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

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

Keywords: Osteoporosis; Rats; Ovariectomy; Physical exercise.

INTRODUCTION
Osteoporosis is a disease with an immense social-economic significance and it has been recognized as a major public health problem. It is a bone disorder, characterized by loss of bone mass, resulting in bone weakness and in an increased susceptibility to fractures[1,2]. Many treatments have been developed with the aim of preventing bone loss and increasing bone mass, including estrogen replacement, bisphosphonate compounds and physical activity programs[3]. Physical activity has been advocated as offering a potential means to increase and to maintain bone activity programs(3). Physical activity has been advocated as offering a potential means to increase and to maintain bone mass, constituting a therapeutic modality to treat bone loss due to osteoporosis. However, some points about the intensity, duration and frequency of the exercises remain confusing and contradictory. Therefore, it is important to study all aspects of bone response to physical exercises thus it can be used with confidence as a treatment within the clinical setting.

Little is known about the effects of a progressive loading exercise programs performed in aquatic environments on bone tissue of osteopenic rats. We hypothesized that muscle contractions performed during jump exercises could be efficient to induce an osteogenic effect, promoting an increase in femoral bone strength and mass, even into the water, where the gravitational force are decreased. This type of exercise could be useful in osteoporotic patients, constituting a safer therapeutic modality instead of traditional exercises, as walking, once the risk of falls and consequently fractures are decreased in the aquatic environment. In this context, the aim of this study was to determine the response of femora of osteopenic rats to a progressive exercise training. Femora were examined anthropometrically, evaluated using a morphometric analysis and a biomechanical test to determine their mechanical strength was performed.
MATERIALS AND METHODS
Forty female Wistar rats (12 weeks, ± 250 g) were used in this experiment. Rats were randomized into 4 groups, with 10 animals each: sham-operated sedentary (SS); ovariec-
tomy-sedentary (OS); sham-operated training (ST) and ovariec-
tomy training (OT).
The remaining animals were kept in a temperature-controlled room (22 ± 2°C) at the Animal Experimentation Laboratory of Federal University of Sao Carlos, with a light-dark cycle of 12/12 hours. The animals were given standard laboratory rat food and were provided with water ad libitum.

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

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

Table 1 - Physical exercise program

<table>
<thead>
<tr>
<th>Weeks</th>
<th>Weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>20% of body mass</td>
</tr>
<tr>
<td>4-7</td>
<td>30% of body mass</td>
</tr>
<tr>
<td>3 and 4</td>
<td>40% of body mass</td>
</tr>
<tr>
<td>5 and 6</td>
<td>50% of body mass</td>
</tr>
<tr>
<td>7 and 8</td>
<td>60% of body mass</td>
</tr>
</tbody>
</table>

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

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

Femoral length: this measure was taken in the left femora, from the femoral major trocanter until the tip of lateral condyle, using a digital caliper (Bron-Sharp, error measure = 0.1%). In sequence, the left femora were submitted to the biomechanical test.

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

Physical Properties: After the mechanical test, both parts of right femora were burned at 800°C for 24 h, to obtain the ash weight (AW), using a digital electronic scale of high precision (Chyo, error measure = 0.1%). The bone density (BD) and volume (BV) were calculated using the Archimedes’ principle (12).

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

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

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

RESULTS
It can be observed in table 2 that the initial body mass of the animals did not show any statistical difference among them.
Ovariectomy produced a statistically significant increase in final body mass and in the variation of body mass during the experiment (Table 2).

Table 2 - Body mass of rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>Initial body mass (g)</th>
<th>Final body mass (g)</th>
<th>Increase of body mass (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Basal</td>
<td>251.66 ± 9.42</td>
<td></td>
<td></td>
</tr>
<tr>
<td>OS</td>
<td>248 ± 6.6</td>
<td>305.5 ± 8.5</td>
<td>57.5 ± 2</td>
</tr>
<tr>
<td>SS</td>
<td>246.5 ± 14.11</td>
<td>285.5 ± 17.9 !</td>
<td>38.6 ± 3.7 !</td>
</tr>
<tr>
<td>ST</td>
<td>255.5 ± 13.54</td>
<td>288.8 ± 16.5 !</td>
<td>33.3 ± 0.2 !</td>
</tr>
<tr>
<td>OT</td>
<td>242.9 ± 5.7</td>
<td>302.4 ± 11.84 *</td>
<td>53.2 ± 6.1 *</td>
</tr>
</tbody>
</table>

* i vs OS; † vs SS; a vs ST, p < 0.05

Ovx led to a significant decrease in cross-sectional area, medullar area and outer diameter when compared to sham-operated and OT groups. Inner-diameter of Sham-operated sedentary animals was higher than the other groups. The medullar area and outer diameter of the osteopenic trained group showed statistically significant higher values compared to the animals of the osteopenic sedentary animals (p = 0.00021 and p = 0.0038, respectively) and showed no difference comparing to the animals of SS and ST groups (Table 3).

Table 3 - Geometrical properties of femora

<table>
<thead>
<tr>
<th>Groups</th>
<th>Cross-section area (mm²)</th>
<th>Medullar area (mm²)</th>
<th>Outer Diameter (mm)</th>
<th>Inner Diameter (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Basal</td>
<td>3.5 ± 0.4</td>
<td>1.8 ± 0.12</td>
<td>3.2 ± 0.2</td>
<td>2.1 ± 0.3</td>
</tr>
<tr>
<td>OS</td>
<td>3.7 ± 0.26</td>
<td>2.0 ± 0.14</td>
<td>3.1 ± 0.26</td>
<td>2.3 ± 0.26</td>
</tr>
<tr>
<td>SS</td>
<td>4.7 ± 0.7 !</td>
<td>2.5 ± 0.95 !</td>
<td>3.7 ± 0.4 !</td>
<td>2.6 ± 1.2 !</td>
</tr>
<tr>
<td>ST</td>
<td>4.5 ± 0.25</td>
<td>2.4 ± 0.39 !</td>
<td>3.4 ± 0.69 !</td>
<td>2.3 ± 0.14 !</td>
</tr>
<tr>
<td>OT</td>
<td>4.7 ± 0.32 !</td>
<td>2.0 ± 0.05 !</td>
<td>3.9 ± 0.16 !</td>
<td>2.3 ± 0.1 !</td>
</tr>
</tbody>
</table>

* i vs basal; † vs SS; a vs ST, p < 0.05

DISCUSSION

In this study, we observed that the ovariectomy-rat model was efficient to induce osteopenia in the animals studied. The animals of the group OS presented a significant decrease in cross-sectional areas, outer diameters, medullar areas, bone weights and bone strength compared to other groups. Osteopenic rats were then examined to determine the effect of aquatic jump exercise on their femora. Jump exercise performed into the water was found to have a stimulatory effect on bone growing. Femoral physical properties (ash weight, bone volume, bone density and mineral density) were significantly lower in osteopenic sedentary animals compared to other groups, excepting the bone density and mineral density of the sham operated sedentary animals (SS). Exercised animals showed higher values of ash weight, bone density and mineral density than SS groups, indicating the positive effect of exercise. No difference was observed between the animals of the ST and OT groups (Table 4).

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

Table 5 - Biomechanical properties

<table>
<thead>
<tr>
<th>Groups</th>
<th>Calcium (mg/ Kg)</th>
<th>Phosphorus (mg/ kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Basal</td>
<td>343.49 ± 21.85</td>
<td>165.04 ± 7.76</td>
</tr>
<tr>
<td>OS</td>
<td>322.4 ± 16.59</td>
<td>159.96 ± 13.65</td>
</tr>
<tr>
<td>SS</td>
<td>327.5 ± 25.80 !</td>
<td>169.27 ± 13.28</td>
</tr>
<tr>
<td>ST</td>
<td>375.7 ± 20.70 !</td>
<td>160.55 ± 8.64</td>
</tr>
<tr>
<td>OT</td>
<td>368.66 ± 17.78 !</td>
<td>153.59 ± 13.71</td>
</tr>
</tbody>
</table>

* i vs basal; † vs SS, p < 0.05

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

Table 6 - Calcium and Phosphorus content
Our results corroborate with other studies which found that a program of exercises can stimulate osteogenesis, increasing the amount of bone density, weight-bearing and cross-sectional area after OVX(4-8,16,17). Moreover, Peng et al.(5) and Hart et al.(6) found that the OVX leads to a significant weakening of bone strength and a program of exercises was able to reverse the decrease. Some of the main concerns about the role of physical activity on stimulating bone tissue are the modality and the intensity of the exercise(5,18). Many studies have shown that weight-bearing exercise can increase bone mineral density (BMD)(19,20). Although weight-bearing is one of the most important factors influencing cancellous bone formation and re-absorption, there is evidence that non-weight-bearing exercises could also benefit bone osteogenesis(19,17). For example, in humans, Yung et al(22) found that swimmers had higher calcaneous bone mass than the sedentary controls. Snyder et al(23) who compare the effects of a running program and a swimming training, observed that the swimmer rats showed higher bone mineral content than runner rats. It is suggested that muscle contractions performed during swimming exercise may have osteogenic effects, been responsible by positive body mass adaptation(16). It seems that more than 70% of the bending moment on a bone is transmitted by muscle force rather than body weight, supporting the idea that muscle strength places greater loads on bones than do gravitational forces associated with weight(24).

We can hypothesize that the stimulatory effects of the present exercise program on femora of the osteopenic rats could be related to the lower limb muscle contractions required to perform jumps. Probably, the forces generated by muscles during the exercise, overloaded sufficiently bone tissue, producing bone adaptation, with an increase of bone metabolism(25,26). Moreover, the exercise regime used in the present study overloaded progressively the animals during the 8-week exercise training and it was consistent with low repetitions. It corroborates with recent theories that suggest that protocols consisting of fewer numbers, several sessions rather than repetitive single bout(27), or a longer interval (30 s) rather than shorter interval between each loading(28) are more effective to increase bone mass and strength(29). In addition, the exercise protocol was performed in an aquatic environment which could be safer for elderly people in improving muscle force and bone quality and body coordination than high impact loading of step aerobic.

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

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

REFERENCES