

37 - RADIOGRAPHIC ANALYSIS OF FEMURS AND SERIC LEVELS OF CALCIUM AND PHOSPHORUS OF OOFORECTOMYZED RATS SUBMITTED TO PHYSICAL TRAINING

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INTRODUCTION

Bone tissue is one of the most metabolic and dynamic of the human body which remains active during the whole life, presenting the capacity of self-regeneration and being able to alter its properties and configurations according to changes in mechanic demands (NORDIN; FRANKEL, 2003).

Osteoporosis is an osteometabolic, systemic and progressive disease characterized by low bone density and microarchitural deterioration of bone tissue, which leads to increased in bone fragility (PIAI et al, 2005; GUARNIERO, 2004), and presents of fractures and the loss of functional autonomy as the most adverse consequences (SILVA, 2003).

In modern society osteoporosis is one of the most prevalent disease that affect the quality of life of people all over the world (ZANETTE et al, 2003) and along with the increase in the number of erdelies, related to an in life expectancy, this situation will tend to be worst in the next years (MOREIRA, 1998). It is estimated that up to 2050 around 6,26 million of osteoporotic fractures of the neck of the femur osteoporosis will occur in world population (ZANETTE, et al. 2003), what will represent increments in the morbidity and mortality related to this disease (AVEIRO et al, 2006).

Osteoporosis is classified as primary or secondary. The primary is subdivided into type I and type II (GALI, 2001). The primary osteoporosis type I, known as pos-menopausal osteoporosis is related to the loss of the ovarian function, with an intensification of bone reabsorption mainly during the first years after the menopause (MARAFON, 2004). The primary osteoporosis type (1) II is related to the process of aging and characterizes by a deficiency in the osseous formation (MARAFON, 2004). The secondary osteoporosis is caused by a base disease that provokes disequilibrium in the mechanisms of bone formation and reabsorption (SILVA, 2002).

There are different methods for diagnosis, which can be through laboratory or radiographic exams or bone biopsy, but the most common method, bone densitometry, is currently regarded as the "golden standard" among the predictive methods of diagnosis (GUARNIERO, 2004). However, (this) it is considered as a static punctual measure that doesn't show the dynamic alterations (that are) occurring in the bone tissue at moment of the exam. So biochemical markers of bone remodelling may be used with the intention of providing the limitative factors (SARAIVA; CASTRO, 2002).

Physical activities, mainly those which offers mechanical impacts are considered as indicators of bone remodeling cycle (MARAFON, 2004). Trainings of strength are in special adopted as preventive measure of osteoporosis (JOVINE et al, 2006), since the bone adapts his strength to the degree of bone stress and bone deposition is proportional to the load of compression to which it is subjected. (GUYTON; HALL, 2002).

Physical exercises offer the mechanical stimulus necessary for changes in bone metabolism and it they represent important factors for the therapy and prevention of osteoporosis (MATSUDO; MATSUDO, 1991). Although sexual steroids hormones are important in the genesis of osteoporosis, physical inactivity constitutes a risk factor (OCARINO, SEAKIDES, 2006). In this sense, the purpose of the present study was to evaluate the effects of high intensity physical training on the bone mass of ooforectomized animals.

METHODOLOGY

Adult female Wistar rats aged approximately 50 days were divided randomly in groups (8-10 animals/group): sedentary control (C); trained control (TC); sedentary ooforectomized (O) and trained ooforectomized (TO). Non-trained groups had their activities limited to own cage from the beginning to the end of the experience.

Animals of groups TC and TO were submitted to a program of strength physical training adapted to the protocol described by Oliveira et al (2002) which consists in jumping inside a tank, whose water reached 150 % of the body size of animal, with overload captured to the body. During twelve weeks the animals of TC and TO groups followed a high intensity physical training protocol which consisted of four sets of 10 jumps/day (interrupted by one minute of resting interval) in a swimming pool, with the water level corresponding to 150% of the body length and overload equivalent to 50% of the body's weight. The training was started after a period of adaptation to the liquid medium, water at temperatures going from 30 to 32 °C, during 20 minutes by day, three times a week, without the use of overload during two weeks.

The oophorectomy was realized in the castrated group under anesthesia with ketamine hydrochloride at 90 mg/kg and xylazine hydrochloride at 10mg/kg through intramuscular administration way (MASSONE, 2003). Median incision of 1.5 cm in the skin and subcutaneous cellular sheet in the back of animal below the last rib and two lateral incisions were realized and the ovaries were exposed and removed.

At the end of training, animals were sacrificed through administration of excessive dose of anesthetics and the femurs of each animal were collected. The femurs were radiographed using digital X-ray equipment through the use of sensor and optic plate (instead of a radiographic film) of the system Digora produced by Soredex, and the images were processed in a software to densitometric analysis by radiographic density, that is, by measure of the radiopacity through their gray scale levels, delimitating (to) the analysis of a square of 23x23 pixels and using the value of the mean density for statistical analysis (SILVA et al, 2007; DUTRA et al, 2007). The delimited area for analysis was the Ward polygon.

At the end of the experimental period, blood samples were collected for dosages of the serum calcium and phosphorus. Urine too was collected 24 hours before sacrificing the animals, for calcium dosage It was collected. The determinations were realized by the enzymatic colorimetric method, using LABTEST reagents.

The data were submitted to descriptive and inferential statistics. The Tukey test was applied for comparison of means between the experimental conditions. Significance level was established in $p < 0.05$.

The study was conducted according to the Ethical Principles on Animal Experimentation adopted by the Brazilian

College of Animal Experimentation (COBEA) and the project was approved by Ethics committee of Federal University of Piauí.

RESULTS

Body weights of the animals of different groups were similar at the beginning of the training, and didn't present significant differences at the end of the 12th week follow-up. However, it was observed that ooforectomized female rats of the O group presented body weight gain higher than female rats non-ooforectomized of the group C (figure 1).

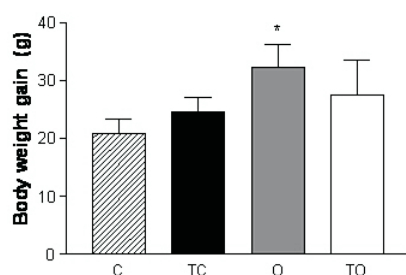


Figure 1. Body weight gain of sedentary control ©, trained control (TC), sedentary ooforectomized (O) and trained ooforectomized (TCA) animals after twelve weeks of strength physical training. * $p < 0.02$ in relation to group C

The result of the bone density of the neck of the femur measured by radiologic analysis using the Digora system to characterize the metabolic state from the osseous tissue of experimental animals is presented in table 1. It was observed that only animals of TO group presented lower radiographic density values than those of C group, demonstrating there was development of osteoporosis in the animals with hypoestrogenism submitted to physical training.

Table 2. Bone density values measured by Digora system in digital radiologic unity, measured as gray levels, and percentages of variation regarding the control group (C) in female rats submitted to strength physical training during 12 weeks.

Group	Osseous Density (Urd) (mean \pm standard error)	Variation(%) in relation to group C
C (n=10)	205.4 \pm 3.01	-
TC (n=10)	197.2 \pm 2.14	-3.99
O (n=8)	200.8 \pm 3.15	-2.24
TO (n=9)	186.2 \pm 3.38*	-9.35

Also there was carried out the analysis of some markers of the bone metabolism to evaluate the effects of the strength training in bone remodelling, searching some relation between measures of bone density by Digora and the variation in the blood concentrations or urinary ones. The analysis of serum calcium and phosphorus and urinary calcium between the experimental groups did not show significant differences in either seric or urinary calcium from those of the control group. However serum phosphorus differed significantly in O ($p < 0.01$) and TO ($p < 0.05$) from those observed for the group C (table 2).

Table 3. Serum calcium, phosphorus and urinary calcium in the animals of the groups sedentary control (C) and trained one (TC) and sedentary ooforectomized (O) and trained (TO)

Group	Serum calcium (mg/dL) (mean \pm standard error)	Urinary calcium (mg/dL) (mean \pm standard error)	Serum phosphorus (mg/dL) (mean \pm standard error)
C (n= 8 a 10)	9.61 \pm 0.17	14.40 \pm 0.83	6.70 \pm 0.28
TC (n= 9 a 10)	10.04 \pm 0.31	14.50 \pm 0.48	5.49 \pm 0.43
O (n= 5 a 9)	9.61 \pm 0.52	11.17 \pm 3.01	4.34 \pm 0.46 ^a
TO (n= 7)	9.28 \pm 0.78	12.42 \pm 0.62	4.81 \pm 0.50 ^b

ANOVA and Tukey multiple comparisons test - a $p < 0.01$ O in relation to C group; b $p < 0.05$ TO in relation to C group.

DISCUSSION

Ovariectomized female rats has been used as model to studies of osteoporosis due to their small size, facility to manipulate and for presenting many similar characteristics with the human as far as osseous tissue is concerned (TEOFILO et al, 2003; PINTO et al, 2006). Phylogenetic similarity between the rat and human turned this animal in to the most appropriate for studies involving the gonads, because it's endocrine activity and the effect of sexual hormones on the body, mainly on the target tissues ex Bones depend on this endocrine action (THORNDIKE; TURNER, 1998). Moreover the same way woman develop osteopeny after menopause female rats develop osteopeny after ovariectomy (MELLO; GOMIDE, 2005), providing similar information as those observed in the pos-menopausal adult skeletal (KALU, 1991 cited by MELLO; GOMIDE, 2005).

Physical activity is considered important for the prevention and treatment of osteoporosis. In spite the fact that there are no doubts about the advantages of exercise to the health, on a general view, there are no evidences that exercise on itself could remove the negative effects of the hypoestrogenism (PINTO NETO et al, 2002). In this study, the effects of high intensity strength exercises were evaluated in animals submitted to physical training after 40 days after oophorectomy. According Carvalho (2001), the induction of osteopeny in mature female rats by ovariectomy needs a resting period of at least of 30 days. The duration of twelve weeks of the training program until the sacrifice of animals took in to consideration the duration of exercises and the progressive application of the overload making sure they would be enough to obtain probable positive effects in the osseous structures affected by osteopeny.

Thus, the ooforectomized female rats not submitted to physical training presented higher body weight than non ooforectomized animals of the non-trained control group. These results are in agreement with informations described in the literature that oophorectomy produces increases of body weight gain (VASCONCELLOS et al, 2005). However, the animals of trained group didn't present body weight gain differently from those of non-trained control group.

The measure of bone density revealed significant loss of bone mass in the group of trained ooforectomized females and variation not significant in control trained female rats and in ooforectomized non-trained ones. This information considered together the fact that in relation to body weight gain too the ooforectomized females submitted to the program of training presented tendency to a lower body weight in relation to non-trained ooforectomized females that could be related to probable

excess of training. According Silva (2003) some factors must be observed beside the physical activity in order to reach a satisfactory result in relation to increasing of bone mass, such as hormonal function and heredity, where the lack of some of these factors could compromise the final result expected from training.

As for what concerns the evaluation of bone density, Digora system used here is a relatively new one used for radiographic dental density analyses which offers good conditions for the interpretation of radiographic density of the tested areas (TAVANO et al, 1999), and matched with rats femurs measurements. Moreover, realization of combined histomorfometric studies could probably more adequate analyses of bone alterations in animals of different groups,

Studies that evaluate the effects of the training with loads in humans have declared positive results in the functional capacity. In this sense, Raso (2000) described increase of the muscular strength induced by adequate exercises program with loads and promoting the improvement of the performance in the activities of daily life as well as proportioning a larger quality of life at old and unable people. Avelino et al (2006) observed that physical training during 12 weeks produced improvement of the functional equilibrium, in the speed of the march and muscular strength of the ankle of old woman porters of osteoporosis.

In this study calcium and phosphorus didn't present differences that could be considered as biochemical markers of bone remodelling. These results are consistent with those obtained by Tenorio et al (2005) in a study evaluating the serum concentration of calcium in ovariectomized mice subjected for physical training. Similarly the study realized by Cecchetti et al (1995, cited by CADORE et al 2005) didn't find significant differences in the serum calcium or phosphorus during the treatment of osteoporosis in women in the pos-menopause. Lets highlighted here that the calcium and phosphorus doesn't constitute markers with sensibility and specificity to clinical use. About the effects of aerobic exercises, Abrahão et al (2006) recently demonstrated that aerobic exercise applied for 30 minutes five days per week during nine weeks was not enough to correct biokinetic alterations of the osseous tissue provoked by ovariectomy in female rats.

Although it is known that estrogens present direct effects on the bone, kidneys and intestine (important organs in the calcium transport) with increasing of the total plasmatic calcium when there is estrogen lack, such as in menopause or after ovariectomy and fall of this concentration with the realization of estrogen reposition (PRINCES, 1994 cited by TENORIO et al, 2005). There are doubts about the influence of the estrogen on the several fraction of calcium that contributes to the concentration of the total plasmatic calcium (TENORIO et al. 2005).

One of the reasons that could justify the lack of alteration of the serum calcium in this study would be the fact that the calcium has a mechanism of physiological regulation that shortens the limits of its plasmatic levels and it doesn't permit variations over 2,4 mmol/L where any tendency of alteration of the plasmatic concentration of this ion is corrected from minutes to hours, mainly by action of the parathormone, in order that the serum calcium and phosphorus must be evaluated together, because the concentration of one influences directly the other. GUYTON, HALL, 2002).

The calcium level in the urine of 24 hours is used to evaluate the renal excretion of calcium and such measures of urinary calcium excretion are greatly influenced by diet, renal function and calcium regulating hormones, such that although widely used their interpretation requires caution since individual variations are very high (SARAIVA; CASTRO, 2002).

Other biochemical parameters represent more specific marker of the bone metabolism. Thus, the intact osteocalcin and bone alkaline phosphatase better represent bone formation, while pyridinoline, deoxypyridinoline and collagen type I cross-linking (amino and carboxi-terminal) telopeptides, the bone resorption and in the follow up of osteoporosis treatment, the bone reabsorption markers are more specific and sensitive than the formation markers where the rate of fall from basal values of resorption markers are related to the increase on bone mass after long-term treatment (SARAIVA; CASTRO, 2007). They could represent, possibly, a more dynamic way of evaluation of the physical exercises in the bone metabolism, because the larger concentration of the markers of bone formation can suggests, an effective training not yet translated by densitometric values. (CADORE et al, 2005).

Several studies show the beneficial effects of physical activity on the bone tissue in the prevention or treatment of (the) osteoporosis however there are contradictions in some results (OCARINO; SERAKIDES, 2006). In this present study it is not possible to verify this fact, so probably an excess of physical activity occurs and could contribute to bone decalcification, because in spite the exercise shows a relevant purpose in the bone remodelling it is not clear what this kind of activities, intensity, duration and frequency of exercises are able to provide a positive influence in the bone mineral density (CARVALHO, 2001). Mechanisms used by physical activity to stimulate the increasing of bone mass need to be better elucidated (OCARINO; SERAKIDES, 2006).

Combined with physical activity another factors that could interfere in the expected effect of physical activity on bone mass in this study include: insufficient nutrition by non-utilization of protein supplements in the animals submitted to training, impeding and/or difficulting the formation of the protein bone matrix or increasing bone resorption by increase of catabolism; non-providing of the necessities of vitamin C with the utilization of the standard diet of rats without use of supplementation; and this vitamin helps in the formation of osteoids by osteoblasts; and the lack of secretion of estrogen that exercises stimulant factors on the activity of the osteoblasts.

The program of physical training with the load, intensity and duration used here doesn't provides protection to loss of osseous mass in ooforectomyzed animals or increase in the osseous mass of intact animals. It is important to rebound the necessity of a larger increment in researches related to the influences of the physical activities on the osseous tissue, because mechanisms used by physical education to stimulate the increasing of the osseous mass need to be better elucidated as well the use of method of analysis more adequate that corroborate to the obtaining of more credible results.

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RADIOGRAPHIC ANALYSIS OF FEMURS AND SERIC LEVELS OF CALCIUM AND PHOSPHORUS OF OOFORECTOMIZED RATS SUBMITTED TO PHYSICAL TRAINING

ABSTRACT

Bone is a multifunctional tissue that answers to a variety of stimuli, such as biological, biochemical and biomechanical ones. Physical activities, mainly those which offers mechanical impacts are considered as initiator of the cycle of bone remodelling. Ooforectomized female rats have been used as animal model to osteoporosis studies. Physical activity is considered important for the prevention and treatment of osteoporosis and there are no doubts about the advantages of the exercise to health, at a general view, there are no evidences that exercise on itself could remove the negative effects of the hypoestrogenism. The purpose of this study was to evaluate the effects of high intensity physical training on the bone mass of ooforectomized animals Wistar female rats aged approximately 50 days were randomly assigned into four groups of 8-10 animals, according ooforectomy and physical training: sedentary control ©, trained control (TC), sedentary ooforectomized (O) and trained ooforectomized (TO). During twelve weeks the animals of TC and TO groups followed a high intensity physical training protocol. The training started 40 days after the ooforectomy, and it consisted of four sets of 10 jumps/day in a swimming pool, with the water level corresponding to 150% of the body length and overload equivalent to 50% of the body's weight. Sedentary ooforectomized female rats presented higher weight gain from those of sedentary control group. There was significant decreasing of bone mass in the group of trained ooforectomized females. The levels of serum and urinary calcium didn't present differences in relation to sedentary control group, while the serum phosphorus was smaller in sedentary ooforectomized group and trained ooforectomized in relation to sedentary control group. Physical training with the load,

intensity and duration used here didn't provide protection to loss of osseous mass in bone mass of female rats with hypoestrogenism.

KEY WORDS: oophorectomy, osteoporosis, physical training.

ANALYSE DE RADIOGRAPHIES DE FEMUR ET DE LES NIVEAUX SÉRIQUES DU CALCIUM ET DU PHOSPHORE DES RATS OVARIECTOMISÉES SOUMIS A L'ENTRAÎNEMENT PHYSIQUE

RÉSUMÉ

L'os est constitué d'un tissu multi-fonctionnel qui répond à une variété de stimulations, entre autres, biologiques, biochimiques et biomécaniques. L'activité physique, en particulier quand elle provoque un impacte mécanique, est considéré comme l'initiateur du cycle d'un remodelage osseux. Rats ovariectomisées ont été utilisées comme modèle animal pour l'étude de l'ostéoporose. L'activité physique a été considérée importante pour la prévention et le traitement de l'ostéoporose et, bien qu'il n'y a pas de doutes sur les bénéfices de l'exercice pour la santé, en général, il n'existe aucune preuve que l'exercice seul peut conjurer les effets négatifs de la ménopause. L'objectif de cette étude était d'évaluer les effets de l'entraînement physique de haute intensité sur la masse osseuse en animaux ovariectomisés. Les rats Wistar, après 50 jours ou plus, ont été assignés à quatre groupes de 8-10 animaux: le contrôle sédentaires (C), le contrôle entraîné (CE), sédentaire ovariectomisées (O) et ovariectomisées entraîné (OE). Pendant douze semaines, les animaux en groupes CE et OE effectué un protocole de l'entraînement physique. L'entraînement a été initié 40 jours après l'ovariectomie et composé de quatre séries de 10 sauts dans le réservoir d'eau avec le niveau correspondant à 150% de la longueur du corps et de surcharge de 50% du poids du corps. Les rats contrôle sédentaire ont présenté un plus grand gain de poids que les animaux contrôle non entraînés. Il y a réduction significative de la masse osseuse dans le groupe ovariectomisée entraîné. Les niveaux de calcium sérique et urinaire n'ont pas présenté de différences concernant le groupe contrôle non entraîné, alors que ceux du phosphore sérique ont été inférieurs aux groupes ovariectomisées entraîné et non entraîné relativement au groupe contrôle non entraîné. L'entraînement physique selon le volume et la durée utilisés n'ont pas produit d'effets positifs sur la masse osseuse des rates avec hypoestrogénisme.

MOTS-CLÉS: ovariectomie, ostéoporose, entraînement physique.

ANÁLISIS DE RADIOGRAFÍAS DE FÉMUR Y NIVELES DE CALCIO Y FÓSFORO EN EL SUERO DE RATONES OOFORECTOMIZADAS SUJETOS DE ENTRENAMIENTO FÍSICO

RESUMEN

El hueso constituyese en un tejido multifuncional que responde a una variedad de estímulos, entre ellos biológicos, bioquímicos y biomecánicos. La actividad física, especialmente por impacto mecánico, es considerada como un iniciador del ciclo de la remodelación ósea. Ratones ovariectomizados se han sido utilizado como modelo animal para el estudio de la osteoporosis, y aunque no hay dudas sobre los beneficios del ejercicio para la salud, en general, no hay pruebas de que el ejercicio aisladamente puede evitar los efectos negativos del hipo-estrogenismo. El objetivo de este estudio fue evaluar los efectos del entrenamiento físico de fuerza de alta intensidad sobre la masa ósea en los animales ooforectomizadas. Ratones Wistar hembras, con 50 días o más, fueron asignados aleatoriamente a cuatro grupos (8-10 animales): control sedentario (C), control entrenado (CE), sedentario ooforectomizado (O) y grupo ooforectomizado entrenado (OE). Durante doce semanas, los animales de los grupos CE y OE han realizado un protocolo de entrenamiento físico de alta intensidad iniciado 40 días después de la ooforectomía. El entrenamiento constaba de cuatro series de 10 saltos en el tanque de agua con el nivel correspondiente al 150% de la longitud del cuerpo y la carga correspondiente al 50% del peso corporal del animal. Las ratas sedentarias ooforectomizadas mostraron mayor ganancia de peso que el grupo controle no sometido a entrenamiento físico. Hubo reducción significativa de la masa ósea en el grupo ooforectomizado. Los niveles de calcio en suero y orina no mostraron diferencias con respecto al grupo controle no entrenado, mientras que los niveles de fósforo sérico fueron menores en los grupos ooforectomizados con respecto al grupo controle no entrenado. El entrenamiento físico en el volumen y duración aquí utilizada no ha representado efectos positivos sobre la masa ósea en ratas con la privación de estrógenos.

PALABRAS CLAVE: ooforectomía, osteoporosis, entrenamiento físico.

ANÁLISE RADIOGRÁFICA DE FÊMURES E NÍVEIS SÉRICOS DE CÁLCIO E FÓSFORO DE RATAS OOFORECTOMIZADAS SUBMETIDAS A TREINAMENTO FÍSICO

RESUMO

O osso constitui-se em tecido multifuncional que responde a uma variedade de estímulos, dentre eles biológicos, bioquímicos e biomecânicos. A atividade física, principalmente de impacto mecânico, é considerada como iniciador do ciclo de remodelação óssea. Ratas ooforectomizadas têm sido utilizadas como modelo animal para estudos de osteoporose. A atividade física constitui-se em importante fator para a prevenção e tratamento da osteoporose e, apesar de não haver dúvidas quanto aos benefícios do exercício para a saúde, de uma forma geral, não existem evidências de que o exercício isoladamente possa afastar os efeitos negativos do hypoestrogenismo. O objetivo deste estudo foi avaliar os efeitos do treinamento físico de força de alta intensidade sobre a massa óssea animais ooforectomizados. Ratas Wistar adultas foram distribuídas aleatoriamente em quatro grupos de 8-10 animais: controle sedentário (C), controle treinado (CT), ooforectomizada sedentária (O) e ooforectomizada treinada (OT). Durante doze semanas os animais dos grupos CT e OT realizaram um protocolo de treinamento físico de alta intensidade iniciado 40 dias após a ooforectomia, e que consistiu na realização de quatro séries de 10 saltos (intercaladas por um minuto de intervalo) em tanque, com o nível da água correspondendo a 150% do comprimento corporal e sobrecarga equivalente a 50% da massa corporal dos animais. As ratas ooforectomizadas sedentárias apresentaram maior ganho de peso que os animais do grupo controle não submetidos a treinamento físico. Houve redução significativa de massa óssea no grupo de fêmeas ooforectomizadas treinadas. Os níveis de cálcio sérico e urinário não apresentaram diferenças em relação ao grupo controle não treinado, enquanto os de fósforo sérico foram menores nos grupos ooforectomizadas não treinadas e ooforectomizadas treinadas em relação ao grupo controle não treinado. O treinamento físico no volume e duração aqui utilizados não produziu efeitos positivos sobre a massa óssea de ratas com hypoestrogenismo.

PALAVRAS-CHAVE: ooforectomia, osteoporose, treinamento físico.

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