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Flaxseed oil during lactation changes milk and body composition in male and female suckling pups rats

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ABSTRACT

We have reported several changes in neonate or adult offspring after the maternal use of whole flaxseed or its components. However, it is unknown the use of higher oil intake in the neonatal period. Here we evaluated the effects of high maternal intake of flaxseed oil during lactation upon milk and body composition in male and female offspring. Lactating rats were divided into: (1) control (C, $n = 10$), 7% soybean oil; (2) hyper 19% soybean oil (HS, $n = 10$); and (3) hyper 17% flaxseed oil + 2% soybean oil (HF, $n = 10$). Dams and offspring were killed at weaning. HS and HF dams, male and female offspring presented lower body weight during lactation. HF mothers presented lower body and visceral fat masses. HF male offspring presented lower body and subcutaneous fat masses. HS and HF milk presented lower triglycerides (TG) and cholesterol. HF male and female offspring showed lower triglyceridemia and insulinemia, but no changes in glycemia and leptinemia. The higher intake of flaxseed oil during lactation reduced the body weight of mothers and offspring, decreases milk lipids and apparently increases insulin sensitivity in this critical period of life. Those changes may explain the previously reported programming effect of maternal flaxseed intake during lactation.

1. Introduction

Functional foods are those that beyond basic nutritional functions can produce beneficial health effects and should be safe for consumption without medical supervision. Flaxseed is the seed from the flax plant (*Linum usitatissimum* L), called linseed, which is a member of the Linaceae family (Oomah, 2001). Flaxseed contains 32–45% of its mass as oil, of which 51–55% is alpha-linolenic acid (ALA, which belongs to omega-3 family) and 15–18% is linoleic acid (omega-6) (Carter, 1993). The ratio of omega-3/omega-6 seems to be an important factor to lipid and glucose homeostasis. Flaxseed and canola oil are rich in omega-3 and lower in omega-6, while soybean oil that is less expensive and because of this, highly used by poor population had a lower omega-3/omega-6 ratio. Beside this, there is a trend of increase the proportion of vegetable oil on the diet (Takahashi and Ide, 2000).

Flaxseed presented potential health benefits associated with decrease in the risk of cardiovascular disease (Cunnane et al., 1995; Jenkins et al., 1999). Several functional properties of flaxseed have been reported including antioxidant activity and the ability to lower blood glucose, serum total cholesterol, LDL-c and triacylglycerol while increasing serum HDL-c (Prasad, 2008). Based in the recommendation to increase the use of oils rich in omega-3, there is an increase in consumption by the population. However, this could lead to an overconsumption of a hyperlypidic diet, which health risk is not completely evaluated. Recently, it was compared the differences in the effects of canola vs. soybean oil in a normal proportion (7%) or hyperlipidic proportion (19%) (Costa et al., 2012, 2013). It is interesting that in the normal proportion canola oil decreases abdominal fat and increases insulin sensitivity, but in the higher proportion canola oil is worse than soybean oil both in relation to obesity and insulin resistance.

Some authors, including our previous data have suggested caution when flaxseed is consumed during pregnancy and lactation (Tou et al., 1998; Troina et al., 2010). Pups whose mothers consumed dietary flaxseed (10%) during gestation and lactation presented lower body weight at birth (Tou et al., 1998). At weaning, the male offspring, whose mothers received whole dietary flaxseed (25%) showed higher body mass but lower body fat mass, lower serum total cholesterol and triglycerides, hyperleptinemia,

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hypoinsulinemia and higher insulin sensitivity, lower serum triiodothyronine (T3) and higher thyrotropin (TSH) (Figueiredo et al., 2009). In another study we reported that the female offspring at weaning showed similar phenotype, presenting lower total fat mass, lower visceral fat mass, lower serum total cholesterol and triglycerides and higher serum leptin, but contrary to the higher body mass observed in the male, the female also presented lower body mass (Troina et al., 2010). Since, the main components of flaxseed is the oil and secoisolariciresinol diglucoside (SDG), we showed in another study, the effects of maternal supplementation with SDG alone or SDG plus 7% flaxseed oil during lactation. In the mother, SDG treatment caused a higher total fat mass, while the oil reduced the fat mass. Also, the oil reduced serum triglycerides and cholesterol in the dams. In the offspring, a lower body fat mass was observed in the oil-treated group both in male and female. Serum triglyceride levels were also lower in both SDG and flaxseed oil + SDG groups, both in male and female (Troina et al., 2012).

Based in the previous reports, we hypothesized that the overconsumption of flaxseed oil during lactation, instead to be health promoting, could be detrimental as was reported for canola oil (Costa et al., 2013). Thus in this study we aimed to evaluate the milk composition, body composition of dams and pups as well as serum leptin, glucose homeostasis and lipid profile. To our knowledge, this is the first study to adress the question of the use of hyperlipidic flaxseed diet during lactation over body composition, glucose homeostasis and lipid profile.

2. Materials and methods

Three-month-old Wistar rats were maintained in a temperature-controlled room (25 ± 1 °C) with a 12:12 dark-light cycle. Virgin female rats (200–220 g) were mated and each female was placed in an individual cage with free access to water and food until parturition. The use of the animals according our experimental design was approved by the Animal Care and Use Committee of the Biology Institute of the State University of Rio de Janeiro (CEUA/060/2011).

At birth, 30 lactating rats were randomly assigned to each of the following groups: (1) control (C, $n = 10$), with free access to a diet containing 7% lipid from soybean oil; (2) hyper soybean oil (HS, $n = 10$), with free access to a diet containing 19% lipid exclusive from soybean oil; and (3) hyper flaxseed oil (HF, $n = 10$), with free access to a diet containing 19% lipid (2% from soybean oil and 17% from flaxseed oil) Table 1. The soybean oil presented 54% of linoleic acid (n-6) and 8% of alpha-linolenic acid (n-3) showing n-6/n3 as 7:1, and the flaxseed oil presented 16% of linoleic acid (n-6) and 57% of alpha-linolenic acid (n-3) showing n-6/n3 as 1:3. In the higher flaxseed oil diet we added 2% of soybean oil to attend the minimum amount of n-6 that has to be offer to growing animals according to the recommendation of the American Institute of Nutrition/AIN 93G (Reeves et al., 1993), since has explained above flaxseed oil present already n-6. The experimental and control diets were offered *ad libitum* and started at birth, which was defined as day 0 (d0) of lactation, and were ended at weaning (d21). After birth, the litters were adjusted to 4 males and 4 female for each dam. When the number of litter from one mother was not enough, we borrow from other randomly selected mother from the same group that gave birth on the same day.

Table 1
Composition of 100 g of diet during lactation.

Ingredients (%)	Control	Hyper soybean oil	Hyper flaxseed oil
Casein[a]	20.00	20.00	20.00
Corn starch[b]	50.29	43.95	43.95
Sucrose[c]	10.00	10.00	10.00
Mineral mix[a]	3.50	3.50	3.50
Vitamin mix[a]	1.00	1.00	1.00
Soybean oil[d]	7.00	19.00	2.00
Flaxseed oil[e]	–	–	17.00
Fiber[f]	5.00	–	–
Choline bitartrate[e]	0.25	0.25	0.25
l-Cystine[e]	0.30	0.30	0.30
Tert-Butylhydroquinone[g]	0.0014	0.0014	0.0014
Macronutrient composition (per 100 g of diet)			
Protein (g)	19.55	19.53	19.53
Carbohydrate (g)	61.14	50.28	50.28
Fat (g)	19.49	49.50	49.50
Total energy (kJ/100 g)	1486.80	1770.80	1770.80

[a] M Cassab Comercio & Industria LTDA (São Paulo, SP, Brazil).
[b] Maisena, Unilever Best Foods Brasil LTDA (Mogi Guaçu, SP, Brazil).
[c] União (Rio de Janeiro, RJ, Brazil).
[d] Liza Cargil Agricultura LTDA (Mairinque, SP, Brazil).
[e] Pragsoluções Biociências LTDA (Jaú, SP, Brazil).
[f] Microcel, Blanver LTDA (Cotia, SP, Brazil).
[g] Vogler Ingredients (Eastman, USA).

At weaning, the dams and 2 pups (1 male and 1 female) for each dam were killed with a lethal dose of pentobarbital (0.06 g/kg/b.w.); blood was collected by cardiac puncture, and the tissues adiposity compartments and carcass were analyzed.

2.1. Nutritional evaluation

Maternal food intake (FI) and body mass (BM) as well as BM of the offspring were daily monitored during lactation. The maternal cumulative FI was calculated through the summation of the daily mother's food intake and the caloric density of the diets was calculated considering 4 kcal/g of protein, 4 kcal/g of carbohydrate and 9 kcal/g of lipid.

2.2. Body composition

Visceral fat mass (VFM) was evaluated weighing the retroperitoneal, mesenteric, and around the uterus and ovaries or testis fat depots.

The carcasses were weighed, autoclaved for 1 h and homogenized in distilled water (1:1). Samples of the homogenate were stored at 4 °C for analysis. Three grams of homogenate were used to determine fat mass content gravimetrically. Samples were hydrolyzed in a shaking water bath at 70 °C for 2 h with 30% KOH and ethanol. Total fatty acids and non-esterified cholesterol were removed using three successive washings with petroleum ether. After drying overnight in a vacuum, tubes were weighed and results were expressed as% fat. The subcutaneous fat mass was estimated from the total fat mass minus the visceral fat mass, and the results were expressed as percentages. The total protein concentrations were determined by the Lowry method. Data were expressed as g protein/100 g carcass (Figueiredo et al., 2009).

2.3. Milk composition

Milk samples were obtained on 14 and 20 days of lactation. Mothers were separated from their pups for 2 h and were injected with oxytocin (5 UI/ml sc – Eurofarm, São Paulo, SP, Brazil). After 30 min, dams were lightly anesthetized with pentobarbital and milk was extracted manually from the thoracic and abdominal teats. All milk samples were analyzed for lactose, total protein, total cholesterol and triglycerides. Lactose was estimated by a colorimetric method using picric acid. Commercial lactose (Sigma, St. Louis, MO, USA) was used as a standard. Protein was estimated by a colorimetric method using bovine serum albumin (Sigma, St. Louis, MO, USA) as a standard. Total cholesterol and triglycerides were determined by an enzymatic and colorimetric method using a commercial kit (Bioclin, Belo Horizonte, MG, Brazil) (Troina et al., 2010).

2.4. Biochemical analysis

The blood samples were centrifuged and the serum was separated to determine the lipid profile. Total cholesterol, triglycerides (TG) and HDL-c were analyzed using Biosystem ® commercial test kits. LDL-c and VLDL-c were obtained using Friedewald calculations: LDL-c (mg/dl) = Total cholesterol − (TG/5) − HDL-c and VLDL-c (mg/dl) = TG/5.

Glucose concentration was determined in blood samples from the tail vein of fasting rats using glucose oxidase reagent strips, and read in a reflectance glucosimeter (ACCU-CHEK Advantage; Roche Diagnostics, Mannheim, Germany). The results were expressed as mg/dl (Figueiredo et al., 2009). The insulin resistance index (IRI) was calculated (fasting insulin × fasting glucose).

2.5. Hormone analysis

Blood samples were centrifuged to obtain serum, which was individually kept at −20 °C until assay. All measurements were performed in one assay. Serum insulin levels were determined by radioimmunoassay (RIA), using a commercial kit (ImmuChem™ ^{125}I, coated tube; ICN Biomedical Inc., Aurora, OH, USA) with an assay sensitivity of 0.1 ng/ml and an intra-assay coefficient of variance of 3.2%. Serum leptin levels were determined by RIA, using a commercial kit (Millipore Corporation, Billerica, MA, USA) with an assay sensitivity of 0.639 ng/ml and intra-assay coefficient of variance of 5.7% (Figueiredo et al., 2009).