EFFECTS OF PHYSICAL ACTIVITY ON THE MECHANICAL PROPERTIES OF OSTEOPENIC FEMALE RATS' FEMURS AND TIBIAE

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SUMMARY

We evaluated the mechanical properties, obtained by means of flexion-compression assays in femurs and flexion assays on three tibial sites of ovariectomized adult female rats submitted to physical activity. Thirty rats were employed and divided into 3 groups: G1: Control. G2: Ovariectomized animals and not submitted to physical activity. G3: Animals trained in a spinning cage for five consecutive days, subsequently submitted to ovariectomy, and allowed to rest during 24 hours. The animals were submitted to physical activity for 30 minutes, 5 days a week, for a period of 9 weeks, at a speed of approximately 0.31 m/s. The values achieved for load and deformation evidenced that ovariectomized rats' femurs presented with a statistically significant reduction on load and deformation mechanical properties, at the maximum limit. Femurs in the group submitted to ovariectomy and physical activity presented with load and deformation values at maximum limit superior to those for the group submitted only to ovariectomy, however, with no statistical significance. The tibiae didn't present significant changes in any of the mechanical properties studied. Physical activity applied for 30 minutes, 5 days a week, during 9 weeks at 0.31 m/s was not enough to correct the biomechanical changes of bone tissue yielded by ovariectomy.

Keywords: *Biomechanics; Bone diseases, Metabolic; Ovariectomy; Physical exercise.*

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INTRODUCTION

The World Health Organization (WHO) defines osteoporosis as a "systemic skeletal disease characterized by mass reduction and microarchitectural deterioration of the bone tissue, with resultant increased bone fragility and susceptibility to fractures"[(1)].

After the fourth decade of life, the amount of bone resorption exceeds the amount of bone formation, leading to a reduction of 0.3% to 0.5% of mineral content a year, both in pre-menopausal women and in men. In this period, a little bone content reduction occurs in regions such as spine, forearm, and total body measures, but a high bone mass loss is found at the proximal region of the femur. The hastened bone tissue loss is a consequence verified after menopause (5% a year), followed by ovariectomy or any other kind of ovarian failure [(2)].

Osteoporosis is a diffuse reduction of bone density that emerges when resorption speed exceeds bone formation. It is the most frequent metabolic change affecting bones, being characterized by a slow and progressive mass reduction, lowering its resistance and allowing for the emergence of fractures on affected bones, even at minimal efforts [(3)].

After sexual hormones cease to produce, women's bone mass reduces quickly during the first 10 years, and slowly during subsequent years [(4)], having, at each remodeling cycle, a lower amount of formed bone and a higher amount of reabsorbed bone [(5)].

The consequences of osteoporosis made this disease become the major public health problem nowadays [(6)].

The influence of physical activity on skeletal dynamics and on osteoporosis prevention has increasingly gaining interest. The mechanisms by which the skeleton responds to physical activity are not clearly explained yet. However, there are evidences showing an increased bone resistance as a response to mechanical loads application and, on the other hand, a reduction of bone mineral density (BMD) when absent [(7)].

In the long-term, exercises can stimulate bone formation[(8)]. This is primarily a result of an increased calcium absorption by intestines, along with a reduction of its elimination by urine and with increased levels of paratyroidal hormone (PTH). Oppositely, immobilization or absolute rest promotes bone reduction [(9)].

It has been observed that athletes have larger bone mass than non-athlete individuals and the comparison between active and sedentary populations corroborates an expressive correlation between physical activity levels and BMD.

Study conducted at the Bioengineering Laboratory, Medical College of Ribeirão Preto - USP.

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Furthermore, it is noticed that physical inactivity causes osteopenia. An example of this is the well-known osteopenia resulting from fracture immobilization [10].

The objective of this study was to evaluate the effects of physical exercise on preventing osteoporosis induced by ovariectomy, by comparing some mechanical properties of femurs and tibias of female rats.

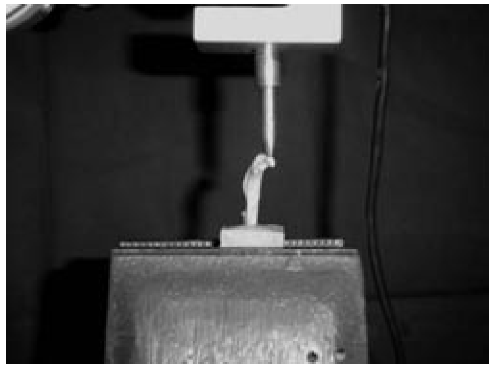

Figure 1 - *Image of a femur with base inserted into acrylic resin, submitted to flexion-compression mechanical assay on the UAM.*

Figure 2 - *Image of a tibia being submitted to flexion mechanical assay at 3 points.*

MATERIALS AND METHODS

Thirty specimens of *Rattus norvegicus albinus*, Wistar variety, have been used for this study. Their ages ranged from 120 to 140 days, presenting with average body weight of 360 - 390g. Those animals were randomly divided into 3 groups, which were maintained within restrain cages. Groups were characterized as G1: control, with 10 animals maintained only within restrain cages for 9 weeks; G2: constituted of 10 animals submitted to ovariectomy procedure; G3: where 10 animals were trained in a spinning cage for five consecutive days and then submitted to ovariectomy, remaining at rest for 24 hours. By the end of rest period, those were submitted to physical activities in a spinning cage for 9 weeks.

Surgical procedure

A cross-sectional straight incision of approximately three centimeters was made at the iliac pit on skin and subdermal cellular tissue at about one centimeter from median line. Muscle wall was divulsed until the abdominal cavity was reached, with the ovary being found surrounded by a fatty mass. Ovary removal was made after uterine tube end ligation, sectioning between ligation and ovary. The wound was closed by planes. The whole procedure was repeated for the opposite side in order to remove the other ovary.

Standard spinning cage

For the execution of physical activity, a spinning cage was built in steel, closed with an aluminum mesh, with 0.96 m perimeter and 0.12 m wide. The cage model is similar to that used by Hoshi et al.[11] and by Wu et al.[12]. A continuous current engine was connected to a source, enabling speed control.

The animals submitted to physical activity had their drills divided into two phases, with the first being considered as training, and the second as treatment. At phase I, the animals were submitted to 5 consecutive days of drills, with duration progressively increased, as follows: 5 minutes on day 1, 10 minutes on day 2, 15 minutes on day three, 20 minutes on day 4, and 25 minutes on day 5, always at average speed of 0.31 m/s. Training was performed prior to surgery. At phase II, physical activity was applied in a spinning cage for 30 minutes, 5 days a week, during 9 weeks, with a speed of 0.31 m/s, beginning 24 hours after surgical procedure.

Specimens preparation

The female rats were sacrificed by excessively administering the anesthetic substance Tiopental® having their femurs and tibias dissected, both right and left. Tibias were soaked in saline solution up to the moment of a mechanical assay of flexion at three points was performed. Femurs had the distal portion inserted in a self-polymerizing acrylic resin, maintaining them pulverized with saline solution during the insertion procedure and soaked until the mechanical assay was performed.

Mechanical assay

Mechanical assays were performed on a universal assay machine (UAM) developed at the Bioengineering Laboratory, FMRP - USP. Load was applied at a speed of 0.1 mm/minute, with 0.02 mm deformation measurement intervals, and recorded by a load cell KRATOS®, KM model, of 50 Kgf, attached to a CAE 201 SODMEX® amplifier. Deformations were recorded by a comparative clock MITUTOYO® with accuracy of 0.01 millimeter. A 2.94 N pre-load was applied, with system adjustment time of 30 seconds.

Right and left femurs were submitted to flexion-compression assay. For the test, the acrylic resin base was attached to a bench vise fixed at the UAM. For load application, a shaft with concave lower end over the femoral head was used (Figure 1).

Right and left tibias were submitted to flexion assay at three points. The load was applied at a cross-sectional plane to the tibia on its posterior surface, by means of a 4 cm long, 0.2 cm wide steel pin on an accessory with 30 mm space between supports (Figure 2).

Values recorded and collected by the extensiometry bridge were transcribed to the software *Microsoft Excel 2000®*, where the construction of graphs representing load vs. deformation was possible, through which the mechanical properties of load at maximum limit, deformation at maximum limit, and stiffness were calculated.

The data obtained were submitted to variance analysis (ANOVA), when compared simultaneously.

For analysis between groups, the *Student – Newman – Keuls* test with 5% significance level was used. All statistical analyses were performed by *Instat - Graph® Pad v. 3.0* software.

RESULTS

Load at maximum limit - femurs

Average values found for load at maximum limit of femurs in the different groups are: G1: (137.95±21.66) N, G2: (108.40 ± 20.56) N and G3: (119.21± 20.8) N.

On simultaneous analysis of groups, a statistically significant difference ($p=0.0003$) was observed. The statistical analysis for comparison between average values for load at maximum limit showed a statistically significant difference ($p< 0.05$) between groups G1 X G2 and G1 X G3. The comparison between G2 X G3 did not show any statistically significant difference.

Deformation at maximum limit - femurs

Average values found for deformation at maximum limit of femurs in the different groups were: G1 (0.78 ± 0.26) mm, G2: (0.53 ± 0.10) mm and G3: (0.63± 0.13) mm.

On simultaneous analysis of groups, a statistically significant difference (p=0,0005) was observed. The statistical analysis for comparison between average values for deformation at maximum limit showed a statistically significant difference ($p< 0.05$) between groups G1 X G2 and G1 X G3. The comparison between G2 X G3 did not show any statistically significant difference.

Stiffness of femurs

Average values found for stiffness of femurs in the different groups were G1: (213.19 ± 51.90) x10^3 N/m, G2: (243.39 ± 62.26) x10^3 N/m and G3: (216,11 ± 55,45) x10^3 N/m.

On the simultaneous analysis of groups, no statistically significant difference was observed ($p=0.2229$).

Load at maximum limit - tibias

Average values for load at maximum limit of tibias in the groups were G1: (54.40 ± 11.45)N, G2: (60.87 ± 6.02)N and G3: (58.30 ± 9.93)N.

Deformation at maximum limit - tibias

Average values for deformation at maximum limit of tibias in the groups were G1: (0.78 ± 0.26) mm, G2: (0.53 ± 0.10) mm and G3: (0.63± 0.13) mm.

Stiffness of tibias

Average values for stiffness of tibias in the groups were G1: (75.40 ± 20.91) x103 N/m, G2: (88.92 ± 12.37) x103 N/m and G3: (80.25 ± 19.73) x103 N/m.

We did not find any statistically significant differences on mechanical properties of tibias in the three experimental groups.

DISCUSSION

For studying the causes, mechanisms of action, and applicable therapies for osteoporosis treatment, a model of castrated female rat has been used for inducing an osteopenic picture. The ovariectomized rat has shown to be a model of great usefulness, especially for presenting similar biological mechanisms to those occurring in osteoporotic women [13].

For the animals to be able to perform physical activities, a spinning cage was made in steel with speed controlled by a source. The cage model was similar to the one used by Hoshi et al.[11] and Wu et al.[12] in their experiments.

Hoshi et al.[11] conducted an experiment where female rats femurs with various ages and submitted to physical activity presented with higher values of load at maximum limit and stiffness than femurs of groups not submitted to physical activity. In that study, periods of 10, 20, and 60 weeks of drills were employed.

Wu et al.[12] analyzed the effects of hormone administration and exercises on ovariectomized rats' bone mass. Femurs of rats submitted to physical exercises presented with a higher bone density when compared to groups not submitted to physical activity.

The time established for physical activity practice is consistent to the study conducted by Wu et al.[12]. However, the speed of spinning in our study is 55% higher than that used by Wu et al.[12] requiring stronger effort from animals.

Kodama[14] claims that mechanical resistance, evaluated by load at maximum limit, from femoral proximal end in a group of ovariectomized rats decreased after 9 weeks of surgery, being comparable to the non-ovariectomized group.

Carvalho[15] states that the osteopenia induction in mature rats due to ovariectomy needs a minimum period of 30 days. Our animals were submitted to exercises since the early period of hormonal action absence and the drills were performed until animals were sacrificed, so that we first considered that this elapsed time would be enough for expressing both hormone absence and the effects of physical activity.

Studies show that different bone regions respond differently to ovariectomy, with regions where cortical bone is prevalent are not affected by significant changes because these are less sensitive to ovarian hormone drop [16].

The femoral flexion-compression assay is efficient to evaluate mechanical properties of the femoral proximal end, especially the region constituted of trabecular bone [14, 17].

The flexion assay at 3 points is preferable for bones of predominantly cortical constitution, such as the tibia, being this kind of test adopted by Mosekilde et al.[16] and by Hogan et al.[18] for evaluating mechanical properties of tibias.

The average values for mechanical properties of tibias for control, ovariectomized, and ovariectomized and submitted to physical activity groups showed no statistically significant differences when compared simultaneously.

Similar results were found in studies conducted by Hogan et al.[18], who submitted tibias of ovariectomized rats to mechanical assay at 3 points, finding no statistically significant difference on biomechanical properties of stiffness and load at maximum limit. Ovariectomy influences cortical tissues, but less expressively, particularly within short periods of time [15].

Thus, we consider that the mechanical tests on tibias in the different groups of animals provided no statistical differences due to the short period of time determined for the effects of ovariectomy on cortical bone to be noticed, or because ovariectomy does not cause changes to the mechanical properties evaluated on tibias, but certainly not as a response to physical exercise, once the ovariectomized group not submitted to physical activity showed results similar to control group.

Regarding assays performed on femurs, we found an average value for load at maximum limit in ovariectomized group significantly lower than the average value in control group, indicating that ovariectomy caused the reduction of this biomechanical parameter.

Similar results were found in studies conducted by Peng et al.[19], which assessed femoral proximal end in female rats 6 weeks after ovariectomy and found a significant reduction of the load at maximum limit in the ovariectomized group compared to the non-ovariectomized one. Kodama[14] assessed the load at maximum limit for femoral proximal region of ovariectomized rats and found a significant reduction of this parameter compared to control.

The average value for load at maximum limit for femurs in the ovariectomy + physical activity group was superior to the average value in the ovariectomized group, although we haven't found statistically significant differences when those results were compared. They per se suggest a beneficial effect of exercises for preventing against bone resistance loss at femoral proximal end in ovariectomized rats. The control group presents with values for load at maximum limit that are statistically superior to that found for ovariectomized groups, and, thus, although there is an indication tending to show a mechanical improvement for femurs of animals submitted to physical activity, our statistically addressed results do not allow us to state that physical activity as used in this study had been enough to reduce deleterious effects of hormone loss.

Different results were found in studies by Hoshi et al.[11] and Chen et al.[20] which reported better biomechanical properties for femurs of animals submitted to physical activity. However, the parameters used for physical activity practice were different from those used in our study. Chen et al.[20] used a slower speed and a longer time of activity practice (60 minutes), Hoshi et al.[11] evaluated the animals in their study during a longer follow-up period (10, 20, and 60 weeks) providing indications that the parameters adopted for physical activity practice or the duration of the experiment were insufficient to assess the mechanical behavior of an osteopenic tissue submitted to physical activity.

The average value for deformation at maximum limit for femurs in control group was higher than in ovariectomized, and ovariectomized + physical activity groups. The ovariectomized +physical activity group presents a superior average value when compared to the non-treated group, but not statistically significant.

The ovariectomy procedure and the practice of physical activities were not able to influence the mechanical property of stiffness, suggesting that estrogen deficiency and physical exercises were not enough to change the ratio load support vs. corresponding material deformation at elastic phase during the adopted period of time.

CONCLUSIONS

Physical activity applied during 30 minutes, 5 days a week, for 9 weeks at 0.31 m/s was not enough to correct the biomechanical changes of bone tissues resulting from ovariectomy at female rats' femoral proximal end.

The tibias of animals submitted only to ovariectomy and of those submitted to ovariectomy + physical activity do not show significant changes in none of studied mechanical properties.

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