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Effects of the hyperlipidic diet, containing saturated fat, on the bone quality of Wistar machos rats in the adult life

Efeitos da dieta hiperlipídica, contendo gordura saturada, sobre a qualidade óssea de ratos Wistar machos na vida adulta

Abstract

Objectives: To evaluate the influence of pork lard intake on the bone composition of male rats at 120 days of age. ***Methods:*** Male Wistar rats from from a laboratory of a federal University of Rio de Janeiro. The animals were kept in a room with temperature (25 to 27°C), light / dark cycle (12-12h), receiving ad libitum water during 8 weeks. The groups, divided into: control, which received casein (GC, n = 12) and experimental fed pig lard (GH, n = 12) rations, were followed up to 120 days. The femur was adequately reserved for future analysis. Statistical analysis was performed using the GraphPadPrism program (version 5.00, 2007, San Diego, USA). ***Results:*** The results were expressed as mean ± standard error of the mean (SEM), considering the level of significance of P<0.05. The GH (6.938 ± 0.030 mm) had a greater (p<0.05) diaphysis width than the GC (4.430 ± 0.040 mm). In the biomechanical test, the maximum strength (GC = 102,200 ± 10,210N and GH = 126,100 ± 4,341N) and the elastic modulus (GC = 471415 ± 44457MPa and GH = 570013 ± 24575MPa) were

higher in the hyperlipid group (p<0.05), respectively. The femoral bone composition was higher (p<0.05) GH BMD (0.15 ± 0.001g / cm²) than the CG (0.144 ± 0.002g / cm²). ***Discussion and Conclusions:*** The hyperlipid diet containing saturated fat influenced the bone structure, but did not reduced bone quality.

Keywords: High fat diet. Rats. Bone. Dietary fats. Adult.

Resumo

Objetivos: Avaliar a influência da ingestão de banha de porco sobre a composição óssea de ratos machos aos 120 dias de idade. ***Métodos:*** Foram utilizados ratos ***Wistar***, machos, provenientes de um laboratório de uma universidade federal do Rio de Janeiro. Os animais foram mantidos em Biotério com temperatura (25 a 27ºC), ciclo claro/escuro (12-12h), recebendo água ***ad libitum*** por um período de oito semanas. Os grupos, divididos em controle, que recebeu ração a base de caseína (GC, n=12) e experimental, que recebeu ração com acréscimo de banha de porco (GH, n=12), foram acompanhados até completarem 120 dias. Ao final do experimento, os animais foram anestesiados. O fêmur foi reservado adequadamente para futuras análises. A análise estatística foi realizada através do programa GraphPadPrism (versão 5.00, 2007, San Diego, USA). ***Resultados:*** Os resultados foram expressos como média ± erro padrão da média (EPM), considerando o nível de significância de P<0,05. O GH (6,938±0,030 mm) apresentou maior (p<0,05) largura da diáfise com relação ao GC (4,430±0,040 mm). No teste biomecânico, foi observada a força máxima dos grupos (GC=102,200 ± 10,210N e GH=126,100 ± 4,341N) e o módulo elástico (GC=471415 ± 44457MPa e GH=570013 ± 24575Mpa) sendo os maiores valores no grupo hiperlipídico (p<0,05). Na composição óssea femural, foi observado maior (p<0,05) DMO do GH (0,15 ± 0,001g/cm²) em relação ao GC (0,144 ± 0,002g/cm²). ***Discussão e Conclusões:*** A dieta hiperlipídica contendo gordura saturada influenciou na estrutura óssea, mas não reduziu a qualidade óssea.

Palavras-chave: Dieta Hiperlipídica. Ratos. Osso. Gordura da dieta. Adulto.

INTRODUCTION

Bone structure continuously changes through adaptive remodeling mechanisms due to mechanical forces and metabolic processes.[1] Multiple factors such as genetics, lifestyle and eating habits are associated with this condition, which results in increased bone fragility, weakness, increased incidence of fractures and reduced quality of life; besides, it has effects on costs with one's health and strongly affects the economy.[2]

Nutrition is a key factor for bone health promotion and maintenance. Diets rich in saturated fat lead to metabolic disorders such as high levels of anabolic hormones, which are strong bone development regulators that act in the osteoblast activity and stimulate bone formation.[3] However, according to other studies, saturated fat can affect the skeletal system and is associated with low bone quality. In addition, increased body mass caused by hyperlipidic diets increases the mechanical action in bone structures and leads to bone microdeformations.[4-6]

In light of inconsistencies between reports in the literature, the aim of the current study was to evaluate the effects of diets rich in saturated fat on the skeletal structure of adult Wistar rats.

MATERIALS E METHODS

The research project followed the Ethical Principles on Animal Experimentation set by the Brazilian College of Animal Experimentation (COBEA - Colégio Brasileiro de Experimentação Animal); it was approved by the Ethics Committee on Animal Research (CEUA - Comitê de Ética no Uso de Animais), under protocol N. 594/2016.

Male Wistar rats bred in colonies held by the Experimental Nutrition Laboratory of Fluminense Federal University were used in the current study. Animals were kept under controlled temperature (25 ºC to 27ºC) and humidity (60 ± 10%) conditions; they were subjected to 12:12h light-dark cycles (6:00 a.m. to 6:00 p.m.).

Control and experimental feed (Table 1) presented the same amount of mineral, vitamin, cellulose and sucrose mix. Diets were manufactured on a weekly basis and stored at 4°C, according to recommendations by the American Institute of Nutrition (AIN-93G). Both experimental groups had free access to water and feed for eight weeks.

Table 1. Nutritional composition of experimental and control feeds to be provided to rats for 120 days. Niterói-RJ, 2018.

Ingredient (g / 100 g)	Control	Experimental
Lard	-	17.0
Casein	20.0	20.0
Maize starch	52.9	35.9
Sucrose	10.0	10.0
Soybean oil	7.0	7.0
Cellulose	5.0	5.0
AIN-93G Mineral Mix	3.5	3.5
AIN-93 Vitamin Mix	1.0	1.0
L-Cystine	0.3	0.3
Choline Bitartrate	0.25	0.25
BHT, mg	14	14
kcal/100g	347.2	468.0

It will be formulated based on recommendations by the American Institute of Nutrition AIN-93G for rodent diets. Control group (CG) and experimental group (treated with feed containing lard (EG). Mineral and vitamin mix; L-cystine; Choline Bitartrate: PragSolutions®; Casein; Maize starch; Cellulose: FARMOS ®; Soybean oil: Lisa® and Sucrose: União®.

Twenty-four (24) male adult (8-weeks old) rats were kept in individual cages. Animals were randomly divided into two groups: the control group (CG), which fed on normal diet; and the experimental (hyperlipidic) group (HG), which fed on hyperlipidic diet. Water and feed were provided ad libitum for eight weeks.

Animals were subjected to 6-hour fasting at the age of 120 days. Next, they were anesthetized with intraperitoneal injection of Thiopentax® (Thiopental Sodium 1G, Cristália Produtos Químicos Farmacêuticos LTDA, Brazil) at 5% (0.15 ml/100g b.w., i.p.); dilution ratio was 50 mL of distilled water to 1 g of anesthetic.

The femurs of the anesthetized animals were collected and stored at -20°C for further analysis. An analytical scale (Bosch S2000, Brazil - 0.0001 precision) was used to weigh (g) the bone pieces, after they were cleaned. A digital caliper (Jomarca, 0.01 mm) was used to

measure femur length (from the distance between the epiphyses, mm) and the width of the diaphysis midpoint (mm).

The bone composition of the femurs was analyzed through Dual-energy X-ray absorptiometry (DXA) at the Nutritional and Functional Evaluation Laboratory (LANUFF) of Emília de Jesus Ferreiro Nutrition School, Fluminense Federal University (UFF - Universidade Federal Fluminense). Bone mineral density - BMD (g / cm²) and bone mineral content - BMC (g) were analyzed.

Biomechanical properties of the femurs were analyzed in the Analytical Laboratory of Restorative Biomaterials of UFF Dentistry School. The analysis was based on a three-point flexural test, which was conducted in the universal testing machine (UTM), whose force was perpendicularly applied to the longitudinal axis of the bone - in the medial portion of it -, at constant speed (0.5 cm/min) until bone breaking at 200 kgf load cell capacity. The tips of the bones were supported by two rolls (3 mm diameter and 21.70 mm radius). Maximum force (N), breaking force (N) and elastic modulus (MPa) were calculated in the EMIC software.

Statistical analysis was performed in the GraphPad Prism software (version 5.00, 2007, San Diego, USA). Results were analyzed through Student's t test and expressed as mean ± standard error of the mean (SEM); significance level was set at $p < 0.05$.

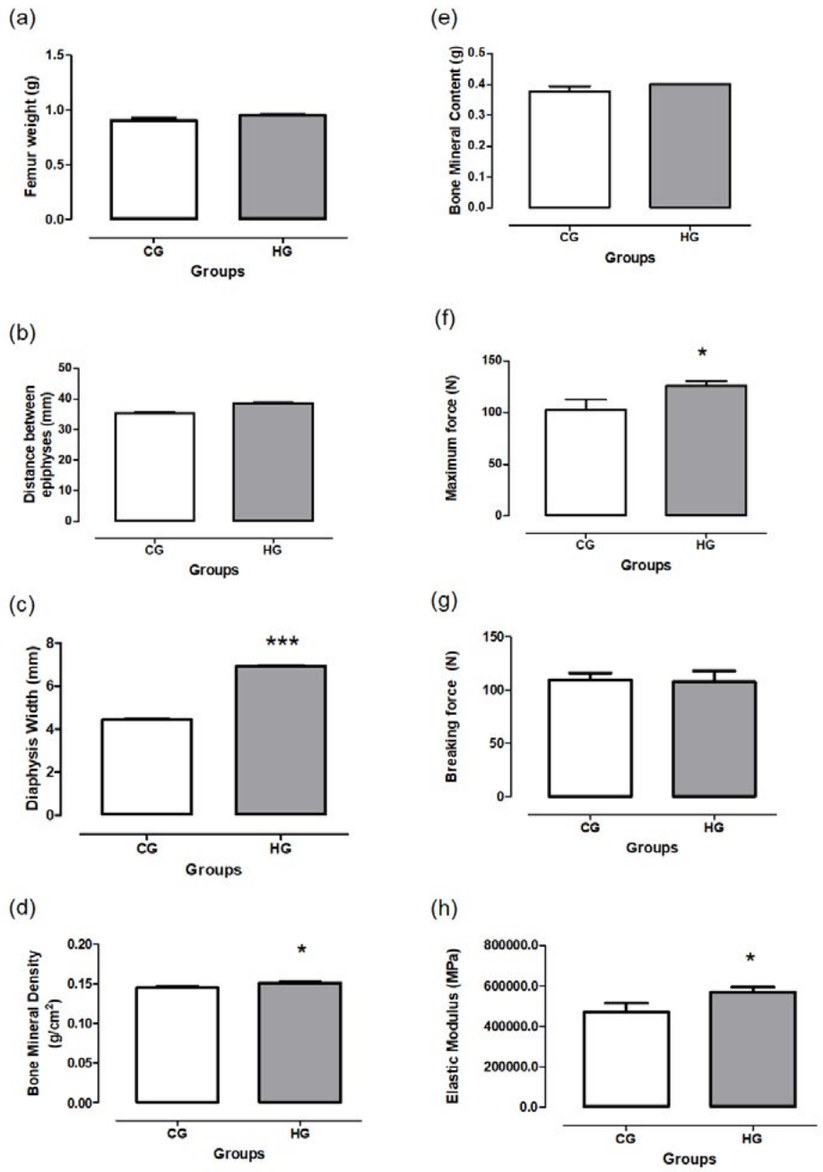
RESULTS

Femur weight in GC (0.902 ± 0.024g) and HG (0.949 ± 0.012g; Figure 1a), and the distance between epiphyses in CG (35.390 ± 0.117mm) and HG (38.66 ± 0.117mm; Figure 1b), did not present significant differences between groups. However, HG (6.938 ± 0.030 mm) presented wider diaphysis ($p < 0.05$; Figure 1c) than CG (4.430 ± 0.040 mm).

With respect to femoral bone composition, HG recorded higher ($p < 0.05$; Figure 1d) BMD (0.15 ± 0.001g/cm²) than CG (0.144 ± 0.002g/cm²). BMC did not show significant difference (Figure 1e) between CG (0.376 ± 0.016g) and HG (0.4 ± 0.0g).

Based on biomechanical test results, the maximum force recorded for CG (102.200 ± 10.210N) and HG (126.100 ± 4.341N) - Figure 1f - and the elastic modulus recorded for CG (471415 ± 44457Mpa) and HG (570013 ± 24575Mpa) -Figure 1h - were higher in the hyperlipidic group ($p < 0.05$) than in the control. Breaking force (CG = 109.200 ± 6.880N and HG = 107.600 ± 10.460N; Figure 1g) did not show significant difference between groups (Figure 1).

Figure 1. Bone parameters (Niterói-RJ, 2018)



DISCUSSION

The present study evaluated the effects of hyperlipidic diets with saturated lard-deriving fat on the bone structure of male Wistar rats. The femurs of HG animals showed increased diaphysis midpoint width, bone mineral density and increased resistance to bone fracture at the end of the experimental period.

These data contradict those recorded in other studies, such as the one conducted by Dong et al.,[7] who found that diets rich in saturated fat resulted in severe bone loss and compromised bone structures. Parhami et al.8 suggested that saturated fat negatively affects bones by inhibiting osteoblastic differentiation. According to Wohl et al.,[9] saturated fat has effects on skeletal health, since it affects bone composition and leads to pathologies such as osteoporosis.

Our data corroborated the study by Wang et al.,[10] who found that high intake of fat containing saturated fatty acids negatively affected total and femoral BMD during animal growth in rat studies, although it could not be associated with impaired bone quality.

CONCLUSION

The present study evidenced that hyperlipidic diets containing saturated fat have affected bone quality. Therefore, we concluded that lard intake by rats during the bone development stage has changed their bone structure, although it did not result in low-quality bone.

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Contributors

Nunes HMV participated in animal care, bone collection, analysis of bone parameters, manuscript writing, and in the last version and design of the study. Pereira AD participated in the statistical analysis of bone parameters, in the elaboration of graphs and in discussions about the collected data. Brazil SC, Armada L and Santos R participated in the conduction of the experimental design. Boaventura GT co-directed the study. da Costa CAS participated in data analysis and interpretation, as well as in the study design and orientation.

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