

Fructooligosaccharides (FOS) and hormonal replacement therapy (HRT) by estrogen suppressed bone resorption in the ovariectomized rat

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Abstract

The present investigation aims to compare the effects of fructooligosaccharides (FOS) and the hormonal replacement therapy (HRT) by estrogen on bone metabolism in ovariectomized rats. Three-month-old (276,0±3,4g body weight) Wistar female rats were submitted to an ovariectomy (OVT) procedure and after 30 days, the animals were randomly assigned (n=10) to treatment groups for 20 days. The animals were treated with FOS (RAFTILOSE® P95) (5g/100g of diet intake/day) through intragastric intubations and/or not with hormonal replacement therapy (HRT) by synthetic β -estradiol (SIGMA/E-4389) (30 μ g/kg of body weight) daily administrated by subcutaneous injection (G1 = OVT + FOS + HRT; G2 = OVT + FOS + saline; G3 = OVT + water + HRT; G4 = OVT + water + saline). The rats were fed with standard diet contend 1.3 % of Ca and received deionized water ad libitum. The data are expressed as the mean values \pm standard error means (SEM) and analyzed by two-way ANOVA Tukey's post-hoc test (p<0.05). Both treatments alone reduced total alkaline phosphatase activity. The FOS treatment increased the tibiae calcium, but the effect of FOS + HRT was more powerful. Radiodensity analysis supports this result too. In femur biomechanical properties, the proportional limit and resilience of FOS + HRT and FOS animals group suggest an increased effect on the bone capacity in absorbing energy. These results are also observed in the qualitative analysis of the SEM. Prebiotics substances consumption should be introduced for prevention or treatment of osteoporosis.

Keywords: Menopause – Ovariectomy – Hormonal Replacement Therapy – Prebiotics – Osteoporosis.

INTRODUCTION

The hormonal ovarian privation induces certain conditions that are implicated in women's health such as sexual dysfunctions, lipoprotein levels change, weight profit and highest cardiac and osteoporosis risk (VASCONCELLOS et al., 2004). Osteoporosis is a systemic skeletal disease characterized by low bone density and microarchitectural deterioration of bone tissue with

a consequent increase in bone fragility. It significantly raises morbidity and mortality, and affects more than 75 million people in Europe, Japan and the USA, causing more than 2,3 million fractures annually in Europe and the USA. The lifetime risk for hip, vertebral and forearm fractures has been estimated to be approximately 40%, similar to that for coronary heart disease. Prevention of such disease and its associated fractures is essential for maintaining health, quality of life, and

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independence amidst elderly people (PREVENTION, 2003).

Functional foods are perspective in the treatment of degenerative diseases, since they modulate biochemical and/or physiological functions in the organism, promoting health protection and retardation of pathological processes (SGARBIERI; PACHECO, 1999).

The functional foods ingredients suggested for the prevention or treatment of osteoporosis are the prebióticos because they stimulate the intestinal absorption of calcium, thus favoring its positive balance (SCHOLZ-AHRENS; SCHREZENMEIR, 2002; GRIFFIN; ABRAMS, 2005; SMEJKAL et al., 2003).

The present investigation aims to compare the effects of fructooligosaccharides (FOS) and the hormonal replacement therapy by estrogen (HRT) on bone calcium metabolism in ovariectomized rats.

MATERIAL AND METHODS

Experimental protocol

Three-month-old (276.0 ± 3.4 g body weight) Wistar female rats ($n=40$) were obtained from Biologic Investigation Multidisciplinary Center of the Campinas State University (Campinas, São Paulo, Brazil). Following 1 week acclimatization period, rats were anesthetized with sodium pentobarbital (40mg/kg body weight) and submitted to an ovariectomy (OVT) procedure. After 30 days of the ovariectomy procedure, the animals were randomly assigned ($n=10$) to treatment groups for 20 days. The animals were treated with FOS (RAFTILOSEÒ P95 – ORAFTI, Tienen, Belgium) (5g/100g of diet intake/day) (OHTA et al., 1998a, b) through intragastric intubations and the solvent given was water, associated or not with hormonal replacement therapy (HRT) by synthetic *b*-estradiol (SIGMA/E-4389) (30mg/Kg of body weight) daily administrated by subcutaneous injection (AZEVEDO et al., 2001; LIU et al., 2004) (G1 = OVT + FOS + HRT; G2 = OVT + FOS + saline; G3 = OVT + water + HRT; G4 = OVT + water + saline). The rats were housed in individual cages at $24^\circ\text{C} \pm 1^\circ\text{C}$ on a 12-hour light/dark cycle, fed with standard pellet diet (LABINA-PURINA / Agribands of Brasil LTDA)

contend 1,3% of Ca and received deionized water ad libitum. The animals were killed through previous anesthesia with sodium pentobarbital (40mg/Kg body weight) (REDDY; LAKSHMANA, 2003) and cardiac puncture exsanguinations. The protocol for animal care procedures received ethics approval from the Campinas State University Animal Experimental Ethical Committee.

Serum calcium

The serum calcium was determined by colorimetric method of end point of the LABORLAB (São Paulo – Brazil).

Total alkaline phosphatase activity in the serum

The total serum alkaline phosphatase activity was determined by kinetic colorimetric method of the BIODIAGNOSTICA (São Paulo – Brazil).

Bone calcium content

The right femurs were kept in formaldehyde solution 100mL/L for 48 hours (CARVALHO; CLIQUET JR, 2003), calcined in muffle (550°C for 6 hours) and the calcium content determined as described for Instituto Adolfo Lutz (1976).

Radiodensity

The left tibiae radiography was done in RX GE 1000 with digital system Gendex Denuptix and analyzed with EMAGO-®/Advanced 3.43 (EM) software (Oral Diagnostic Systems, ACTA, Netherlands) (HAITER-NETO; WENZEL, 2005). The images were exported in TIF (tagged image file) format.

Biomechanics properties

The breaking force and breaking energy of the femoral diaphysis were determined by using a three-point bending rheolometer (hydraulic serv equipment; 810 TestStar II MTS model – USA) according to the method described by Carvalho (2001). The measurement conditions were the following: sample space, 18mm; plunger speed, 2 mm/min.; and load range, 100kN.

Scanning electron microscopy (SEM)

The right tibiae was prepared by a procedure similar as that described elsewhere by Carvalho and

Cliquet (2003), and Reddy and Lakshmana (2003). Then it was examined on a Scanning Microscope JSM-5800LV/Jeol Serving Advanced Technology.

Statistical analysis

The data are expressed as the mean values \pm standard error means (SEM). The effects of FOS and HRT were analyzed by two-way ANOVA and the specific differences were determined by the Tukey's post-hoc test (STATISTICA 6.0 for windows - (STATSOFT, 2000) ($p < 0.05$).

RESULTS AND DISCUSSION

The serum calcium concentration was kept constant, despite of the different treatments (0.019 ± 0.001 mol/L). Takada et al. (1997) described that only in critically situations as undernutrition or hyperparathyroidism that calcium serum concentration it suffers alterations being above or below the normal one (1.1-1.3 mmol/L).

The total alkaline phosphatase activity of G4 animals groups was higher ($G4 = 25.4 \pm 1.0$ mol/L) if compared to the other groups ($G1 = 13.9 \pm 0.6$; $G2 = 13.1 \pm 0.8$; $G3 = 14.0 \pm 0.9$ mol/L). This result indicates an increase in the process of bone loss.

These results support those of Vieira ([2003]) and Yoshikubo et al. (2005), who also describe an increase of total alkaline phosphatase in conditions where the bone metabolism increases. It suggests that the managed treatments of isolated or associated form were efficient in the reduction of the bone loss caused by the ovariectomy, indicating normalization in the process of bone loss.

The results of bone calcium content showed that the treatment with FOS isolated ($G2 = 354.9 \pm 17.6$ g Calkg bone) or associated with HRT ($G1 = 380.4 \pm 26.6$ g Calkg bone) was efficient in the process of bone calcium incorporation (Figure 1).

However, the bone calcium of G2 animals group ($G2 = 354.9 \pm 17.6$ g Calkg bone) did not display difference to the G3 animals group ($G3 = 281.4 \pm 6.9$ g Calkg bone), suggesting that the isolated treatments (G2 and G3) are efficient in the process of bone calcium maintenance, but the

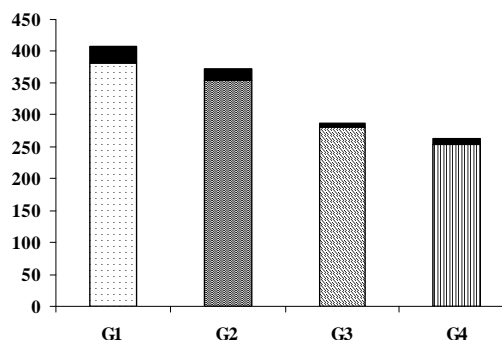


Figure 1 - Bone calcium content.

Notes: G1=OVT+FOS+HRT; G2=OVT+FOS+saline solution; G3 =OVT+water+HRT; G4=OVT+water+saline solution. The different letters indicate statistical difference ($p < 0.05$) between the groups.

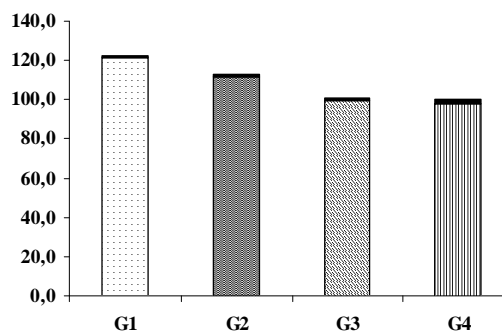


Figure 2 - Mean grey shade and standard error mean for the radiography of left tibiae epiphysis.

Notes: G1=OVT+FOS+HRT; G2=OVT+FOS+saline solution; G3 =OVT+water+HRT; G4=OVT+water+saline solution. The different letters indicate statistical difference ($p < 0.05$) between the groups.

association of FOS and HRT (G1) is more efficient in the suppressed bone. This result is supported by the radiodensity analysis (Figure 2) and qualitative analysis for SEM (Figure 3) too. The G4 animals group (255.1 ± 6.9 g Calkg bone) did not present differences as compared to the G3 animals group, emphasizing that the HRT alone was not efficient in the bone calcium deposition.

Zafar et al. (2004) also concluded that ovariectomized rats feeding with non-digestible oligosaccharides (NDO) (5.5g/100g of diet) for 21 days had a positive effect on calcium absorption and retention in bone. Ohta et al. (1998b) already showed that the dietary FOS enhanced calcium absorption and prevented the changes indicative of postgastroctomy osteopenia, such as decreases in bone calcium content and bone mineral density in

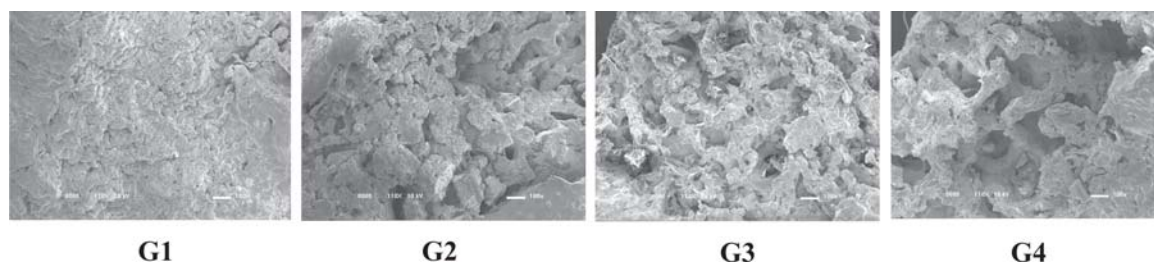


Figure 3 - The right tibiae scanning electron microscopy (SEM).

Note: G1=OVT+FOS+HRT; G2=OVT+FOS+saline solution; G3=OVT+water+HRT; G4=OVT+water+saline solution.

gastrectomized rats. These results show that an improvement in calcium absorption caused by prebiotics substances can prevent or treat post ovariectomy and postgastrectomy osteopenial osteoporosis.

As showed in Figure 2, our results of the radiodensity support what we had previously described for the result found in the bone calcium content. The ovariectomized rats did not receive any kind of treatment (G4) and the isolated HRT treatment (G3) did not show difference. However, the FOS isolated administration increased the calcium in the epiphysis of the tibiae. With the FOS plus HRT (G1) administration this effect is more pronounced.

The biomechanical properties of bone results of the animals to the proportional limit (the end point of the elastic region) and resilience (capacity of the bone in absorbing energy of elastic form, then allowing to return it to its previous form, with the withdrawal from the load) suggest that the treatment with hormone associated with FOS (G1 = 126.7 ± 6.1 N; $28.6 \pm 3.7 \times 10^{-3}$ J respectively) or not (G3 = 137.3 ± 6.8 N; $34.2 \pm 3.8 \times 10^{-3}$ J) has an increased effect on the bone capacity in absorbing energy. G3 animals group, though, was

the highest. The rupture limit of bone (the biggest value of load necessary to reach the final resistance of the bone) within the G3 animals group was higher (G3 = 168.1 ± 5.3 N) than in the other groups (Table 1).

The biomechanical properties of bone results can be explained for the fact that the analyses were carried out in diaphysis of a bone such as femur, which is the most compact portion of the bone, representing only 3% of its remodelling in adult individuals (RIGGS; MELTON, 1995; BROUNS; VERMEER, 2000).

The analysis of rats proximal epiphysis of the right tibiae through scanning electron microscopy showed the harmful effect of the estrogen privation in the bone structure. The ovariectomized rat (Figure 3,d) presented several and deep resorption lacunae inducing to a fragilized bone. The intragastric intubation of FOS (G2) (Figure 3,b) or the HRT one (G3) (Figure 3,c) separately restored the partial form of the bone structure, but not with a complete reorganization, as observed in the G1 animals group (Figure 3, a). The groups treated with FOS and HRT presented a bone with continuous surface and without resorption lacunae.

Table 1 - Biomechanic properties (1).

Experimental Groups	Proportional limit (N)	Resilience ($\times 10^{-3}$ J)	Rigidity ($\times 10^3$ N/m)	Rupture of load (N)
G1	$126,69 \pm 6,15^{ab}$	$28,59 \pm 3,66^{ac}$	$306,98 \pm 21,96^a$	$156,33 \pm 2,92^a$
G2	$113,46 \pm 3,75^c$	$25,38 \pm 3,78^{ab}$	$294,63 \pm 19,61^a$	$149,41 \pm 5,86^a$
G3	$137,27 \pm 6,80^b$	$34,20 \pm 3,76^c$	$289,81 \pm 11,53^a$	$168,10 \pm 5,30^b$
G4	$110,73 \pm 4,38^c$	$17,95 \pm 1,59^b$	$291,03 \pm 11,26^a$	$147,96 \pm 5,60^a$

(1) Mean values \pm SEM ($p < 0,05$). Notes:- G1=OVT+FOS+HRT; G2=OVT+FOS+saline solution; G3 =OVT+water+HRT; G4=OVT+water+saline solution. The different letters indicate statistical difference ($p < 0,05$) between the groups.

The ovariectomized rat (Figure 3, d) presented several and deep resorption lacunae, as reported by Reddy and Lakshmana (2003) too. However, the intragastric intubations with FOS (Figure 3, b) separately restored the partial form of the bone structure, as described by Reddy and

Lakshmana (2003) also in ovariectomized rats treated also by OST-6 herbal preparation.

Our findings show that the FOS consumption should be introduced as an alternative for prevention and/or treatment of osteopenia or osteoporosis.

Fructooligosacarídeos (FOS) e terapia de reposição hormonal (TRH) com estrogênio impedem a reabsorção óssea em ratas ovariectomizadas

Resumo

O objetivo deste trabalho foi comparar os efeitos dos fructooligosaccharídeos (FOS) e da terapia de reposição hormonal (TRH) com estrogênio no metabolismo ósseo de ratas ovariectomizadas. Ratas Wistar com 3 meses de idade (276.0 ± 3.4 g de peso corpóreo) foram submetidas a ovariectomia e, após 30 dias, foram randomizados (n=10) em 4 grupos. Os animais foram tratados com FOS (RAFTILOSE® P95) (5g/100g de dieta consumida/dia) através de intubação intragástrica e (ou) não submetidos à TRH com β -estradiol sintético (SIGMA/E-4389) (30 μ g/kg of body weight), administrado diariamente por injeção subcutânea (G1 = OVT + FOS + HRT; G2 = OVT + FOS + saline; G3 = OVT + water + HRT; G4 = OVT + water + saline) por 20 dias. Os ratos foram alimentados com dieta padrão que continha 1.3% de cálcio e receberam água deionizada ambos *ad libitum*. Os resultados foram expressos em média \pm erro padrão da média (EPM) e foram submetidos à análise de variância ANOVA e Tukey ($p < 0.05$). O FOS e a TRH reduziram a atividade da fosfatase alcalina total. O tratamento com FOS aumentou a concentração de cálcio na tíbia e sua radiodensidade. Em relação ao limite proporcional bem como à resiliência do fêmur dos animais, os resultados sugerem que os tratamentos com FOS e FOS + TRH aumentaram a capacidade de esse osso absorver energia. Os resultados quantitativos são confirmados pela análise óssea qualitativa (microscopia eletrônica de varredura). Com os resultados obtidos, os FOS podem ser introduzido nos protocolos de prevenção e (ou) tratamento da osteoporose.

Palavras-chave: Menopausa – Ovariectomia – Terapia de reposição hormonal – Prebióticos – Osteoporose.

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