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Assessments of body composition and bone parameters of lactating rats treated with diet containing flaxseed meal *(Linum usitatissinum)* during post-weaning period

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Abstract

Introduction: **There are few studies on body composition and the effects of diet on weight postpartum women. The aim was to evaluate the body composition and bone parameters in lactating rats treated with diet containing flaxseed flour during postweaning period.**

Methods: **After weaning, the lactating rat were divided in control (n = 6) and experimental (F, n = 6) group, treated with 25% flaxseed flour diet. After 30 days, body composition by dual-energy X-ray absorptiometry, serum analysis, organs and intra-abdominal fat mass, femur and lumbar vertebra parameters were determined.**

Results: **The groups showed similar food intake, body mass and bone parameters. While F group showed the following: lower body (-5%), gonadal (-17%), mesenteric (-23%) and intra-abdominal (-6%) fat mass. Increase of HDL-cholesterol (+10%) and lower glucose (-15%), triglycerides (*P* < 0.05, -37%) and cholesterol (*P* < 0.05, -21%).**

Conclusions: **The findings highlight the effects of flaxseed for control of adiposity and to maintain a healthy biochemical profile during the postnatal period.**

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Key words: *Flaxseed flour. Rats. Postweaning period. Adiposity. Bone.*

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EVALUACIONES DE COMPOSICIÓN CORPORAL Y PARÁMETROS ÓSEOS EN RATAS LACTANTES TRATADAS CON DIETAS A BASE DE LINAZA *(LINUM USITATISSINUM)* DURANTE EL PERIODO DE DESTETE

Resumen

Introducción: **Hay pocos estudios sobre la composición corporal y los efectos de la dieta en mujeres en el periodo postparto. El objetivo consistió en evaluar la composición corporal y los parámetros óseos en ratas lactantes tratadas con dietas a base de linaza durante el periodo de destete.**

Métodos: **Después del destete, las ratas lactantes fueron divididas en un grupo de control (n = 6) y un grupo experimental (F, n = 6), tratadas con una dieta a base de harina de lino al 25%. Al cabo de 30 días, se midieron los parámetros corporales mediante absorciometría de rayos X de doble energía, se realizó un análisis sérico, y se evaluó órganos y masa grasa intra-abdominal así como los parámetros en fémur y vértebras lumbares.**

Resultados: **El grupo mostró una ingesta alimenticia similar, así como parámetros óseos y de masa corporal. Mientras que el grupo F mostró los porcentajes siguientes en masa grasa: parte inferior del cuerpo (-5%), gonadal (-17%), mesentérica (-23%) e intra-abdominal (-6%). Aumento de HDL-colesterol (+10%) y disminución de glucosa (-15%), triglicéridos (P < 0,05, -37%) y colesterol (P < 0,05, -21%).**

Conclusiones: **Los resultados destacan los efectos del lino para el control de la adiposidad y para mantener un perfil bioquímico sano durante el periodo postnatal.**

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Palabras clave: *Harina de lino. Ratas. Periodo pos-destete. Adiposidad. Huesos.*

Introduction

According to the World Health Organization (WHO), there are periods of life considered vulnerable to the development of future obesity, as women in the reproductive period, with successive pregnancies.[1] The period of pregnancy and lactation are phases that produce a redirection of nutrients to the maternal tissues. The relationship between pregnancy and changes in adipose tissue was analyzed in a longitudinal study in which the results indicated that there is an association between pregnancy and increased adiposity in women.[2,3] Reports and studies of women with severe obesity have pointed to pregnancy as a cause of their excess weight.[4]

It is too often mothers face challenges related to changes common to postpartum period, such as weight gain, decreasing levels of physical activity and increased food consumption.[5-7] Furthermore, during pregnancy occur changes in bone physiology to compensate for the increasing needs of minerals and developing fetuses. During lactation, there is an increase in the rate of bone resorption, and loss of calcium from the skeleton feeding. Although the rate of bone formation is increased during this time, is exceeded by the rate of bone resorption, resulting in a rapid decrease in bone mass.[8] However, there are few studies on body composition and the effects of diet on weight postpartum women.

Preventive and therapeutic strategies, including nutritional interventions are possible ways to avoid the appearance of changes in metabolism.[2,9] In this context, flaxseed (*Linum usitatissimum*) has distinguished between the effects foods with disease prevention, in addition to being an adjunct in combating obesity and overweight.[10,11]

Flaxseed (Linum usitatissimum) is made up of 41% lipids (50-55% as α-linolenic acid, and 15-18% as linoleic acid), 28% fibers, 21% protein, 4% minerals and 6% carbohy-drates distributed among phenolic acids, sugars, lignan and hemicelluloses.[12] Previous studies showed protective effect of flaxseed intake in experimental models.[13-16] Nevertheless, there is a lack of data on the lactating rats. Thus, the aim of this study was to evaluate the body composition and bone parameters in lactating rats treated with diet containing flaxseed flour during postweaning period.

Materials and methods

The protocol used to deal with experimental animals was approved by Ethics Committee on Animal Research of Fluminense Federal University, Niteroi-RJ, Brazil. All procedures are in accordance with the provisions of Brazilian Society of Science and Laboratory's Animals.

Wistar rats were kept in a room with controlled temperature (23 ± 1° C) and with an artificial dark-light cycle (lights on from 07·00 to 19·00 hours). Virgin female rats (3 months old) were caged with male rats and after mating each female was placed in an individual cage with free access to water and food. Within 24h of birth excess pups were removed, so that only six pups were kept per dam. This procedure maximizes lactation performance.[17] During 21 days of lactation, rat dams were continued on an *ad libitum* diet of standard laboratory food (Nuvilab®, Paraná, Brazil).

After the 21 days of lactation, at weaning, the lactating rat were randomized divided in control (C, n = 6) and experimental (F, n = 6) group. Both groups were treated with semi-purified diet based on American Institute of Nutrition (AIN-93M) recommendations.[18] The control diet containing 14 g of casein, 4 ml of soybean oil and 5 g of fiber/100 g. While the experimental diets containing 8 g of casein and 25 g of flaxseed flour. The groups received the same amounts of vitamins and minerals per gram of diet (table I). The flaxseeds were crushed to obtain the flour that was weighed and used immediately for the diet preparation. The experimental diet had a concentration of 25% of flaxseed that aimed to meet the entire recommended fiber intake and it was not necessary to add oil because this seed is source of this component. Food intake (g) and body mass (g) were evaluated every 3 days. The female rats had free access to diet and water.

Table I
Composition of experimental diets

Ingredient (g/100 g)	*C*	*F*
Casein	14.0	8.0
Flaxseed flour	–	25.0
Cornstarch	62.07	52.07
Sucrose	10.0	10.0
Soybean oil	4.0	–
Fiber	5.0	–
AIN-93M Mineral Mix	3.5	3.5
AIN-93 Vitamin Mix	1.0	1.0
L-Cystine	0.18	0.18
Choline Bitartrate	0.25	0.25

C: Control group; F: Experimental group fed with diet containing 25 g/100 g of Flaxseed flour.
Casein, Mineral and Vitamin Mix, L-Cystine and Choline Bitartrate: Pragsoluções®; Cornstarch and Fiber: FARMOS®; Soybean: Liza®; Commercial Sucrose: União®; Flaxseed: Armazen®.
Formulated to meet the American Institute of Nutrition AIN-93M recommendation for rodent diets.[18]

At the end of the nutritional period, 30 days post-weaning, after 8 h of fasting, rats were anesthetized with Thiopentax® (*Tiopental*, 0.1 mg/100 g) and subjected to dual-energy X-ray absorptiometry (DXA)[19,20] using a Lunar IDXA 200368 GE instrument (Lunar, Wisconsin, USA) with specific software (encore 2008. Version 12.20 GE Healthcare). Total lean (g), fat mass (g), and bone analysis (bone mineral density- BMD (g/cm^2); bone mineral content- BMC (g); and bone area (cm²)) were measured for each rat. The DXA technician did not know about the experimental protocol.

After DXA, the length (cm, measured as the distance from tip of the nose to the tip of the tail) was evaluated. And blood was collected by cardiac puncture. Blood samples were centrifuged and serum was stored at -80º C for posterior analyze of glucose, triglycerides, cholesterol, HDL-cholesterol, calcium, phosphorus, magnesium (mg/dL, respectively) and albumin (g/dL) by colorimetric method (Bioclin BS-120, Belo Horizonte, MG, Brazil). Liver, heart, kidney, pancreas and intra-abdominal fat mass (retroperitoneal, mesenteric and gonadal) were excised and weighted (g).

Right femur and lumbar vertebra (LV4) were collected and cleaned of soft tissue and preserved at -80° C for posterior analyze. Bone dimension: the distance between epiphysis and the medial point width of the diaphysis were measured using calipers with a readability of 0·01mm. After drying overnight, femur and LV4 were weighed. Before, bone mineral density (BMD), in each femur and LV4, was determined by DXA.[21] After DXA analyses the bones were carried out in order to make the mineral composition where the bone samples were dried at 105° until reaching stable weight, then it were submitted to higher temperatures to 550º in Muffle Quimis Microprocessor - Q318M until they become ashes and liquefied them in thermostat at 80º with nitric acid to 70% in order to analysis minerals of calcium, phosphorus and magnesium by colorimetric method (Bioclin, Belo Horizonte, MG, Brazil).

Statistical analyses were carried out using the Graph Pad Prism statistical package version 5.00, 2007 (San Diego, CA, USA). Food intake and body mass were analyzed by two-way ANOVA, followed by Bonferroni post-test. The other data were analyzed by Student's *t* test. All results are expressed as means ± SEM with significance level of 0.05.

Results

During the nutritional period, food intake and body mass no differs between groups. In regard to body length, control (41.05 ± 0.63 cm) and experimental (42.42 ± 1.37 cm) groups showed no significantly differences in the end of experimental period (fig. 1).

In regard to body composition, total lean, body BMD, BMC and bone area the groups showed similar results. Meantime, the experimental group showed lower fat mass (-5%) to control (table II).

When evaluated the organs mass at 30 days post-weaning, liver, heart, kidney and pancreas mass were similar between control and experimental groups. The adiposity showed lower gonadal (-17%) and mesenteric (-23%) and intra-abdominal (-6%) fat mass in the experimental group (table III).

Serum analyzes showed no differences to calcium, phosphorus, magnesium and albumin. However, the experimental rats showed increase of HDL-cholesterol (+10%) and smaller glucose (-15%), triglycerides ($P < 0.05$, -37%) and cholesterol ($P < 0.05$, -21%) compared to control group (table IV).

Femur and lumbar vertebra (LV4) analyzes showed no differences to mass, bone dimensions and bone mineral density. When evaluated the bone mineral composition in femur and lumbar vertebra, the groups

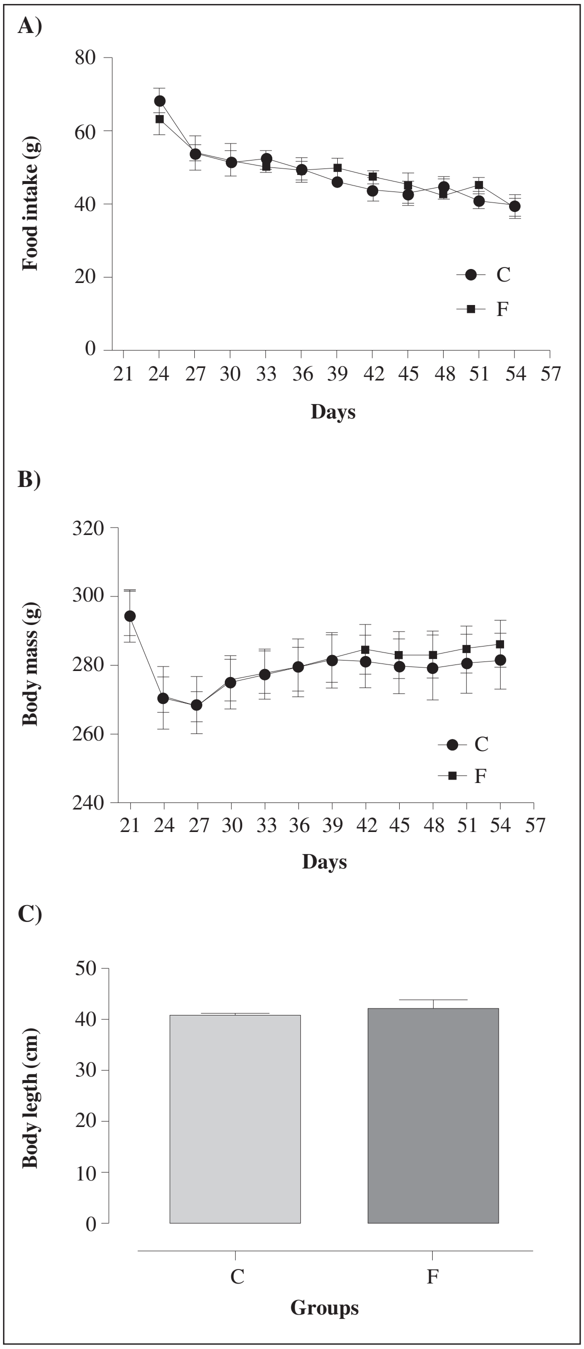

Fig. 1.—Food intake (A) and body mass (B) of lactating rats during 30 days post-weaning. Body length (C) in the end of experimental period. Control group, fed with control diet (◦, C, n = 6) and experimental diet, containing 25 g/100 g of flaxseed flour (•, F, n = 6). Values are means (A and B, twoway ANOVA. C, Student's t test).

Table II
Body compartments analyzed by DEXA, at 30 days post-weaning

	C (n6)		*F (n6)*	
	Mean	*SEM*	*Mean*	*SEM*
Total lean (g)	176.20	2.86	182.30	4.63
Fat mass (g)	79.50	7.02	75.17	6.60
Body BMD (g/cm^2)	0.15	0.01	0.15	0.01
Body BMC (g)	8.38	0.32	8.15	0.25
Body bone area (cm^2)	52.83	1.07	53.00	0.96

C: Group fed with control diet; F: Group fed with experimental diet, containing 25 g/100 g of flaxseed flour. Values are means (Student's t test).

Table III
Organ and intra-abdominal fat mass (g) at 30 days post-weaning

	C (n6)		*F (n6)*	
	Mean	*SEM*	*Mean*	*SEM*
Liver	8.31	0.66	7.93	0.53
Heart	1.40	0.35	1.47	0.43
Kidney	1.32	0.37	1.22	0.36
Pancreas	2.37	0.50	2.08	0.57
Retroperitoneal fat	4.21	0.56	5.51	1.19
Gonadal fat	7.83	0.98	6.46	0.80
Mesenteric fat	4.24	0.46	3.23	0.28
Intra-abdominal fat	16.29	1.88	15.21	2.01

C: Group fed with control diet; F: Group fed with experimental diet, containing 25 g/100 g of flaxseed flour. Values are means (Student's t test).

showed no differences to calcium, phosphorus and magnesium concentrations (table V).

Discussion

To our knowledge, this study to evaluate the effects of diet containing flaxseed flour on the maternal physiology after lactation in rats. Our results showed that, associated with a significant reduction in the serum cholesterol and triglycerides, the flaxseed flour intake contributes to control of body and intra-abdominal adiposity.

The nutritive demands of lactation are considerably greater than those of pregnancy. The nutrient intake of lactating women affects the nutrient content of breast-milk and maternal health. Thus, nutritional requirements for lactating women are higher compared to women who do not breastfeed.[2,22] However, there is little information that evaluates the nutrient intake and body composition of women after lactation period. In the present study, both groups received diet based on AIN-93M, to be used during adult maintenance.[18] And

Table IV
Serum analyzes at 30 days post-weaning

	C (n6)		*F (n6)*	
	Mean	*SEM*	*Mean*	*SEM*
Glucose (mg/dL)	166.20	16.82	140.70	12.34
Triglycerides (mg/dL)	75.80	11.67	47.50*	1.50
Cholesterol (mg/dL)	55.67	2.60	43.67*	2.01
HDL-Cholesterol (mg/dL)	19.83	0.94	21.83	1.66
Calcium (mg/dL)	11.30	0.36	11.38	1.50
Phosphorus (mg/dL)	6.70	0.43	6.86	0.40
Magnesium (mg/dL)	2.68	0.09	2.71	0.11
Albumin (g/dL)	3.58	0.04	3.40	0.08

C: Group fed with control diet; F: Group fed with experimental diet, containing 25 g/100 g of flaxseed flour. Values are means.
*Significantly different from the control group (Student's t test, $P < 0.05$).

Table V
Femur and LV4 mass, dimensions, bone mineral density (BMD) and bone mineral composition at 30 days post-weaning

	C (n6)		*F (n6)*	
	Mean	*SEM*	*Mean*	*SEM*
Femur:				
Mass (mg)	737.30	32.59	681.10	35.61
Distance between epiphysis (mm)	141.80	20.23	140.60	16.07
Width of the diaphysis (mm)	162.00	3.46	163.70	3.75
BMD (g/cm^2)	0.16	0.004	0.15	0.003
Calcium (mg/dL)	29.28	0.27	28.98	0.80
Phosphorus (mg/dL)	6.67	0.31	6.55	0.18
Magnesium (mg/dL)	15.29	0.96	13.75	0.96
LV4:				
Mass (mg)	219.30	11.25	206.30	16.88
BMD (g/cm^2)	0.14	0.005	0.13	0.004
Calcium (mg/dL)	18.43	0.50	18.15	0.67
Phosphorus (mg/dL)	4.79	0.45	6.01	0.56
Magnesium (mg/dL)	3.82	0.80	3.14	0.93

C: Group fed with control diet; F: Group fed with experimental diet, containing 25 g/100 g of flaxseed flour. Values are means (Student's t test).

to our surprise, regardless of flaxseed meal in the experimental diet, the groups showed similar food intake and body mass development. Previous studies related that the presences of fibers from flaxseed, as their bioactive compounds, are able to promote the satiety sensation, helping to reduce body mass.[14,23] Nevertheless, seems that the diet containing flaxseed did not affect satiety and the balance of the body mass, in the post-lactation period.

Excessive weight gain during pregnancy and retention of weight in the post-partum period are risk factors for obesity in later life.[24,25] Strategies to prevent post-partum obesity include behaviors associated with improved diet and weight control.[26] In present study, the fat tissue analyzes, highlighted a lower percentage of body, gonadal, mesenteric and intra-abdominal fat mass in the female rats treated with experimental diet. Flaxseed contain relevant concentration of lipids, and previous studies support the concept that fats do not affects body fat compartments equally.[27-29] Thereby, the flaxseed flour intake may be associated with a decrease of adipose mass in body and intra-abdominal compartments.

In regard to bone structure, during lactation there is an increase in the rate of maternal bone resorption and in calcium losses from the maternal skeleton. However, when offspring are weaned and milk production is halted, the bone mineral density (BMC) recovers by ~6 months after lactation. In the experimental models, BMC returns to the prepregnancy baseline value after 2-3 weeks in mice,[30] 2-6 weeks in rats.[31,32] Nevertheless, the multi-parity and lactation were risk factors for osteoporosis.[8,32,33] Several studies have documented the link between the intake of dairy foods and osteoporosis and indicate that polyunsaturated fatty acids may influence bone health.[21,34-36] The flaxseed contains high concentration of α-linolenic acid and some studies have reported a bone protective effect of this fatty acid; whereas, others have reported no effect.[37-40] The body composition, femur and lumbar vertebra (LV4) analysis demonstrated that female rats of the experimental group followed during ~4 weeks (30 days) after lactation, probably showed the bone parameters recovery regardless of the flaxseed flour.

Among the women, the cardiovascular diseases are responsible for almost half of deaths. Pregnancy brings a physiological stress that can uncover an underlying propensity for chronic diseases, addition to osteoporosis. Thus, the access to postnatal care constitutes a good opportunity for disease prevention.[41,42] In this context, the diet containing flaxseed flour contributed to the reduction of cholesterol and triglycerides. Although no significant differences have been observed, the experimental group showed higher HDL-cholesterol and lower glucose serum concentration. The findings corroborate previous reports[12,23] that whole flaxseed contributes to the health lipid profile in hyperlipidemic subjects and in rats with normal biochemical parameters, reducing risks for chronic diseases.

In summary, despite of preliminary analysis, this study described the changes in body composition, bone structure and growth of maternal rats after weaning. Furthermore the findings highlight the contribution of flaxseed flour intake for control of adiposity and to maintain a healthy biochemical profile during the postnatal period.

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