

Maternal consumption of flaxseed during lactation affects weight and hemoglobin level of offspring in rats

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Abstract

Objective: To investigate the effects of maternal flaxseed consumption during lactation on the body weight, hematological indicators and visceral fat mass of male offspring in adulthood.

Methods: Sixteen female Wistar rats were divided into two groups after giving birth. During lactation the control group (CG) was fed a casein-based diet and the flaxseed group (FG) was fed a casein-based diet containing 25% flaxseed. After weaning, male offspring were fed on commercial chow until adulthood and euthanized at 170 days for blood collection and visceral fat mass assessment.

Results: Offspring of rats in the FG had lower body weight (FG = 42.69±3.06 g; CG = 47.31±4.72 g; $p = 0.036$) at weaning. At 170 days, lower hemoglobin levels were observed in the FG (FG = 12.30±1.28 g/dL; CG = 13.88±0.91 g/dL; $p = 0.02$). There was no statistically significant difference in visceral fat mass between groups.

Conclusions: Maternal consumption of a flaxseed-based diet during lactation resulted in lower body weight at weaning and lower hemoglobin levels in adulthood, when compared with the control group.

J Pediatr (Rio J). 2010;86(2):126-130: Flaxseed, rats, programming, lactation, hemoglobin.

Introduction

Disease prevention should take place throughout life, starting with the mother's diet during gestation, through lactation and into adulthood.¹ Silveira et al. report that epidemiological studies conducted in different places around the world have found relationships between certain environmental factors at the start of life and variations in individuals' genetic load expression.² This phenomenon is known as "programming" and is a process through which a given factor acting at the start of life, during a critical or sensitive period, can have effects on adult health.³ Many different studies have indicated that prenatal and initial postnatal nutrition have an influence on the predisposition

to chronic diseases associated with diet, including obesity, hypertension and cardiovascular diseases in adulthood.⁴ The results of these investigations demonstrate the importance of preventing chronic diseases and of health promotion during the different phases of life.²

The search for functional foods, such as flaxseed, has been increasing because of their beneficial effects and their role in the prevention of diseases,⁵ in addition to their ability to aid in reducing weight and body mass index (BMI).⁶ Flaxseed is taken by people of all ages and both sexes, by pregnant women, nursing mothers and women going through the menopause.⁷ This oleaginous seed is rich

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in dietary fiber and protein and contains 41% lipids, 50-55% of which is made up of the polyunsaturated fatty acid alpha-linolenic acid (n-3 or ALA), with 15-18% alpha-linoleic acid (n-6 or AL) and 18% monounsaturated omega-9 fatty acids. It also contains potassium, calcium, phosphorous, magnesium, sulfur and vitamins.⁸

Due to the limitations to the population's consumption of fish, alpha-linolenic acid of vegetable origin could be an important option for providing sufficient concentrations of polyunsaturated fatty acids during lactation. The n-3 fatty acids are essential nutrients and one of their primary functions is to provide the docosahexanoic acid (DHA) needed for nervous system development and function. Ground flaxseed is therefore a good option that can easily be incorporated into the diet in bread, cereals, muffins, margarine and salads.^{9,10}

Despite flaxseed's high nutritional value, it is appropriate to mention that its composition also includes anti-nutritional components that can have adverse effects. Linatine can interfere with vitamin B6 absorption and can lead to deficiencies, while cyanogenic compounds and phytic acid can chelate minerals such as zinc, iron and calcium.¹¹ It has also been demonstrated that oil rich in n-3 fatty acids reduced iron absorption and retention, leading to a reduction in hemoglobin concentration.¹²

The benefits of flaxseed consumption has been demonstrated in adults, reducing cholesterol and glucose,¹³ but little research has been conducted into the effects of consumption exclusively during lactation. The objective of this study was to evaluate the effects of maternal flaxseed consumption during lactation on the body weight, hematological indicators and visceral fat mass of offspring when adults.

Materials and methods

Study design

The research protocol was approved by the Animal Research Ethics Committee of the Faculty Office of Research & Postgraduate Studies at the Universidade Federal Fluminense (UFF), Niterói, Brazil, under hearing number 24-08. All procedures were in accordance with Brazilian School of Animal Experimentation (Colégio Brasileiro de Experimentação Animal, COBEA) standards.

Sixteen nulliparous female Wistar rats were obtained from the colony kept by the experimental nutrition laboratory (LabNE) at UFF at 90 days of age and were mated at a ratio of one male per three females and fed on Nuvilab® commercial chow (Nuvital Ltda., Paraná, Brazil). After giving birth the mothers were divided into two groups at random and fed on one of the following two diets for the entire lactation period: the control group (CG) was fed a casein-based diet, while the flaxseed group (FG) was fed

a casein-based diet containing 25% flaxseed. At weaning, 8 male offspring from each group (one from each mother) were fed on commercial chow until they reached adulthood, when they were euthanized at 170 days. Body weight (g) and food intake (g) were recorded three times a week. All animals were kept in an animal house with controlled temperature (21-23 °C) and light-dark cycle (12/12 h), with water and chow ad libitum. The rats were anesthetized with an intraperitoneal injection of Thiopentax (Sodium thiopental 1 g, Cristália Produtos Químicos Farmacêuticos Ltda., Brazil) at 5% (0.15 mL/100 g body weight) and blood was taken via cardiac puncture into tubes containing EDTA for hemoglobin and hematocrit assays.

After euthanasia, the retroperitoneal, mesenteric and epididimal fat were weighed using an analytic balance (Bosch S2000, Brazil).

Experimental diets

The flaxseed was ground in a liquidizer, weighed, put into sealed bags and stored in a refrigerator until it was used to make up the rats' diet. The experimental diets prepared at LabNE were isocaloric, containing 17% protein and fortified with vitamins and minerals, as laid out by the Committee on Laboratory Animal Diets, 1979, and modified according to the American Institute of Nutrition-93 G diet (AIN-93G).¹⁴ The diet given to the FG contained 25% flaxseed, with the intention of meeting the entire fiber recommendation (AIN-93G). The ingredients of the experimental diets (Table 1) were weighed and homogenized in an industrial food mixer made by Hobart® (São Paulo, SP, Brazil) with boiling water to gelatinize the starch. The resulting dough was formed into pellets and dried in a Fabbe-Primar® ventilated oven (São Paulo, SP, Brazil) at 60 °C for 24 hours, labeled and stored in a refrigerator until used.

The commercial chow contained 23% protein, 67.6% starch, 4% minerals, 0.4% vitamins and 5% soy oil.

Biological assessment of diets

The "lactation value" (LV) was used to evaluate the biological value of the diets. This is based on increase in body weight, which is related to protein quality and milk production. This phase of the experiment lasts as long as the offspring are feeding exclusively on their mothers' milk, since after the 14th day they open their eyes and begin to feed on the mothers' diet as well. The LV is calculated from the change in maternal weight (g) + weight gain of offspring (g) / maternal protein intake (g).¹⁵

Biochemical tests

Blood samples were centrifuged (Sigma centrifuge) at 3500 rpm for 15 minutes to separate serum. Hemoglobin was assayed with a commercial test kit (BIOCLIN,

Table 1 - Composition of 100 g of each experimental diet given during lactation

Ingredient	Control (g/100 g of mix)	Flaxseed (g/100 g of mix)
Casein*	20	14.11
Flaxseed†	0	25
Cornstarch‡	52.95	45.84
Refined sugar §	10	10
AIN 93G* mineral mixture	3.50	3.50
Vitamin mixture*	1	1
Soybean oil **	7	0
Cellulose††	5	0
Choline bitartrate*	0.25	0.25
L-Cystine*	0.30	0.30
Tert-butylhydroquinone	0.0014	0.0014
Total	100	100

AIN-93G = American Institute of Nutrition-93 G.

* Ingredient used to prepare diet supplied by M. Cassab Comércio e Indústria Ltda. (São Paulo, SP, Brazil).

† Ingredient used to prepare diet supplied by Arma Zen Produtos Naturais Ltda. (Rio de Janeiro, RJ, Brazil).

‡ Ingredient used to prepare diet supplied by Maizena, Unilever Bestfoods Brasil Ltda. (Mogi Guaçu, SP, Brazil).

§ Ingredient used to prepare diet supplied by União (Rio de Janeiro, RJ, Brazil).

**Ingredient used to prepare diet supplied by Liza, Cargill Agricultura Ltda. (Mairinque SP, Brazil).

††Ingredient used to prepare diet supplied by Microcel, Blanver Ltda. (Cotia, SP, Brazil).

Indústria Quibasa - Química Básica Ltda., Belo Horizonte, MG, Brazil). Hematocrit was assayed in whole blood using the microhematocrit technique and disposable microcapillaries.

Statistical analysis

Data are reported as means and standard deviations. The results were tested for normal distribution using the Shapiro-Wilk test. For results with normal distribution, comparisons between groups were carried out using Student's *t* test for independent data. Where results did not have normal distribution, the Mann-Whitney nonparametric test was employed. In all tests, significance was set at $p \leq 0.05$. SPSS for Windows, version 10.0, was used for statistical analysis.

Results

There were no differences between the mothers given control and flaxseed diets during lactation in terms of dietary intake (FG = 634.0 ± 43.81 g; CG = 661.89 ± 64.69 g, $p = 0.364$) or protein intake (FG = 109.28 ± 8.09 g; CG = 115.33 ± 12.91 g, $p = 0.280$). The variation in body weight was similar for the female rats in both groups throughout lactation, with $p = 0.161$ at 21 days. The mothers in the FG and the CG exhibited similar lactation values during the first 14 days of lactation (FG = 1.93 ± 0.17 g; CG = 1.90 ± 0.22 g, $p = 0.764$).

The birth weight of offspring in both groups was similar (FG = 6.14 ± 0.41 g; CG = 5.86 ± 0.39 g, $p = 0.194$). Mothers

on the flaxseed diet gained less weight during lactation ($p = 0.043$), while their offspring had lower body weight ($p = 0.036$) after weaning only (Table 2). At euthanasia, at 170 days, there was no difference in body weight between the two groups (FG = 379.33 ± 38.04 g; CG = 408.07 ± 23.17 g, $p = 0.114$). Chow consumption up to 170 days was similar for both groups (FG = $3,096.21 \pm 281.09$ g; CG = $3,084.59 \pm 243.04$ g, $p = 0.935$).

Hemoglobin levels at 170 days were lower in the FG compared with the CG ($p = 0.02$), whereas there was no difference in hematocrit levels (Table 2).

The visceral fat mass at euthanasia was 21.43% lower in the FG than the CG, but this difference did not reach statistical significance (Table 2).

Discussion

No differences were observed in terms of change in weight, protein intake or dietary intake between the female rats given experimental diets during lactation. Intakes were recorded and analyzed in order to guarantee that any alteration observed among the offspring could be attributed to the presence of flaxseed. It has been demonstrated that the fatty acid profile of the diet did not affect dietary intake, when rats' consumption of a diet rich in n-3 was compared with consumption of a diet rich in n-6.¹⁶ During the last century, consumption of diets rich in n-6 increased, with unknown implications for the n-3 and n-6 content in the brain. This elevated consumption has doubled the concentration of n-6 in human milk and, as a result, in infant

Table 2 - Data on offspring, at different points during the experiment

Group	CG (n = 8)	FG (n = 8)	p
Weight gain during lactation (g)*	41.45±4.81	36.55±3.82	0.043
Weight at weaning (g)*	47.31±4,72	42.69±3,06	0.036
Hematocrit (%)†	46.71±1.38	44.43±3.86	0.259
Hemoglobin (g/dL)*	13.88±0.91	12.30±1.28	0.020
Visceral fat mass (g)*	14.56±6.46	11.44±4.39	0.312

CG = control group; FG = group flaxseed.

* Student's *t* test.

† Mann-Whitney test.

Significance is $p \leq 0.05$ for all tests.

formulae, since their nutritional composition is based on the composition of human milk.^{17,18}

Vegetable sources of protein are widely used for human nutrition because of their low cost and lower fat content, when compared with foods of animal origin.¹⁹ The adequacy of flaxseed protein for lactation was confirmed by the lactation value, since the result was similar to that for the group fed only on casein. Casein is a protein of animal origin, recommended as the standard protein for experimental research with laboratory animals.¹⁴ When flaxseed was used as the only protein source for growing Wistar rats, it did not demonstrate good performance, with results inferior to those for a group fed on casein.²⁰

Maternal exposure to a diet containing 25% flaxseed did not affect body weight gain of the mothers over a 21-day period, but did result in reduced body weight of offspring at weaning, since the FG body weight increased less during lactation. A similar result was observed by Collins et al., when the investigated diets containing flaxseed or defatted flaxseed at a range of concentrations.²¹ Another study found that 5% flaxseed or 1.5 mg/day of secoisolariciresinol diglucoside (SDG) reduced the plasma concentration of insulin-like growth factor (IGF-I),²² which acts as a mediator of growth hormone (GH), since the effects of GH on body mass gain are mediated by IGF-I.²³ Although we did not assay GH or IGF-I in this study, they may have caused the lower body weight at weaning.

No changes in hematocrit were observed, but lower hemoglobin levels were observed in the FG, probably due to the presence of anti-nutritional factors contained in the flaxseed. According to Harkness & Wagner, mean hemoglobin levels for rodents vary from 11 to 18 g/dL.²⁴ Taking this as a reference, even though the FG had lower levels, both groups had hemoglobin levels within this range. An opposite effect was observed by Babu et al. when they fed rats on 10% flaxseed for 56 days and observed an increase in hematocrit percentage but unaltered hemoglobin levels.²⁵

Although there was no difference in nutritional intake or in body weight at 170 days, a 21.43% reduction was observed in visceral fat mass in the FG. A tendency towards lower visceral fat mass has been shown in rats given a diet rich in flaxseed oil, when compared with rats on a diet rich in saturated fat, which is similar to the results of this study.²⁶ Maternal nutrition during lactation has already been confirmed as a determinant factor in programming the offspring's weight when adults.²⁷ The results of our study appear to show that it also programs body composition. Researchers have investigated the relationship between childhood weight and risk of disease in adulthood. Yliarsila et al. found that, in humans, a rapid increase in BMI after the age of 2 years is related to increased accumulation of adipose tissue in adulthood.²⁸

Research with animals has also demonstrated the influence of prenatal and postnatal nutrition on the development of diseases in adulthood. An experimental study based on a model of fetal malnutrition during gestation followed by a hypercaloric diet after birth demonstrated that hyperphagia during adulthood is the result of fetal programming and results in permanent changes to plasma insulin and leptin and body weight in adults rats.²⁹ It has also been shown that a hyperlipidic diet during pregnancy and lactation affects offspring's susceptibility to diet-induced obesity, which leads to adverse metabolic consequences.³⁰

A small number of studies have evaluated the effects of nutritional programming during lactation in isolation and demonstrated that this appears to be the most critical period.²⁷

Although the results of studies on animals should be extrapolated to humans with caution, our results suggest that transfer of compounds from the flaxseed via milk during lactation programs offspring to have lower weight at weaning and lower hemoglobin concentration when

adult. More studies are needed to elucidate the effects of programming with flaxseed on reduced visceral fat mass of offspring.

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