

EVALUATION OF FEMUR OF ORCHIECTOMIZED GUINEA PIGS BY BONE DENSITOMETRY USING DUAL-ENERGY X-RAY ABSORPTIOMETRY (DXA) AND MECHANICAL TESTING¹

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ABSTRACT

The aim of this study was to evaluate the effects of castration on bones in the male guinea pigs and to observe whether mechanical testing correlates with dual-energy X-ray absorptiometry (DXA). Twelve male guinea pigs (*Cavia porcellus*), aged 21-27 days, and with average initial weight of 279 grams were used. The animals were equally allocated to two groups: GI - orchietomized animals and GII - intact control animals. They underwent euthanasia at seven months following surgery. DXA measurement was performed at the mid-third of the right femoral diaphysis in the cortical region and at the left femoral neck in order to verify its correlation with results of mechanical testing. Three-point bending test of right femur and axial compression test of left femur were performed. Bone mineral density of GI was significantly lower only at femoral neck. No differences were observed in the maximum load values between GI and GII for both bending and axial compression tests. The bending test revealed lower bone stiffness in GI compared to GII, but in the axial compression test no differences between groups were observed. Only left femur showed positive correlation coefficient between maximum load and bone mineral density according to Pearson's correlation coefficient. The results suggest that hormonal deprivation in guinea pigs induces reduction of bone mineral density, especially in the femoral neck area and reduction of bone stiffness in the mid-femoral diaphysis.

Key words: bone, DXA, mechanical testing, guinea pigs.

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DENSITOMETRIA ÓSSEA POR ABSORTOMETRIA RADIOLÓGICA DE DUPLA ENERGIA (DXA) E TESTES MECÂNICOS NA AVALIAÇÃO DO FÊMUR DE COBAIOS ORQUIECTOMIZADOS

RESUMO

O objetivo do estudo foi avaliar os efeitos da orquiectomia nos ossos de cobaios e observar se havia correlação entre os testes mecânicos e a absorptometria radiológica de dupla energia (DXA). Foram utilizados 12 porquinhos-da-índia (*Cavia porcellus*), machos, com idade variando de 21 a 27 dias e peso inicial médio de 279 gramas, distribuídos em dois grupos experimentais equitativos: GI - castrado e GII – inteiro (controle). Todos os animais foram submetidos à eutanásia sete meses após a realização do procedimento cirúrgico. Foi realizada aferição pelo DXA na região cortical do terço médio da diáfise femoral direita e no colo femoral esquerdo, a fim de verificar correlação com os resultados dos ensaios mecânicos. No fêmur direito realizou-se ensaio de flexão de três pontos e no fêmur esquerdo ensaio de compressão axial. A densidade mineral óssea do GI foi significativamente menor somente no colo do fêmur. Não foram observadas diferenças nos valores de carga máxima entre GI e GII, tanto para os ensaios de flexão como para os de compressão axial. O ensaio de flexão revelou menor rigidez do osso no GI em relação ao GII, mas no ensaio de compressão axial não foram observadas diferenças entre os grupos. Apenas o fêmur esquerdo demonstrou um coeficiente de correlação positiva entre a carga máxima e densidade mineral óssea, de acordo com o coeficiente de correlação de Pearson. Os resultados sugerem que a privação hormonal em porquinhos-da-índia induz a redução da densidade mineral óssea, especialmente no colo femoral, e redução da rigidez óssea na porção média da diáfise femoral.

Palavras-chave: osso, DXA, teste mecânico, porquinho-da-índia.

DENSITOMETRÍA ÓSEA POR ABSORCIOMETRÍA DE RAYOS X DE ENERGÍA DUAL (DXA) Y PRUEBAS MECÁNICAS EN LA EVALUACIÓN DE FÉMUR DE COBAYOS ORQUIECTOMIZADOS

RESUMEN

El objetivo de este estudio fue evaluar los aspectos efectivos de la castración en huesos de cobayos y observar si pruebas mecánicas se correlacionan con la absorciometria radiológica de energía dupla (DXA). Fueron utilizados doce conejillos de india (*Cavia porcellus*) machos, entre 21 a 27 días de edad y con peso inicial de 279 gr. los animales fueron distribuidos igualmente en dos grupos: GI – animales orquiectomizados y GII – animales enteros (control). Estos fueron sometidos a la eutanasia siete meses posteriores a la cirugía. Se realizó la medición de DXA en la región cortical del tercio medio de la diáfisis femoral derecha y en el cuello femoral izquierdo, a fin de verificar su relación con los resultados de las pruebas mecánicas. Fueron ejecutadas pruebas de flexión en tres puntos del fémur derecho y la prueba de compresión axial en el fémur izquierdo. La densidad mineral ósea del GI fue significativamente menor apenas en el cuello del fémur. No fueron observadas diferencias en los valores de carga máxima entre GI y GII, tanto para las pruebas de flexión como para las de compresión axial. La prueba de flexión reveló menor rigidez del hueso en el GI con relación al GII, sin embargo en las pruebas de compresión axial, no fueron observadas diferencias entre los grupos. Apenas el fémur izquierdo demostró un coeficiente de correlación positivo entre la carga máxima y densidad mineral ósea de acuerdo con el coeficiente de correlación de Pearson. Los resultados sugieren que la privación hormonal en los cobayos induce a una reducción de la densidad mineral ósea, especialmente en la región del cuello femoral y reducción de la rigidez ósea en la diáfisis femoral media.

Palabras-clave: hueso, DXA, prueba mecánica, cobayo.

INTRODUCTION

Several methods are available to evaluate bone density and bone mineral mass. Bone densitometry by DXA (1-5), quantitative computed tomography (6,7), and optical radiographic densitometry (6,8) are some of the imaging methods.

Optical radiographic densitometry has advantages of low cost and technical simplicity, but bone mass measurement must be performed at peripheral skeletal sites and depending on the type of radiographic film used, variations concerning degree of radiographic exposure and processing may occur (6,8). Quantitative computed tomography provides higher resolution images and has applicability for selective measurement of the trabecular bone. Besides the high cost of equipment, this method presents some critical factors, such as, high radiation doses, interference from fatty tissue and intestinal gas (8,9). Bone densitometry by DXA is one of the most widely used methods especially for human patients due to its high precision and accuracy, high-resolution image, practicability, low radiation dose, and short scanning time. Moreover, this method enables deep measurement which is crucial for studies of fractures (1,5,6,10).

Of the invasive techniques for measuring bone density, biomechanical tests provide true bone strength (11,31). However, it requires cadaver bones (12) or animals which underwent specific surgical procedure or treatment (13). Bone reacts to the applied force according to its magnitude, direction and duration. Force may be enough either to fracture the bone or to alter its three-dimensional relationship with skeletal mass (14).

Osteoporosis is a skeletal disorder characterized by compromised bone strength, thereby making them brittle and prone to breakage (10,15). Several processes may be involved in this disorder including hormonal depletion (15), nutritional secondary hyperparathyroidism (16), hypovitaminosis D and renal dystrophy (15,16). Animals such as rats, mice, rabbits, guinea pigs, ewes, non-human primates, dogs and cats have been tested as experimental models for osteoporosis (4,16-20). However, the rats considered modeling or remodeling species, according to age progression in both cancellous and cortical bone (31), are one of the most currently used to evaluate benefits from diet or mineral supplementation (13), drugs and hormones (21-23) for osteoporosis prevention and treatment, among others.

Due to high rates of osteoporosis in women as a consequence of estrogen deprivation after menopause (15), female animals have been used as the standard model in most experimental studies (13,17,19,23,31). However, osteoporosis has also been observed in male individuals (7,18,19,21,22,24). In addition, although the rats are a suitable model for osteoporosis the small size may be a problem to evaluate some types of implants in osteoporotic bone. Therefore, the aim of this study was to evaluate the effects of castration on bones in the male guinea pigs and to observe whether mechanical testing correlates with DXA.

MATERIALS AND METHODS

This study followed the Guide for Care and Use of Laboratory Animals and it was given the approval of the Ethical Committee of our Veterinary School (38/2008, 02/15/2008). Twelve male Dunkin-Hartley guinea pigs, aged 21-27 days, and with average initial weight of 279 grams were used. The animals were equally and randomly allocated to two groups: GI – orchietomized animals and GII – intact control animals. The animals were housed in

100x68x28 cm polyethylene cages, and they were fed water and a commercial chow diet *ad libitum*.

Surgical procedure and post-operative care

To perform orchietomy, GI animals were anesthetized with an association of ketamine (40 mg/kg) and xylazine (5 mg/kg) administered via intraperitoneal route. Guinea pigs were placed in dorsal recumbency, and the caudal ventral abdomen was clipped, prepared, and draped using a sterile technique. A caudal ventral midline abdominal incision was made. Testicles were displaced carefully and removed by open castration. Parietal tunic was incised and spermatic cord exposed, ligated and transected. Parietal tunic and inguinal canal were closed with a cruciate mattress suture. Subcutaneous tissue and skin incision were closed using simple continuous pattern and simple interrupted pattern, respectively. Nylon monofilament 4-0 was the suture material used for all tissue layers. Buprenorphine hydrochloride 0.05 mg/kg, SC, was administered during anesthetic induction and after 12 hours. Skin suture was removed 10 days after surgery.

Bone densitometry and mechanical testing

Animals were euthanized at 31 or 32 weeks of age which represents seven months postoperatively, and femur bones were immediately frozen until analysis, when they were defrost. Bone densitometry exam was performed using DXA (Lunar Densitometer DPX Alpha). Bones were evaluated individually in craniocaudal position. To allow comparison with mechanical testing, measurements from the right femur were obtained at the mid-third region of cortical diaphysis and those from the left femur were obtained at the neck region. Bone mineral content (g), bone surface area (cm²) and bone mineral density (g/cm²) were determined in this study.

Mechanical testing was performed on an EMIC[®] universal testing machine. System precision is 0.018 + F/3700 kN in accordance with NBR 6156 and NBR 6674 specifications of ABNT (Brazilian Association of Technical Norms). Calibration is periodically checked by manufacturers. Universal machine was fitted with a load cell of 2000N capacity at a loading rate of 20 mm/min. The graphic register enabled assays according to parameters and unities previously defined.

In the bending test of right femur, distance between two points of the support was standardized at two thirds of the specimen and load application was equidistant from each end. For axial compression test of left femur, bones were placed in a vertical position and axial load was applied to femoral neck. Tests were performed considering the following structural-mechanical properties: maximum energy (N/10⁻³m) was defined as maximum load capacity multiplied by deformation value of specimen; maximum load (N) was defined as load-bearing capacity of specimen until its rupture; stiffness (N/mm) was defined as the relationship between load and deformation (diagram) indicating the rigidity of the structure; elastic limit (N/mm), also defined by load-deformation diagram, indicating the end of elastic region.

Statistical analysis

Student's *t* test for independent samples was used as statistical methodology. Associations between variables were also studied using Pearson Linear Correlation. Differences were considered statistically significant at $p < 0.05$.

RESULTS

No complications were observed during surgery procedure or postoperative period. Means and standard deviations for bone mineral density are reported in Table 1. Bone mineral density of the orchietomized group was significantly lower only at femoral neck.

Means and standard deviations of mechanical testing are reported in Table 2. No differences were observed in the maximum load values between orchietomized and intact control groups for both three-point bending and axial compression tests. The three-point bending test revealed lower bone stiffness in the orchietomized group compared to the intact control group, but in the axial compression test no differences between groups were observed.

Only left femur showed positive correlation coefficient (Table 3) between maximum load and bone mineral density (Figure 1), according to Pearson's correlation coefficient performed between maximum load and bone mineral density, and between stiffness and bone mineral density of right and left femurs.

Table 1. Means and standard deviations for bone mineral density (g/cm²) of femoral diaphysis and femoral neck in guinea pigs: GI – orchietomized animals and GII – intact control animals.

Groups	Femoral diaphysis	Femoral neck
I	0.293±0.008	0.266±0.013
II	0.308±0.030	0.300±0.035
<i>p</i> values	<i>p</i> >0.05	<i>p</i> <0.05

Table 2. Means and standard deviations for mechanical properties using bending test (right femur) and axial compression test (left femur) in guinea pigs: GI – orchietomized animals and GII – intact control animals.

Groups	Bending test (RF)		Axial compression (LF)	
	Maximum Load (N)	Stiffness (N/mm)	Maximum Load (N)	Stiffness (N/mm)
I	118±21	148±19	151±22	269±46
II	152±43	183±21	177±43	225±31
<i>p</i> values	<i>p</i> >0.05	<i>p</i> <0.01	<i>p</i> >0.05	<i>p</i> >0.05

Table 3. Pearson's correlation coefficient between bone mineral density (g/cm²) of femoral neck and mechanical testing performed in left femur of guinea pigs.

Bone area	Maximum load (N)	Stiffness (N/mm)
Femoral neck density	0.85	0.26
<i>p</i> values	<i>p</i> <0.001	<i>p</i> >0.05

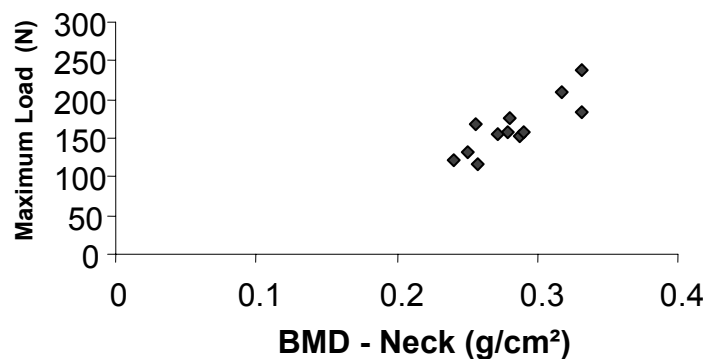


Figure 1 Pearson's correlation between maximum load and bone mineral density (BMD) of the left femur.

DISCUSSION

In human patients, bone mineral density is most frequently studied in bones with a higher incidence of fractures due to their brittleness. Spine, proximal third of the femur, distal third of the radius and proximal third of the humerus (2,10) are examples of these types of bones. Experimental studies with animals have evaluated long bones, such as femurs (3,7,21-23), tibiae (7,17,18), lumbar vertebrae (4,18,22), and mandibles (3,24). As the present study used *ex vivo* models, no difficulties were encountered in the analysis of proximal femurs by DXA. However, quadruped position, with exception of that in non-human primates, may be a limiting factor when analyzing proximal femurs of *in vivo* models, due to difficulties in keeping the femur parallel to the spine. DXA uses X-ray beams as energy source to measure body surface area and bone mass in g/cm³ (5,10). Interpretation must be based on comparison of results from the subject with others from the same gender, ethnic group and age, because different ethnic groups have different eating habits, physical exercise habits, sun exposition and are exposed to different levels of fluoride in water (2). As the present study used animals at similar ages, with the same pattern of eating and kept under the same environmental conditions, a reliable comparative standard between groups was possible to establish.

Measurements of bone mineral density were performed in femoral neck and diaphysis from orchietomized and intact control groups. Bone mineral density of the orchietomized group was significantly lower only at femoral neck. These results are in agreement with those of other authors who reported that orchietomized male rats or guinea pigs may present reduction in bone mineral density, showing its effects on skeleton in case of androgen deficiency (21,22,24). However, unaltered bone density at femoral diaphysis may be related to measurements in the cortical region. Sites of trabecular bone are more likely to be affected by hormonal deprivation (1). Therefore, the skeletal site to be analyzed plays an important role in lesion characterization (31).

Bone strength is determined by geometry, cortical density, porosity, trabecular bone morphology and intrinsic properties of bone tissues (25). True bone strength may be determined by mechanical testing (11, 31), which provides values for maximum load, yield point and stiffness (26). Maximum load and stiffness were the chosen variables for analysis, since they are structural properties defining limit of usage and structure stability, respectively (27,28). No differences were found in the maximum load values between orchietomized (GI=118 ± 21) and intact control groups (GII=152 ± 43) using the three-point bending test, in which the specimen is subject to bending until fracture (27,28). Using the same mechanical test, a study reported similar findings for tibia of sedentary female rats that had undergone

oophorectomy (27). According to the author the cortical bones are more stable and resistant to hormonal deprivation effects, which explain the lower incidence of fractures in diaphysis even in advanced osteoporosis.

In the axial compression test, no differences were found in the maximum load values between orchietomized (GI=151 ± 22) and intact control (GII=177 ± 43) groups. These findings are not in agreement with densitometric values, which presented a decrease in bone mineral density at femoral neck of the orchietomized group. Considering that maximum load (load-bearing capacity) may be directly associated with bone mineral density (29), a bone density loss of about 12% is probably not enough to promote any differences in the load-bearing capacity of bones. However, in a study on female rats was observed a decrease in the maximum load of femoral neck three months after oophorectomy (30). The three-point bending test revealed significant differences in relation to stiffness, indicating lower bone stiffness in the orchietomized group (GI=148 ± 19) when compared to the intact control group (GII=183 ± 21). In the axial compression test no differences between groups were observed. The results suggest that bone diaphysis of orchietomized animals becomes less elastic due to structural alterations, which is in agreement with a study that reported decreased rigidity of tibia from female rats, twelve months after oophorectomy (28).

When values of maximum load versus bone mineral density and values of stiffness versus bone mineral density of right and left femurs were correlated (Pearson's correlation), a positive correlation ($r = 0.85$; $p < 0.001$) between maximum load (N) and bone mineral density (g/cm^2) was observed only in left femurs. This finding indicates that maximum load-bearing capacity of femoral neck increases as bone mineral density increases.

CONCLUSION

The results suggest that hormonal deprivation in guinea pigs induces reduction of bone mineral density, especially in the femoral neck area, as well as reduction of bone rigidity in the femoral diaphysis.

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