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# Maternal flaxseed oil intake during lactation changes body fat, inflammatory markers and glucose homeostasis in the adult progeny: role of gender dimorphism

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**Abstract**

We evaluated maternal flaxseed oil intake during lactation on body composition, lipid profile, glucose homeostasis and adipose tissue inflammation in male and female progeny at adulthood. Lactating rats were divided into the following: control 7% soybean oil (C), hyper 19% soybean oil (HS) and hyper 17% flaxseed oil + 2% soybean oil (HF). Weaned pups received a standard diet. Offspring were killed in PN180. Male HF presented higher visceral adipose tissue (VAT) and triacylglycerol, and female HF showed insulin resistance. Both male and female HF had hyperleptinemia, and only male HF had hyperprolactinemia. In VAT, male HF presented lower PPAR-γ expressions and higher TNF-α, IL-6, IL-1β and IL-10 expressions; in subcutaneous adipose tissue (SAT), they presented lower PPAR-γ and TNF-α expressions. Female HF presented higher leptin, as well as lower adiponectin, TNF-α, IL-6 and IL-1β expressions in VAT and lower TNF-α in SAT. Flaxseed oil during lactation leads to gender-specific effects with more adiposity and dyslipidemia in male and insulin resistance in female. Higher prolactin and inflammatory cytokines in male could play a role in these gender differences. We suggest that the use of flaxseed oil during lactation increases metabolic syndrome risk in the adult progeny.


*Keywords:* Flaxseed oil; Lactation; Adipose tissue inflammation; Metabolic programming; Insulin resistance; Rats

## 1. Introduction

Flaxseed (*Linum usitatissimum L.*) is a rich source of oil (32–45%), of which 51–55% is alpha-linolenic acid (ALA, n-3) and 15–18% is linoleic acid (LA, n-6) [1]. The flaxseed oil consumption is associated with potential health benefits due to the presence of n-3 fatty acids. Among its benefits are lower adiposity and improvement of lipid profile and insulin sensitivity, related to a reduction in proinflammatory status [2–5]. Because of these putative advantages, the consumption of flaxseed and its components, especially the oil, is increasing worldwide [6]. Thus, flaxseed oil higher consumption has been suggested as a potential alternative for salad dressings or an enrichment strategy for industrially processed foods [7].

The prevalence of obesity has increased during the last decades despite no significant change in fat intake but with changes in the fatty acid composition of dietary fats. There was a marked shift in fatty acid composition from saturated fats to fats enriched with PUFAs of the n-6 series in the typical western diet [8]. Soybean oil is still more consumed because it is less expensive than n-3 rich oils, such as flaxseed. Recently, among some vegans, flaxseed oil and flaxseed are chosen as one of the major sources of ALA [9]. Thereby, this trend is leading to an overconsumption of flaxseed oil, which health risk is not completely evaluated, especially during lactation. It is known that imprinting factors acting in this critical window may program the intermediary metabolism and body composition during development [10].

When whole flaxseed is consumed during critical periods of development such as pregnancy, lactation or puberty may cause hormonal and metabolic disorders, supposedly by the flaxseed lignan (secoisolariciresinol diglucoside — SDG) content [11,12]. Our previous studies with whole flaxseed during lactation showed higher body mass but lower body fat mass, lower serum total cholesterol and triglycerides, hyperleptinemia, hypoinsulinemia and higher insulin sensitivity in male pups at weaning [13]. Also female offspring at weaning showed similar phenotype, but contrary to the male, the female presented lower body mass [12]. At adulthood, the male offspring whose mother received whole dietary flaxseed had lower glycemia, higher insulinemia and lower adiponectin and an increase in the size of subcutaneous and visceral adipocytes, while the female offspring presented higher body fat and visceral fat mass and higher serum total cholesterol, triglyceride and 17β estradiol [12–14].

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Recently, we demonstrated that higher intake of flaxseed oil during lactation decreases milk lipid contents (cholesterol and triacylglycerol) and reduces the body weight, serum triglycerides and insulin, as well as increases insulin sensitivity at weaning in both gender offspring [15]. As several imprinting factors can act during lactation, such as the mentioned above, it is expected that programming effects of flaxseed oil intake could be acting during offspring development.

Therefore, based in our previous findings with whole flaxseed and with flaxseed oil, we hypothesized that the higher consumption of flaxseed oil during lactation may cause hormonal and metabolic alterations at adulthood that can differ from the use of soybean oil and can be gender specific. Thus, we aimed to evaluate in this study the body composition, glucose homeostasis, lipid profile, serum hormones concentrations (insulin, leptin, adiponectin, corticosterone and prolactin) and adipose tissue inflammation in male and female offspring at adulthood, whose mothers received flaxseed oil during lactation.

## 2. Materials and methods

### 2.1. Animal research

Three-month-old Wistar rats were maintained in a temperature-controlled room (25±1°C) with a 12:12 h 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).

After birth, the litters were adjusted to 4 male and 4 female for each dam, and four pups (two male and two female) from each dam were killed at weaning and were studied in previous experiment [15]. The 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 exclusive 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 LA (n-6) and 8% of ALA (n-3) showing n-6/n-3 as 7:1, and the flaxseed oil presented 16% of LA (n-6) and 57% of ALA (n-3) showing n-6/n-3 as 1:3. The soybean and flaxseed oils were added as part of the diets, and in the 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 [16]. The high fat diets with vegetal oils presented 19% of oil, which correspond to 42% of total daily energy from lipid (Table 1). In this experimental design, we decided to include a control group with 7% of lipid exclusive from soybean oil (19% of total daily energy from lipid) because this amount of oil represents the quantity and the source of oil, which was recommended in the AIN 93G and we thought that was important to present data of, a group that received a normal intake of oil. The flaxseed oil used in the diet was extract by cold pressed (PRAGSOLUÇÕES LTDA, Brazil). The experimental diets started at birth, which was defined as day 0 (d0) of lactation, and were ended at weaning (d21). During the lactation, fresh diet was offered daily to prevent rancidity lipid and the maternal food intake was monitored every day, previously described in Guarda *et al.* [15]. The milk composition was studied at 14 and 20 days of lactation and the results were presented in a previous manuscript [15].

Table 1
Composition of 100 g of diet used in the experimental 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 | 5.00 | 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 |
| **Energy intake of macronutrient (per 100 g of diet)** | | | |
| Protein (%) | 19.51 | 16.37 | 16.37 |
| Carbohydrate (%) | 61.03 | 42.14 | 42.14 |
| Fat (%) | 19.46 | 41.49 | 41.49 |
| Total energy (kJ/100 g) | 1488.20 | 1772.55 | 1772.55 |

[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, all remaining pups were separated in male and female, with 4 animals per cage, accordingly the mother treatment and received a standard laboratory diet (Nuvilab, NUVITAL, PR, Brazil) containing 22% of protein, 66% of carbohydrate and 4% of lipid until they were 180 days old. All pups were killed with a lethal dose of pentobarbital (0.06 g/kg/b.w.) when they were 180 days old; blood was collected by cardiac puncture, and the tissues' adiposity compartments and carcass were analyzed.

### 2.2. Nutritional evaluation

The food intake and body mass were evaluated once every 4 days after weaning until they were 180 days old. The animals were grouped in 4 animals per cage and the food intake was measured per cage and corrected by the number of animals per cage according to the formula: Food intake (g)=[(*Ci*−*Cf*)/4]/*n*, where *Ci* is the total chow offered to the cage; *Cf* is the total chow left in the cage; 4 represents the number of days between the day that we offer the chow and the day that we check the left diet; and *n* represents the number of animals per cage. The cumulative food intake for each animal was calculated through the summation of the food intake after weaning until 180 days old. The weight body mass gain was calculated from the body mass at 180 days old subtracted from the initial weight (at weaning). The data were expressed in grams (g).

### 2.3. Body composition

Visceral fat mass was evaluated by weighing the retroperitoneal, mesenteric and perigonadal fat pads.

The animals were eviscerated (all organs were removed) and the carcasses were weighed with visceral fat mass, 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 was 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 nonesterified 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 total protein concentrations were determined by the Lowry method. Data were expressed as gram (g) protein/100 g carcass [13,15].

### 2.4. Biochemical and hormonal analysis

Blood samples were centrifuged and serum was separated to determine the lipid profile. Total cholesterol and triglycerides were analyzed using Biosystem commercial test kits.

The total protein and albumin were determined using Biosystem commercial test kits. The results were expressed as grams per deciliter (g/dl). The globulin was calculated by the total protein subtracted of albumin and the result was expressed as grams per deciliter (g/dl).

Glucose concentration was determined in blood samples from the tail vein of fasted (12 h) rats using glucose oxidase reagent strips and read in a reflectance glucometer (ACCU-CHEK Advantage; Roche Diagnostics, Mannheim, Germany) 5 min before they were killed [17]. The results were expressed as milligrams per deciliter (mg/dl). The insulin resistance index (IRI) was calculated by the product of fasting insulin (μUI/ml)×fasting glucose (mmol/l) [18–20].

Blood samples were centrifuged to obtain serum, which was individually kept at −20°C until assay. All measurements were performed in one assay. The hormonal analysis was determined by radioimmunoassay (RIA), using the following commercial kits: serum insulin levels (ImmuChem $^{125}$I, coated tube; ICN Biomedical Inc, Aurora, OH, USA), leptin levels (Millipore Corporation, Billerica, MA, USA), adiponectin levels (LINCO Research, St. Charles, MI, USA) and corticosterone levels (ImmuChem $^{125}$I, double antibody; MP Biomedicals LLC, Orangeburg, NY, USA); prolactin levels were measured by RIA of double antibody using murine reagents provided by the National Institutes of Health (National Institutes of Health — National Institute of Diabetes and Digestive and Kidney Diseases, Bethesda, USA) [13,15,17].

### 2.5. Reverse transcription polymerase chain reaction analysis

For mRNA studies, visceral abdominal fat was dissected and immediately frozen in liquid nitrogen and stored at −80°C until RNA extraction. Total RNA was extracted from 100 mg of the tissue, under RNase-free conditions, with TRIzol Reagent (Cat. No. 15596-018) (Invitrogen, Carlsbad, CA, USA), and quantified *via* NanoVue Plus Spectrophotometer (GE Healthcare, Buckinghamshire). The cDNA was prepared from the total RNA using the Moloney Murine Leukemia Virus Reverse Transcriptase (M-MLV RT) for reverse transcription polymerase chain reaction (PCR) (Cat. No. 1158862) and Oligo(dT)15 Primer (Promega, Madison, WI, USA). The mRNA levels of leptin (F: 5′-CAT CTG CTG GCC TTC TTC AA-3′; R: 5′-ATC CAG GCT CTC TGG CTT CTG-3′) were conducted in 40 cycles of 95°C for 15 s, 53°C for 30s and 70°C for 45 s; Adiponectin (F: 5′-CAA GGG AAC TTG TGC AGG-3′; R: 5′-CAC CCT TAG GAC CAA GAA-3′), Peroxisome