-coated glass slides. Measurements were performed on the cancellous bone of the right proximal tibial metaphysis, 195-μm distal to the epiphyseal growth plate; a total of 20 fields were assessed using a semiautomatic method (OsteoMeasure, OsteoMetrics®, Atlanta, GA, USA), a digitizing table with a cursor, and a camera. The 5-μm sections were stained with 0.1% toluidine blue (pH 6.4) and viewed under polarized light at 40× magnification. The histomorphometric indices were as follows: bone volume (BV/TV, percent), osteoid volume (OV/BV, percent), osteoblast surface (Ob.S/BS, percent), osteoid surface (OS/BS, percent), osteoid thickness (O.Th, micrometer), osteoblast number per bone perimeter (N.Ob/B.Pm, per millimeter), osteoclast number per bone perimeter (N.Oc/B.Pm, per millimeter), osteoclast surface (Oc.S/BS, percent), eroded surface (ES/BS, percent), trabecular separation (Tb.Sp, micrometer), trabecular number (Tb.N, per millimeter), and trabecular thickness (Tb.Th, micrometer). All data were obtained by a blinded assessor. The histomorphometric indices were reported according to the standardized nomenclature recommended by the American Society of Bone and Mineral Research [24].

Biochemical marker

Blood samples were kept on ice and immediately centrifuged for 10 min at 4 °C (4500 × g). The serum was obtained and used for osteocalcin, osteoprotegerin, and receptor activator of nuclear factor-kappa B ligand (RANKL) determinations. The samples were stored at 20 °C until analysis.

Serum osteocalcin (nanogram per milliliter) was determined using the MicroVue Osteocalcin enzyme immunoassay (QUIDEL Corporation, San Diego, CA). The osteoprotegerin (OPG) ELISA kit and soluble receptor activator of nuclear factor-κ B ligand (sRANKL) kit were purchased from Biomedica (Wien, Austria).

Statistical analysis

Data are presented as means ± SD. Two-way ANCOVA using the body weight as a covariate was used to analyze densitometry and mechanical data. Two-way ANOVA was used to analyze other results because of the two different categorical independent variables (ovariectomy and jumping exercise). We used SPSS statistical software (version 20.0; IBM Corporation, Armonk, NY) for all statistical analyses. If a significant interaction was found, data were assessed by Bonferroni's analysis. Significance level was set at $p < 0.05$.

Results

No rats died during the experiments.

Experiment 1—prevention program

Body mass

Initially, all groups had a similar initial body mass (mean 199.75 ± 7.45 g). By the end of the experiment, the OVX group was significantly heavier ($p < 0.05$, Table 1) than the sham group, with gains in body mass of the OVX group observed until week 6. Jump training affected body mass; trained rats showed a decrease in body mass.

Hormonal dosage

The OVX group showed significant higher values of FSH than the sham groups ($p < 0.05$, Table 1).

The effects of ovariectomy on bone

Ovariectomy induced a significant decrease in BMD of the left femur ($p < 0.05$, Table 1, Fig. 2) and L5 vertebra ($p < 0.05$, Table 1, Fig. 2) compared to the sham group. We observed reductions of 9.6% in bone density in the left femur and 30.9% in the L5. In the femur, ovariectomy induced a significant decrease in ultimate load ($p < 0.05$, Table 1, Fig. 2); in the femur and L5 vertebral body, ovariectomy induced a significant decrease in stiffness ($p < 0.05$, Table 1) compared to the sham group.

There was a significant deformation in the femur of OVX compared to that in the sham group ($p < 0.05$, Table 1).

Ovariectomy induced significant differences in BV/TV, Tb.Sp, and Tb.N structural variables ($p < 0.05$, Table 1) and OS/BS and O.Th formation variables compared to the sham group. ES/BS and Oc.S/BS resorption variables showed significant higher values in the OVX compared with those in the sham group.

Serum osteocalcin ($p < 0.05$, Table 1) levels in the OVX group were not significantly different from those in the sham

Table 1 Weight, BMD, mechanical tests, histomorphometry, osteocalcin, osteoprotegerina, and ampli-RankL marker and FSH (follicle-stimulating hormone) dosage analysis of prevention groups

	Groups			
	OVX-SEDp	SHAM-SEDp	OVX-EXp	SHAM-EXp
Weight (g)				
Initial	197.00 ± 7.52	196.50 ± 3.37	203.50 ± 9.44	202.00 ± 9.48
Final	503.00 ± 25.84	432.50 ± 41.24*	451.00 ± 47.42*	390.50 ± 34.83***
BMD (g/cm^2)				
Femur	0.21 ± 0.01	0.23 ± 0.01*	0.23 ± 0.01*	0.24 ± 0.02**
L5 vertebra	0.11 ± 0.01	0.14 ± 0.02*	0.14 ± 0.01*	0.15 ± 0.02
Mechanical tests				
Ultimate load (*N*)				
Femur	124.96 ± 14.60	132.72 ± 19.03	134.63 ± 29.94	140.20 ± 21.76
L5 vertebra	181.36 ± 26.12	252.55 ± 51.24*	282.56 ± 70.58*	318.65 ± 60.50*
Stiffness (*N*/mm)				
Femur	186.12 ± 39.07	294.07 ± 73.10*	251.34 ± 69.55*	329.59 ± 62.93***
L5 vertebra	436.86 ± 99.64	580.53 ± 111.11*	569.33 ± 158.47*	706.24 ± 176.59***
Deformation (mm)				
Femur	0.79 ± 0.17	0.53 ± 0.06*	0.61 ± 0.17*	0.52 ± 0.13
L5 vertebra	0.56 ± 0.15	0.65 ± 0.17	0.74 ± 0.30*	0.58 ± 0.08
Histomorphometry				
Structural				
BV/TV (%)	16.02 ± 2.49	36.04 ± 9.16*	25.51 ± 6.68*	41.22 ± 7.90***
Tb.Th (μm)	55.86 ± 5.74	60.55 ± 9.19	56.57 ± 10.44	57.55 ± 9.98
Tb.Sp (μm)	300.00 ± 61.70	117.53 ± 41.93*	173.49 ± 48.61*,**	83.54 ± 18.33***
Tb.N (/mm)	2.89 ± 0.53	5.89 ± 1.48*	4.54 ± 1.05*,**	7.20 ± 0.97**,***
Formation				
OV/BV (%)	0.33 ± 0.24	0.49 ± 0.26	0.31 ± 0.26	0.23 ± 0.10
OS/BS (%)	3.23 ± 2.19	5.36 ± 2.57*	2.92 ± 2.33**	2.12 ± 0.80**
Ob.S/BS (%)	0.93 ± 1.14	1.23 ± 0.68	0.83 ± 0.66	0.68 ± 0.24
O.Th (μm)	7.60 ± 5.94	57.64 ± 56.23*	26.08 ± 28.24	13.60 ± 18.83**
N.Ob/B.Pm	0.40 ± 0.37	0.60 ± 0.29	0.51 ± 0.38	0.42 ± 0.11
Resorption				
N.Oc/B.Pm	0.22 ± 0.13	0.16 ± 0.05	0.05 ± 0.05*	0.07 ± 0.05**
ES/BS (%)	1.03 ± 0.59	0.62 ± 0.25*	0.16 ± 0.18*	0.17 ± 0.15**
Oc.S/BS (%)	0.63 ± 0.38	0.38 ± 0.15*	0.13 ± 0.14*	0.14 ± 0.11**
Biochemical markers				
Osteocalcin (ng/ml)	2.61 ± 0.56	2.20 ± 0.32	3.29 ± 0.75*	3.55 ± 0.67**
Osteoprotegerin (ng/ml)	0.06 ± 0.06	0.05 ± 0.07	0.07 ± 0.08	0.04 ± 0.04
Ampli-RANKL (ng/ml)	0.21 ± 0.25	0.75 ± 0.35*	0.38 ± 0.49	0.27 ± 0.12**
Hormonal dosage				
FSH (mIU/ml)	108.65 ± 36.64	29.52 ± 4.68*	161.87 ± 29.72*	33.48 ± 10.70***

All values are mean ± SD. BMD and mechanical tests used ANCOVA statistical test with body weight as a covariate

OVX-SEDp OVX sedentary prevention (n = 10), *SHAM-SEDp* Sham-operated sedentary prevention (n = 10), *OVX-EXp* OVX exercised prevention (n = 10), *SHAM-EXp* Sham-operated exercised prevention (n = 10), *BV/TV* bone volume ratio, *ES/BS* eroded surface, *N.Ob/B.Pm* number of osteoblasts, *N.Oc/B* number of osteoclasts, *Ob.S/BS* osteoblastic surface, *Oc.S/BS* osteoclastic surface, *OS/BS* osteoideal surface, *OV/BV* osteoideal volume, *O.Th* osteoideal thickness, *Tb.N* trabecular number, *Tb.Sp* trabecular separation, *Tb.Th* trabecular thickness

*indicates statistical difference with OVX-SEDp group ($p < 0.05$), **indicates statistical difference with SHAM-SEDp group ($p < 0.05$), ***indicates statistical difference with OVX-EXp group ($p < 0.05$)

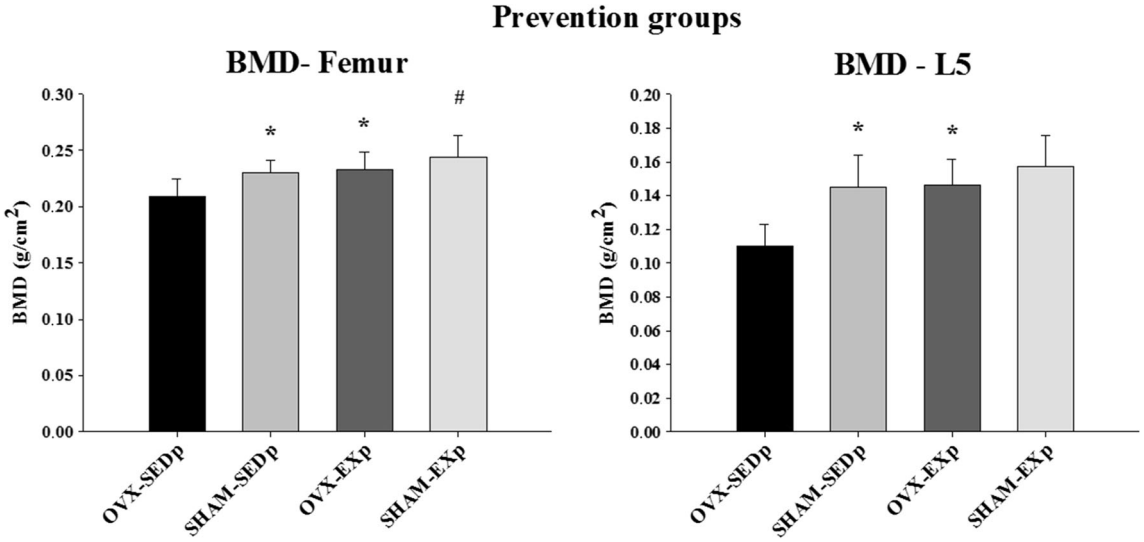

Fig. 2 Bone mineral densitometry (BMD) comparison between the following four prevention groups: OVX-SEDp, SHAM-SEDp, OVX-EXp, and SHAM-EXp. The values are presented as the mean ± standard deviation. OVX-SEDp: OVX sedentary prevention (n = 10), SHAM-SEDp: sham-operated sedentary prevention (n = 10), OVX-EXp: OVX exercised prevention (n = 10), SHAM-EXp: sham-operated exercised prevention (n = 10). * indicates statistical difference with OVX-SEDp group ($p < 0.05$). # indicates statistical difference with SHAM-SEDp group ($p < 0.05$). § indicates statistical difference with OVX-EXp group ($p < 0.05$). ANCOVA with body weight as a covariate

group. OPG did not alter levels by ovariectomy ($p > 0.05$, Table 2). OVX significantly decreased serum RANKL levels ($p > 0.05$, Table 2).

Preventive effects of physical exercise on bone loss

Jumping exercise preserved bone mineral density at the skeletal sites tested ($p < 0.05$, Table 1) compared to OVX, showing similar values to those observed in the sham group. Jumping exercise preserved stiffness (L5 vertebra) and deformation (femur) of the skeletal sites tested ($p < 0.05$, Table 1) compared to OVX, showing similar values to the sham group.

Prevention jump training induced a significant difference in BV/TV, Tb.Sp, Tb.N, and OS/BS variables and decreased N.Oc/B.Pm, ES/BS, and Oc.S/BS resorption variables ($p < 0.05$, Table 1) compared with the OVX.

Jump training significantly increased serum osteocalcin ($p < 0.05$, Table 1) compared to the controls. OPG did not alter levels by exercise ($p > 0.05$, Table 2). Jump training significantly decreased serum RANKL levels ($p > 0.05$, Table 2) in the sham groups.

Experiment 2—treatment program

Body mass

Initially, all groups had a similar body mass (mean 191.00 ± 7.50 g). By the end of the experiment, the treatment OVX group had significantly higher body mass ($p < 0.05$) than the sham group. The gains in body mass of the OVX group were observed until week 6. Jump training did not affect significantly these parameters in the treatment groups (Table 2).

Hormonal dosage

The OVX group showed significant higher values of FSH than the sham group ($p < 0.05$, Table 2).

The effects of ovariectomy on bone

Ovariectomy did not induce a significant decrease in BMD of the left femur and L5 vertebra ($p > 0.05$, Table 2, Fig. 3). In the L5 vertebral body, ovariectomy induced a significant decrease in stiffness ($p < 0.05$, Table 2) compared to the sham group.

Ovariectomy induced a significant difference in BV/TV, Tb.Sp, and Tb.N structural variables ($p < 0.05$, Table 2). Serum osteocalcin ($p < 0.05$, Table 2) levels in the OVX group were significantly different from those in the sham group. OPG did not alter levels by ovariectomy ($p > 0.05$, Table 2). OVX significantly decreased serum RANKL levels ($p < 0.05$, Table 2).

Effects of physical exercise on osteopenic bone

Jumping exercise increased bone mineral density in the L5 vertebra ($p < 0.05$, Table 2). Jump training significantly increased ultimate load compared to the ovariectomized group ($p < 0.05$, Table 2).

Jump exercise in the OVX group induced a significant difference in BV/TV, Tb.Sp, Tb.N, and N.Ob/B.Pm variables

Table 2 Weight, BMD, mechanical tests, histomorphometry, osteoprotegerina, and ampli-RankL marker and FSH (follicle-stimulating hormone) dosage analysis of treatment groups

	Groups			
	OVX-SEDt	SHAM-SEDt	OVX-EXt	SHAM-EXt
Weight (g)				
Initial	188.50 ± 8.57	192.50 ± 4.83	189.50 ± 9.48	193.50 ± 7.14
Final	509.50 ± 50.79	436.50 ± 64.24*	483.50 ± 68.60	422.50 ± 37.06***
BMD (g/cm^2)				
Femur	0.23 ± 0.01	0.23 ± 0.01	0.24 ± 0.02	0.26 ± 0.02**§
L5 vertebra	0.12 ± 0.01	0.13 ± 0.02	0.14 ± 0.02*	0.15 ± 0.01**
Mechanical tests				
Ultimate load (*N*)				
Femur	126.01 ± 21.89	143.31 ± 15.32	158.97 ± 32.31*	141.56 ± 20.96
L5 vertebra	215.96 ± 44.25	217.40 ± 46.73	242.38 ± 62.02	277.86 ± 62.26***
Stiffness (*N*/mm)				
Femur	285.88 ± 61.60	357.61 ± 39.26	323.07 ± 58.23	323.59 ± 65.59
L5 vertebra	436.03 ± 113.26	562.34 ± 202.58*	444.48 ± 66.44	532.77 ± 66.50
Deformation (mm)				
Femur	0.55 ± 0.12	0.47 ± 0.05	0.60 ± 0.06**	0.51 ± 0.09***
L5 vertebra	0.65 ± 0.15	0.65 ± 0.19	0.79 ± 0.16	0.66 ± 0.15
Histomorphometry				
Structural				
BV/TV (%)	18.38 ± 9.56	31.76 ± 5.54*	36.16 ± 6.55*	21.39 ± 8.49**,***§
Tb.Th (μm)	52.66 ± 6.65	55.24 ± 6.93	62.44 ± 10.68	70.84 ± 22.44**
Tb.Sp (μm)	304.42 ± 189.28	122.68 ± 34.20*	111.65 ± 20.61*	288.17 ± 127.73**,***
Tb.N (/mm)	3.42 ± 1.69	5.80 ± 0.98*	5.82 ± 0.75*	3.13 ± 1.07**,***
Formation				
OV/BV (%)	0.09 ± 0.07	0.14 ± 0.10	0.17 ± 0.11	0.41 ± 0.26**,***
OS/BS (%)	1.72 ± 1.40	1.51 ± 1.51	1.79 ± 0.82	3.90 ± 2.62**,***
Ob.S/BS (%)	0.30 ± 0.24	0.42 ± 0.26	0.54 ± 0.26	1.00 ± 0.72**,***
O.Th (μm)	3.19 ± 2.17	8.84 ± 13.66	12.10 ± 16.07**	7.96 ± 4.08**
N.Ob/B.Pm	0.13 ± 0.09	0.21 ± 0.12	0.37 ± 0.16*	0.59 ± 0.41**,***
Resorption				
N.Oc/B.Pm	0.1 ± 0.05	0.07 ± 0.04	0.04 ± 0.04*	0.03 ± 0.05*
ES/BS (%)	0.31 ± 0.26	0.27 ± 0.28	0.15 ± 0.12	0.12 ± 0.25
Oc.S/BS (%)	0.25 ± 0.20	0.20 ± 0.17	0.18 ± 0.19	0.17 ± 0.29
Biochemical markers				
Osteocalcin (ng/ml)	1.68 ± 0.42	2.15 ± 0.36*	2.24 ± 0.45*	2.61 ± 0.37**,***
Osteoprotegerin (ng/ml)	0.07 ± 0.09	0.03 ± 0.08	0.07 ± 0.07	0.07 ± 0.06
Ampli-RANKL (ng/ml)	0.12 ± 0.11	0.97 ± 0.55*	0.28 ± 0.42	0.36 ± 0.59**
Hormonal dosage				
FSH (mIU/ml)	108.13 ± 44.28	25.753 ± 6.029*	208.942 ± 78.677*	27.023 ± 6.335***

All values are mean ± SD. BMD and mechanical tests used ANCOVA statistical test with body weight as a covariate

OVX-SEDt OVX sedentary treatment (n = 10), *SHAM-SEDt* Sham-operated sedentary treatment (n = 10), *OVX-EXt* OVX exercised treatment (n = 10), *SHAM-EXt* Sham-operated exercised treatment (n = 10), *BV/TV* bone volume ratio, *ES/BS* eroded surface, *N.Ob/B.Pm* number of osteoblasts, *N.Oc/B* number of osteoclasts, *Ob.S/BS* osteoblastic surface, *Oc.S/BS* osteoclastic surface, *OS/BS* osteoideal surface, *OV/BV* osteoideal volume, *O.Th* osteoideal thickness, *Tb.N* trabecular number, *Tb.Sp* trabecular separation, *Tb.Th* trabecular thickness

* indicates statistical difference with OVX-SEDt group ($p < 0.05$), **indicates statistical difference with SHAM-SEDt group ($p < 0.05$), ***indicates statistical difference with OVX-EXt group ($p < 0.05$)

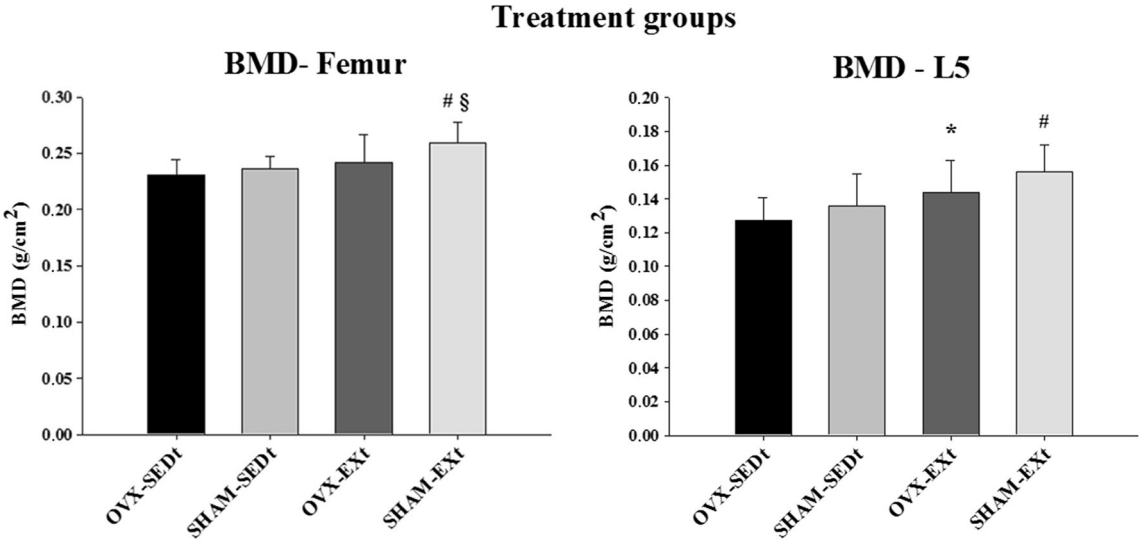

Fig. 3 BMD comparison between the following four treatment groups: *OVX-SEDt*, *SHAM-SEDt*, *OVX-EXt*, and *SHAM-EXt*. The *values* are presented as the mean ± standard deviation. *OVX-SEDt* OVX sedentary treatment (n = 10), *SHAM-SEDt* sham-operated sedentary treatment (n = 10), *OVX-EXt* OVX exercised treatment (n = 10), *SHAM-EXt* sham-operated exercised treatment (n = 10). * indicates statistical difference with *OVX-SEDt* group ($p < 0.05$). # indicates statistical difference with *SHAM-SEDt* group ($p < 0.05$). § indicates statistical difference with *OVX-EXt* group ($p < 0.05$). ANCOVA with body weight as a covariate

and decreased N.Oc/B.Pm resorption ($p < 0.05$, Table 2) compared with the OVX-SED.

Jump training significantly increased serum osteocalcin levels ($p < 0.05$, Table 2). OPG did not alter levels by exercise ($p > 0.05$, Table 2). Jump training significantly decreased serum RANKL levels ($p > 0.05$, Table 2) in the sham groups.

Discussion

Physical exercise stimulates bone formation. Physical exercise can maintain and increase BMD [10, 21, 25–28], bone mechanical properties [29, 30], and bone formation and reduce bone resorption [31–34]. Our investigation confirmed the osteogenic effects of jump training and its beneficial effect on bones in ovariectomized estrogen-deficient rats. The increases in bone mass and mechanical force induced by jump training were similar in the sham and OVX groups. The increase in ultimate breaking force in the two jump training groups was achieved via improvement in the mechanical properties of the bones tested. Moreover, serum osteocalcin, an index of bone formation, was significantly higher in both training groups. These data indicate that jump training stimulated bone formation and increased bone mass and mechanical properties in the OVX rats, as well as in the sham-operated rats.

Serum estrogen concentrations exhibit an inverse relation with FSH concentrations. Serum FSH levels are related to the number of follicles present in the ovaries: when levels are low, the serum concentration of FSH is high. In our study, as expected, ovariectomized rats showed high values of FSH [35].

The OVX rat is an established model of estrogen deficiency-induced bone loss and has been validated for the bone loss seen in human menopause. Several animal studies have used OVX rat models to examine the effects of mechanical loading on the skeletal system [36–42]. Thus, the 20- to 32-week-old rats used in that study would be hormonally active and relevant for modeling estrogen deficiency by ovariectomy. Ovariectomy leads to sex hormone deficiency and decreases in bone thickness, bone density, and bone hardness [43].

In the present study, the rats in the prevention group exhibited a significant decrease in body weight, while those in the treatment groups exhibited no significant changes in body weight. In previous studies on the rat jump model, there were no marked differences in the effects on bone mass and mechanical properties when 5, 10, 20, or 40 jumps per day were performed, although a slight tendency to and increase according to the number of jumps per day was observed [19]. We choose 20 jumps because this number produced well-defined increases in bone mechanical properties [19].

Honda at el. [44] also imposed a large mechanical stress via jump training. However, the number of daily repetitions was small (ten jumps per day). From these studies, high-intensity low-repetition exercise had a beneficial effect on OVX as well as the sham rats. These authors explained that jump training induced great mechanical stresses because of the high ground reaction force on the lower leg imposed by a 40-cm jump. This increases the ultimate breaking force mainly by geometric change in the bones but not by changes in mechanical properties: there was an increase in the moment of inertia but no significant change in bending stresses [45].

Impact exercise has beneficial effects on the bone [29]. Bone responds to mechanical loading by stimulating bone formation where strain is higher [46]. The skeleton is constantly subjected to mechanical loading because of gravitational force and muscle contraction, which stimulate bone remodeling through mechanotransduction. Osteocytes are able to detect the mechanical signals and modulate bone formation and resorption by signaling osteoblasts and osteoclasts, respectively [8].

Jump training is effective to increase bone mass and ultimate stress to failure of bones in animals [11, 18, 19, 47] and also to increase BMD in the human lumbar spine and femoral regions [48, 49]. Ten jumps per day or 30 jumps per week with maximum effort are an effective bone stimulus [8].

Both the total bone volume (TV) and the trabecular BV were increased by the exercise. BV/TV (percent) was significantly higher in the exercise versus in the sedentary groups, indicating that modeling-dependent new bone formation in the proximal tibia also was increased by the exercise, as shown by other parameters of bone formation and resorption.

The results of this study showed that jumping 20 times per session on a daily basis increased bone mass and mechanical properties in estrogen-deficient rats and control rats in both prevention and treatment groups. Our results also suggest increase in BMD, formation parameters of histomorphometry, and osteocalcin. Jumping exercise might therefore improve bone and provide a useful model to compare exercise prescriptions to help define the most efficient and effective exercise recommendations for bone health of premenopausal and menopausal women. The optimal exercise prescription in humans needs further investigation.

Low serum RANKL is a predictor of fragility fracture [50, 51]. Postmenopausal women's OPG correlates with age; however, free RANKL-positive correlation with age and negative correlation with vertebral BMD were found [52]. In postmenopausal osteoporosis, the reduction in estrogen levels may also remove an important control on RANKL action and decrease the synthesis of OPG [53, 54]. Our results suggest that, in both the ovariectomized and in exercised rats, the expression of RANKL soluble in serum decreased.

There are some methodological limitations of this study. First, we used a relatively low number of rats, too few to adequately power statistical interactions between the sham and OVX groups in some analysis (i.e., biomechanical tests). Second, we used different periods of age for the prevention and treatment programs: because of this, we cannot compare their efficiency. Third, we did not perform a qualitative analysis of the nature of the bones (i.e., micro CT or scanning electron microscopy). However, within the limitations outlined above, the results obtained are univocal and greatly encouraging.

Conclusion

Daily jumping exercise increases bone mineral density, mechanical properties, and formation/resorption. Some effects of training were observed in both the prevention and treatment models, suggesting that this exercise regimen is effective in preventing/reversing bone loss after ovariectomy in rats in whom osteopenia is already established.

Perspectives

Increase in life expectancy is accompanied by a higher incidence of osteoporosis, with deleterious consequences. Therefore, experimental models of bone loss represent an important tool to investigate the therapeutic effects of bone-enhancing treatments that may be applicable to humans. Mechanical loads play an important role in bone health and jumping physical exercise applied to ovariectomized rats prevents or ameliorates the loss of bone induced by decreased estrogen. We investigated whether jumping physical activity could prevent or reverse deterioration of the bone. Jumping prevented bone loss and was effective in improving bone resistance. However, considering that the target human population that would benefit from such exercise modalities consists of the elderly and individuals with multiple comorbidities, other modalities of exercise should be tested, as jumping in these patients may be dangerous and cause falls and induce other injuries.

Compliance with ethical standards The protocols for animal experimentation were previously approved by the Institutional Animal Care and Use Committee of the university (protocol number CETEA 182/2008).

Conflicts of interest None.

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