65 - EFFECT OF AEROBIC TRAINING PROGRAM ON STRENGTH AND BONE MINERAL DENSITY OF OVARIECTOMIZED RATS

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INTRODUCTION

Physical activity, especially of mechanical impact has been considered as an initiator of the remodeling bone cycle (MARAFON, 2004), and strength training in particular are adopted as a preventive measure against osteoporosis (JOVINO; BUCHALA, 2006).

The strength training has been appointed as the principal agent to induce bone remodeling and metabolism, but not yet fully understood the mechanisms involved in the effects of exercise on bone health and on the type, intensity and duration of exercise (MOSER et al , 2004; JOVINE; BUCHALA, 2006; JAMES; CARROLL, 2008), although the resulting osteogenic effect of physical activity appears to require training characterized by large volume and intensity (CADORE et al, 2005).

The ovaries are the main source of circulating estrogen, and there is clear evidence that the decrease or absence of estrogen leads to progressive bone loss (AMADEI et al, 2006). So after ovariectomy in young or in early menopause, it can be observed accelerated bone loss with faster installation of osteoporosis (KEEN, 2007).

Ovariectomized rats, due to its small size, ease of handling and the similarities with humans, have been widely used as an animal model to simulate the accelerated loss of bone mass observed in postmenopausal osteoporosis studies in humans (TEÓFILO et al, 2003).

The exercises are presented as an important factor in both the treatment and prevention of osteoporosis, providing the mechanical stimulus necessary to changes in bone metabolism (MATSUDO; MATSUDO, 1991), because although the sex steroids are important in the pathogenesis of osteoporosis, physical inactivity is a risk factor (OCARINA; Serakides, 2006).

The bone demineralization can be prevented by hormone replacement therapy, calcium intake and physical exercise (HALL, TARANTO, 2000; KEEN, 2007).

Several authors (MOSER et al, 2004; ALMEIDA; RODRIGUES, 1997; IWAMOTO, TAKEDA, ICHIMURA, 1998) highlight the importance of physical activity during the period of maturation to optimize peak bone mass which results in a positive balance of bone mass to adulthood or remodeling stimulus in elderly individuals. Thus, this study aimed to compare the effects of aerobic exercise protocol and strength exercise on bone mineral density of ovariectomized rats

MATERIALS AND METHODS

Female Wistar rats (200 to 250g) underwent oophorectomy and after 40 days were randomly divided into three groups (8 animals / group), as follows: G1 - Control (limited activities inside the cage), G2 - program swimming of moderate intensity; G3 - program of strength exercises. Ovariectomy was performed after anesthesia intraperitoneally with a solution composed of ketamine and 40mg/kg xylazine 5mg/kg body weight (MASSONE, 2003).

Midline incision of 1.5 cm in length on the skin and subcutaneous cellular fabric in the back of the animal below the last rib and two lateral incisions were made, and the ovaries were exposed and removed.

The training consisted of swimming sessions conducted 50 minutes daily five days a week for four weeks, while resistance training consisted of four sets of ten jumps with one minute between sets and overload of 50% of body weight for five weekly sessions for four weeks.

The weight in grams was determined at baseline, weekly and end of the experiment.

Upon completion of the training period, were sacrificed and all animals were collected their femurs. Blood samples were obtained to determine serum levels of calcium, phosphorus, albumin, total protein and alkaline phosphatase. Measurements were performed by enzymatic colorimetric method, using reagents Labtest

Radiographic analysis was performed in the right femur using the system Digora (Soredex Orion Corporation). For this, digital images were obtained from femurs using a sensor system and optical plate Digora, the images were processed in software for densitometric analysis by radiographic density in areas of 10x10 pixels in the region of the trigone of Ward, by using the value of average density for statistical analysis (SILVA et al, 2007; DUTRA et al, 2007).

Radiographs were obtained using a device Dabi Atlante, model Spectro 70X, with anteroposterior view and time of exposure to X-ray beams of 0.1 seconds.

Data analysis was performed by paired t test for comparison within groups, and ANOVA followed by post-Tukey test for comparison between groups. The significance level was set at $p < 0.05$.

All procedures were performed according to established ethical principles for animal experimentation by the National Control of Animal Experimentation (CONC) and national legislation in force for animal vivisection (Federal Law 11,794, of 08.10.2008). The study was approved by the Ethics Committee of the School through the protocol Novafapi 002/2009.

RESULTS

The mean body weight of animals of different groups was similar at the beginning of the training period, and also showed no statistically significant difference at the end of the training period of four weeks.

However, the ovariectomized rats of group G1 showed body weight gain significantly higher when compared with G2 and G3 (Figure 1).

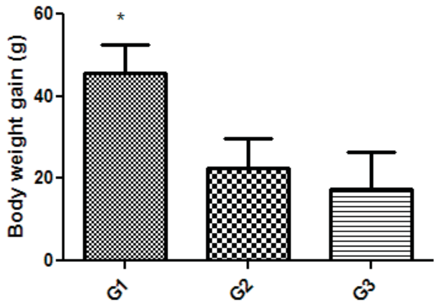

Figure 1. Body weight gain of animals in groups sedentary control (G1), aerobically trained (G2) and trained anaerobically (G3) at the end of 4 weeks of training.* t test, p <0.05 as compared to G2 and G3.

Regarding the biochemical markers evaluated (Table 1) showed that aerobic training programs (G2) and strength (G3) produced no changes in serum albumin, total protein and calcium, but increased the activity of alkaline phosphatase (p <0.001) compared to the ovariectomized control group without exercise (G1), suggesting increased activity on bone metabolism. The serum phosphorus levels were significantly higher (p <0.005) in G1 compared to G2 and G3. Serum creatinine was significantly increased (p <0.05) in G3 compared to G1.

Table 1 - Biochemical dosages of Rattus norvegicus underwent oophorectomy after four weeks of aerobic or anaerobic physical training, which started forty days after ovariectomy. Teresina, 2010.

	G1 (8 animals)	G2 (8 animals)	G3 (8 animals)
Albumin (g / dL)	3,14±0,25	3,126±0,21	3,455±0,22
Total Protein (g / dL)	7,80±0,35	6,993±0,14	7,426±0,36
Calcium (mg / dL)	10,50±0,53	9,856±0,15	9,985±0,28
Phosphorus (mg / dL)	7,06±0,36	4,971±0,47[a]	4,955±0,34[a]
Alkaline Phosphatase (U / L)	37,14±2,94	75,04±7,66[b]	69,13±6,47[b]
Creatinine (mg / dL)	0,43±0,044	0,500±0,06	0,629±0,035[c]

ANOVA and Tukey's post-test: ap<0,005 compared to G1; bp<0,001 compared to G1; cp<0,05 compared to ao G1.

Figure 2 shows that the two training regimes used produced similar effect of increase (p <0.01) in the relative weight of the femurs in the control group (G1).

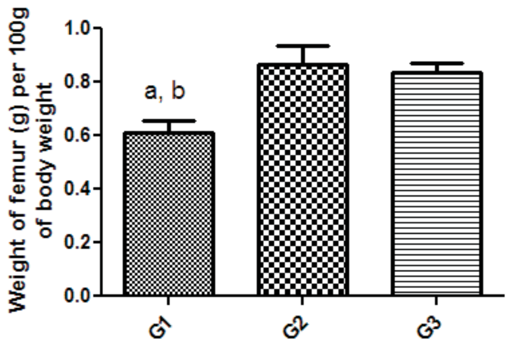

Figure 2 - Relative weight of the left femur (g/100g body weight) of Rattus norvegicus after four weeks of aerobic or anaerobic physical training, begun forty days after ovariectomy. Teresina, 2010. ANOVA and Tukey post-test: ap <0.005 when compared to G1, bp <0.01 compared to G1.

It was observed that the ovariectomized groups submitted to aerobic training (G2) and strength (G3) showed a significant increase (p <0.01) in bone density determined by X-ray analysis at the level of the trigone of Ward when compared with the control group. However, no differences were found between the two types of training.

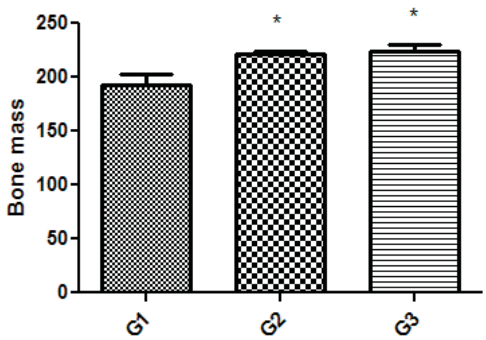

Figure 2 - Bone mineral density determined by X-ray analysis of the trigone of the right femur Ward of Rattus norvegicus after four weeks of aerobic or anaerobic physical training, which started forty days after ovariectomy.aerobic. Teresina, 2010. ANOVA and Tukey post-test: * p <0.01 as compared to G1.

DISCUSSION

The drop in estrogen levels increases the risk of cardiovascular disease, osteoporosis, sexual dysfunction, and increases plasma lipoprotein and weight gain. In this study, physical training (swimming, G2) and strength (G3) produced no increase in body mass of groups submitted to swimming protocol (G2) or jump (G3) in ovariectomized animals, while ovariectomy resulted in significant increase body mass. In this sense, Rennó et al (2007) evaluating ovariectomized animals and pseudo-trained and untrained ovariectomized exercise program with progressive loading, observed greater weight gain and increased body mass in sedentary animals ovariectomized. Hoegh-Andersen et al (2004) in a study with castrated rats and castrated pseudo-significant increase in body weight of castrated animals compared to the pseudo group after nine weeks. These results corroborate those found in this study revealed a tendency to gain weight in ovariectomized animals due to ovarian hormone deprivation.

The analysis of biochemical markers of bone remodeling done in the present study showed effects of training programs conducted in G2 and G3 increased activity of alkaline phosphatase and reducing serum phosphorus levels, although they have not changed the levels of serum calcium. Accordingly, Netto et al (2006) in a study evaluating ovariectomized animals found no differences in serum calcium between the groups, and showed increased activity of alkaline phosphatase, and have seen diminished concentration of bone calcium. Additionally, Iwamoto, Takeda, Ichimura (1998) found higher levels of alkaline phosphatase and osteocalcin in animals submitted to aerobic training on a treadmill one hour per day to 12 m / min compared to sedentary control group. Moreover, Tenorio (2005) study with female mice ovariectomized and ovariectomized and normal pseudo-observed decrease in serum calcium in ovariectomized trained group compared to other groups, and Finkelman et al (1992) observed reduction in serum calcium and increased phosphorus concentration in the group of ovariectomized animals compared to control. The increase in alkaline phosphatase activity suggests increased osteoblastic activity indicating osteoid formation and deposition of collagenous matrix, which can be a better indicator of bone formation than the serum levels of calcium, which are kept firmly under control mainly by parathyroid hormone and are not specific for this process (LAPIN, 2007).

The measurement of bone density showed that the training programs used here produced a significant increase in bone mass in ovariectomized compared to control group, which is also evidenced by the increased weight of the femur of trained animals. Tenorio (2005) and ovariectomized mice pseudoovariectomized evaluating whether or not subjected to aerobic training of walking on a treadmill for 30 days with constant speed of 20m/min and lasting progressive (increasing duration of exercise) for 5 weeks, observed decrease in serum calcium and increased weight of the femur in ovariectomized trained group compared to other groups. Iwamoto, Takeda, Ichimura (1998) found a significant increase in bone mineral density of the tibia with aerobically trained animals walking on a treadmill five times a week (EXP 1: 12m/min, 1 hour / day: 2 EXP: 18m/min, 1 hour / day: 3 EXP: 12m/min, 2 hours / day) after 12 weeks in the control group. Netto et al (2006) by evaluating the grayscale observed a lower density in the ovariectomized group compared to non-ovariectomized group. Abraham, Shimano, Picado (2006) conducted a study with normal rats, ovariectomized and ovariectomized exercised on treadmill (0.31 m / s) for 30 minutes, 5 times a week for nine weeks and reported a greater burden and greater strain on the ceiling femurs compared to normal animals. In contrast, Rennó (2007) found no significant difference in bone mineral density values measured by the ashes and bone density between the ovariectomized animals and pseudo-ovariectomized.

In the systematic review by Jovine; Buchalla (2006) with items included in the period 1985 to 2005 in two databases predominated work with better outcomes for bone health with resistance exercise for long periods of training in detriment to the work activity aerobic. Physical activity showed direct effects on bone mineral density especially in studies on the lumbar vertebrae, whereas in studies that analyzed the bone density of femoral neck were modest results revealing values in the gain in bone density is not significant from a biological and clinical standpoint (MOAYYERI, 2008). A study of 100 women aged between 36 and 76 years in the process of menopause or postmenopausal on which it was observed that calcium intake and physical activity, although they have not produced an increase in bone mass minimized the bone loss, showing a protective effect rather than forming bone as assumption (ALMEIDA; RODRIGUES, 1997).

The groups of ovariectomized animals submitted to swimming training or force had higher bone mass and biochemical profile suggestive of bone remodeling, particularly increased activity of alkaline phosphatase in ovariectomized animals compared with sedentary. However, no differences in bone mass in animals undergoing training aerobic or anaerobic. We stress the importance of further studies using larger training period in order to confirm the trends revealed in this research, including assessments and biochemical markers of bone metabolism such as osteocalcin, and histological evaluations to determine the effects on bone architecture.

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EFFECT OF AEROBIC TRAINING PROGRAM ON STRENGTH AND BONE MINERAL DENSITY OF OVARIECTOMIZED RATS

ABSTRACT

Introduction: Hypoestrogenism or abstinence of estrogen causes rapid bone loss faster installation of osteoporosis. Moreover,exercise represent an important factor in preventing osteoporosis,since it consists of mechanical stimulation induces remodeling and osteogenesis. Objectives: To compare the effects of aerobic exercise protocol and strength exercise on osteopenia in ovariectomized rats. Methods: Adults rats (250 to 300g) divided into three groups (8/group): control (G1) - oophorectomy; G2-oophorectomy + swimming, G3-oophorectomy + strength exercise. The training started 40 days after ovariectomy and a week of adaptation to the aquatic environment. G1 had its activities restricted to the cage. At the end of the trial determined the serum levels of calcium, phosphorus, alkaline phosphatase, creatinine, total protein and albumin. The left femur was weighed and determined the radiographic bone density. Results: G1 showed higher body mass and increased weight gain after the trial period. Phosphorus concentrations and alkaline phosphatase were higher in G2 (4.97 ± 0.47, 75.04 ± 7.66) and G3 (4.95 ± 0.33, 69.13 ± 6.47) compared with G1 (7.06 ± 0.36, 37.14 ± 2.94) and creatinine was higher in G3 (0.63 ± 0.035 p <0.05). The weight of the femurs (0.61 ± 0.04, 0.8681 ± 0.066, 0.84 ± 0.03) and bone density was lower in G1 (192.2 ± 10.78) and no difference between G2 (220.9 ± 3.27) and G3 (224.5 ± 5.91). Conclusion: The physical training programs used produced similar effects, which consisted in increasing bone density and reduction in body weight gain in ovariectomized rats.

KEY WORDS: ovariectomy, osteoporosis, physical activity

EFFET DE PROGRAMME DE FORMATION AEROBIC SUR LA FORCE ET LA DENSITÉ MINÉRALE OSSEUSE DES RATS AYANT SUBI UNE OVARIECTOMIE

RESUMÈ

Introduction: L'œstrogène est faible ou manque d'œstrogènes entraîne une perte osseuse accélérée avec une installation plus rapide de l'ostéoporose. En outre, l'activité physique représente un facteur important dans la prévention de l'ostéoporose, car elle se compose d'une stimulation mécanique induit le remodelage et l'ostéogenèse. Objectifs: comparer les effets du programme d'exercice aérobie et de musculation sur la densité minérale osseuse des rats ayant subi une ovariectomie. Méthodes: Des rats adultes (200 à 250g) ont été divisés en groupes (8/groupe): contrôle (G1) - ovariectomie, G2 - natation + ovariectomie, G3 - l'exercice de force + ovariectomie. La formation a commencé 40 jours après ovariectomie et une semaine d'adaptation à l'environnement aquatique. G1 avait restreint ses activités à la cage. À la fin du procès déterminé les concentrations sériques de calcium, de phosphore, la phosphatase alcaline, de créatinine, protéines totales et l'albumine. Le poids du fémur gauche et de la densité osseuse radiographique a été déterminée. Résultats: G1 montré supérieur de masse corporel et du gain de poids a augmenté après la période d'essai. Les concentrations de phosphore et la phosphatase alcaline ont été plus élevés dans le G2 (4,97 ± 0,47, 75,04 ± 7,66) et G3 (4,95 ± 0,33, 69,13 ± 6,47) comparativement à G1 (7,06 ± 0,36, 37,14 ± 2,94) et de la créatinine était plus élevé dans le G3 (0,63 ± 0,035 p <0,05). Le poids des fémurs (0,61 ± 0,04, 0,87 ± 0,066, 0,84 ± 0,03) et la densité osseuse plus faible dans le G1 (192,2 ± 10,78) et aucune différence entre G1 (220 , 9 ± 3,27) et G2 (224,5 ± 5,91). Conclusion: Les programmes de formation physique utilisée produit des effets similaires, qui consistait à augmenter la densité osseuse et la réduction du gain de poids chez les rats ayant subi une ovariectomie.

MOTS-CLÉFS: ovariectomie, ostéoporose, entraînement physique.

EFECTO DE AEROBIC PROGRAMA DE FORMACIÓN DE LA FUERZA Y LA DENSIDAD MINERAL ÓSEA DE RATAS OVARIECTOMIZADAS

RESÚMEN

Introducción: El bajo nivel de estrógeno o la falta de estrógenos provoca la pérdida acelerada de masa ósea con una instalación más rápida de la osteoporosis. Por otra parte, la actividad física representan un factor importante en la prevención de la osteoporosis, ya que consiste en la estimulación mecánica que induce la remodelación y la osteogénesis. Objetivos: comparar los efectos del programa de ejercicios aeróbicos y entrenamiento de fuerza sobre la densidad mineral ósea de ratas ovariectomizadas. Métodos: Las ratas adultas (de 200 a 250g) fueron divididos en grupos (8/grupo): control (G1) – ooforectomía; G2 - ooforectomía + natación G3-ooforectomía + entrenamiento de la fuerza . El entrenamiento comenzó 40 días después de la ovariectomía y una semana de adaptación al medio acuático. G1 tuvo sus actividades limitadas a la jaula. Al final del experimento se determinaron los niveles séricos de calcio, fósforo, fosfatasa alcalina, creatinina, proteínas totales y albúmina. El peso del fémur izquierdo y la densidad ósea radiográfica se determinaron. Resultados: G1 presentaron mayor masa corporal y aumento de peso después del período de prueba. Las concentraciones de fósforo y fosfatasa alcalina fue mayor en el G2 (4,97 ± 0,47, 75,04 ± 7,66) y G3 (4,95 ± 0,33, 69,13 ± 6,47) en comparación con G1 (7,06 ± 0,36, 37,14 ± 2,94) y la creatinina fue mayor en el G3 (0,63 ± 0,035 p <0,05). El peso del fémur (0,61 ± 0,04, 0,87 ± 0,066, 0,84 ± 0,03) y la densidad ósea menor en el G1 (192.2 ± 10.78) y ninguna diferencia entre G1 (220 , 9 ± 3.27) y G2 (224,5 ± 5,91). Conclusión: Los programas de entrenamiento físico utilizados ha producido efectos similares, que consistía en el aumento de la densidad ósea y la reducción en la ganancia de peso en ratas ovariectomizadas.

PALABRAS CLAVE: ooforectomía, osteoporosis, entrenamiento físico.

EFEITO DE PROGRAMAS DE TREINAMENTO AERÓBIO E DE FORÇA SOBRE A DENSIDADE MINERAL ÓSSEA DE RATAS OOFORECTOMIZADAS

RESUMO

Introdução: O hipoestrogenismo ou a ausência de estrógenos provoca acelerada perda óssea com instalação mais rápida da osteoporose. Por outro lado, os exercícios físicos representam fator importante na prevenção da osteoporose, uma vez que consistem em estímulo mecânico indutor de remodelação e osteogênese. Objetivos: comparar os efeitos de programa de exercício aeróbio e de exercício de força sobre a densidade mineral óssea de ratas ooforectomizadas. Métodos: Ratas adultas (200 a 250g) foram distribuídas em grupos (8/grupo): Controle(G1) – ooforectomia; G2– ooforectomia+natação; G3– ooforectomia+exercício de força. O treinamento foi iniciado 40 dias após ooforectomia e uma semana de adaptação ao meio aquático. G1 teve suas atividades restritas à gaiola. Ao fim do período experimental foram determinados os níveis séricos de cálcio, fósforo, fosfatase alcalina, creatinina, proteínas totais e albumina. O peso do fêmur esquerdo e a densidade óssea radiográfica foram determinados. Resultados: G1 apresentou massa corporal maior e maior ganho de peso ao fim do período experimental. As concentrações de fósforo e fosfatase alcalina foram maiores em G2 (4,97±0,47; 75,04±7,66) e G3 (4,95±0,33;69,13±6,47) quando comparados com G1 (7,06±0,36; 37,14±2,94) e a creatinina mostrou-se maior em G3 (0,63±0,035 com p<0,05). O peso dos fêmures (0,61±0,04; 0,87± 0,066; 0,84±0,03) e a densidade óssea menor no G1 (192,2±10,78) e não houve diferença entre G1(220,9±3,27) e G2(224,5±5,91). Conclusão: Os programas de treinamento físico utilizados produziram efeitos semelhantes, que consistiram em aumento da densidade óssea e redução do ganho de peso corporal em ratas ooforectomizadas.

PALAVRAS-CHAVE: ooforectomia, osteoporose, atividade física.