Acta of Bioengineering and Biomechanics
Vol. 10, No. 2, 2008

Effect of treadmill exercise on lumbar vertebrae in ovariectomized rats: anthropometrical and mechanical analyses

P.A. SIMÕES*, A. ZAMARIOLI, P. BLÓES, F.C. MAZZOCATO, L.H.A. PEREIRA, J.B. VOLPON, A.C. SHIMANO

Laboratory of Bioengineering and Department of Biomechanics, Medicine and Rehabilitation of the Locomotor Apparatus, Faculty of Medicine of Ribeirão Preto, University of São Paulo.

The purpose of this study was to examine the preventive effect of exercise on lumbar vertebrae in ovariectomized rats. Three-month-old female Wistar rats were divided into 3 groups: control group (A, n = 10); non-exercised ovariectomized group (B, n = 7) and exercised ovariectomized group (C, n = 7). The rats from group C were subjected to treadmill exercise (15 m/minute in the initial six weeks and 19 m/minute in the next six weeks, 1 hour/day, 4 days/week) for 12 weeks. At death, the fourth lumbar vertebrae were removed and an anthropometrical analysis by a paquimeter and a mechanical compression test by a universal test machine were performed. After 12 weeks, the ovariectomy decreased the superior–inferior vertebral height and the maximal braking load in group B compared to group A, while the exercise increased the vertebral mass in group C compared to both groups A and B ($p < 0.01$) and the stiffness compared to group B. We concluded the physical activity has an important role to prevent the osteopenia in lumbar vertebrae.

Key words: *spine, ovariectomy, osteopenia, biomechanics*

1. Introduction

Bone is a dynamic tissue in the continuous processes of formation and resorption which are stimulated by osteoblasts and osteoclasts, respectively [1]. After menopause, the reduction of estrogen concentration increases the osteoclastic resorption that exceeds the rate of osteoblastic formation, resulting in a bone mass loss (osteopenia or osteoporosis). In the last years, these diseases have extensively been researched all over the world, since they became a serious health problem – one of the most dangerous diseases of the women and elderly people. The most usual fractures caused by the osteoporosis are those of hips and vertebrae [2].

Physical exercises are widely used as a preventive method that minimizes the decrease of the bone mineral density [3]–[10]. The physical activity increases the bone mineral density in rats and in women after menopause [11].

2. Purpose

This study is aimed to verify the influence of the treadmill running on mechanical and structural properties of the forth lumbar vertebrae by means of biomechanical analysis with compression test and anthropometrical measurements of ovariectomized rats.

3. Material and methods

Three-month-old female Wistar rats (n = 30), weighing approximately 250 g, were used. Animals

* Corresponding author: P.A. Simões, University of São Paulo, Faculty of Medicine of the Departament of Biomechanics, Medicine and Rehabilitation of the Locomotor Apparatus, Santos Dumont Street, 564 – Bairro Vila Tibério, CEP 14050-060 Ribeirão Preto – São Paulo – Brazil, e-mail: arianezamarioli@usp.br
Received: April 22, 2008
Accepted for publication: June 4, 2008

were kept under constant living conditions (25 ± 2 °C, 12 h light–dark cycle) and were allowed free access to standard food and water. Three groups were established: group A, control group, with normal rats; group B, rats ovariectomized at the age of 3 months and kept sedentary; group C, rats ovariectomized at the age of 3 months and performing the established exercise program three days after the surgery. The ovariectomy was based on the procedure used by ZARROW [12] whose goal was to develop the osteopenia. The physical exercise program consisted in training rats on a flat-bed treadmill. Rats ran 4 days a week, 1 hour per day at the speed of 15 m/min from the beginning of the training until the sixth week when the speed was increased to 19 m/min during the other six weeks.

At the end of the experiment, the animals were killed under anaesthesia and their fourth lumbar vertebrae were dissected out. The L4 vertebra was cleaned off soft tissues and separated into two parts, i.e. the pedicle and the lamina, and then the vertebral body was collected. The L4 vertebral bodies were weighed, measured with a paquimeter and subjected to a test of compression in the universal test machine using a load cell of 500 N, in the Laboratory of Bioengineering. In the compression test, a careful procedure was adopted to maintain a parallelism between the extremities of the vertebral body. The test was performed at a speed of 0.1 millimeter per minute. Thus, the values of the maximal braking load and stiffness were obtained.

The data collected were analyzed using the analysis of covariance – ANOVA One Way Analysis of Variance – to test the differences between groups. The level of significance was set at 5%.

4. Results

The results were based on the tests carried out on 24 rats (A = 10, B = C = 7). There were 6 dropouts during the study because of three deaths as a result of the ovariectomy in group B and the improper performance of three rats on the treadmill.

The means of the measurements from anterior to posterior vertebra were: A (3.73±1.96) mm; B (3.17±0.23) mm and C (3.28±0.28) mm. There was no statistically significant difference between the groups. The means of the measurements from superior to inferior vertebra were: A (8.93±0.35) mm; B (8.43±0.51) mm and C (8.86±0.57) mm. There was a significant difference between groups A and B. The means of the weight values were: A (0.49±0.04) g; B (0.47±0.03) g and C (0.57±0.04) g. There was a significant difference between groups A and C and also between B and C.

The means of the values of the maximal braking load were: A (153.2 ± 10.8) N; B (112.5±8.9) N and C (131.1±10.5) N. There was a statistical difference between groups A and B ($p < 0.01$). In spite of the fact that we have not found a statistical difference between the other groups, the ovariectomized group, which underwent the exercise, had higher values of force than ovariectomized sedentary. The means of the values of the stiffness of the vertebrae were: A (461.6±66.5) N/mm, B (338.7±57.6) N/mm and C (490.7±44.3) N/mm. There was a significant statistical difference between groups B and C ($p \le 0.05$).

5. Discussion

Previous studies have shown that the ovariectomy in rats is a suitable experimental model for postmenopausal osteopenia that faithfully reflects the changes observed in women [4], [7], [8].

BONNET et al. [4] made use of dual X-ray analysis and observed that 10 weeks after ovariectomy the bone mineral density and trabecular strength decreased in ovariectomized rats. In our study, we found that the ovariectomy reduced the superior–inferior measurement of vertebral body and the maximal strength in group B when compared to group A.

Many authors reported that the exercise had a positive effect on bone tissue [3], [6], [7], [9], [13]. In the study of NOTOMI et al. [7], the rats did a tower climbing exercise, but in the most of the current studies the running exercise in a treadmill was used based on different protocols [3]–[6], [8]–[10].

PENG et al. [9] used the following protocol: 30-minute running at 10 m/min twice a day for 7 weeks. They noticed that the exercise restores the bone mass in cancellous bone from ovariectomized rats, but it does not return to its original state. PENG, VAANANEN and TUUKKANEN [10] using a protocol of the exercise twice a day, 30 min/day for 8 weeks at two different speeds: 10 m/min and 18 m/min concluded that the running induced an increase in the bone mineral density of the femur and vertebrae. CHEN et al. [5] with a 17 m/min for 1 h/day, 5 days/week for 9 weeks treadmill protocol noticed that the physical activity increased the bone mineral density and the cancellous bone neoformation. BONNET et al. [4] observed via histomorphometric analysis that the exercise increased the number of trabeculae. In their study, the rats were subjected to a treadmill running at 13 m/min, 60 min/day, 5 days/week for 10 weeks. Based on the anthropometrical measurements carried out we found that the exercise allowed an increase of the vertebrae mass. This seems to be due to the enhancement of the bone mineral density which was proved by GALA et al. [6] via the densitometric measurements. OCARINO et al. [8] via the following protocol: 15 m/min, 5 days a week, in the first week the exercise lasted 15 min/day and after the first week for 30 min/day for 3 months reported that the physical activity did not only stop bone loss caused by ovariectomy, but it also increased the trabecular bone volume and thickness in vertebrae. Therefore, their findings provide evidence, by means of bone histomorphometry, that exercise due to its therapeutic effects suitably protects the bones from osteopenia. IWAMOTO et al. [14] observed no significant difference in a maximum breaking force of the 5th lumbar vertebral body among rats that ran at different speed and with different frequency of the exercise (12 m/min, 1 h/day; 18 m/min, 1 h/day, 12 m/min, 2 h/day). They concluded that beneficial effects of treadmill running on bone strength became evident only in weight-bearing bones. In order to provide a weigh-bearing to the vertebrae, NAKAJIMA et al. [13] inclined the treadmill by up to 11% and found the small but significant effect of this inclination on vertebral bone. However, BARENGOLTS et al. [3] even using a 21 m/min, 7% grade, 40 min/day, 4 days/week for 3 months protocol did not find any improvement in L4 vertebra weight. On other hand, based on the paper by IWAMOTO et al. [14], OCARINO et al. [8] studied the effect of treadmill on ovariectomized rats and found that the nasal bone, a non-weight-bearing bone, was significantly thicker in active ovariectomized rats than in the sham-operated or sedentary ovariectomized ones. We did not find any significant difference in the maximal strength in the lumbar vertebrae between the active and sedentary rats; however, this difference appeared in the bone mass and the stiffness.

6. Conclusion

The ovariectomy induced a decrease both in superior–inferior vertebral height and in maximal braking load when compared to the intact group. The exercise increased the vertebral mass when compared to the intact and to the ovariectomized and sedentary group and increased the stiffness when compared to the sedentary ovariectomized group.

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