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Flaxseed flour diet during lactation until 180 days results in an increase in body adiposity in adult male rats

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ABSTRACT

This study evaluated the influence of flaxseed flour intake on body adiposity in Wistar male rats at 180 days. At birth, we randomly assigned male Wistar rats to control (C) and experimental (FF) groups treated with a control or flaxseed flour diet until 180 days. We evaluated food and energy intake, body mass and length during 21–180 day period. We also evaluated oral glucose tolerance test (OGTT), lean and fat mass by dual-energy X-ray absorptiometry, serum glucose, insulin, HOMA-IR and leptin, intra-abdominal fat mass and retroperitoneal adipocyte area. Groups showed similar food, energy intake, body length, insulin, total lean mass and adipocyte area. FF group showed the following ($P < .05$): lower concentrations of glucose in OGTT and serum glucose (−15%), leptin (−56%), higher body mass, total (+38%), trunk (+35%), intra-abdominal (+52%), mesenteric (+36%) and retroperitoneal (+42%) fat mass. At 180 days, flaxseed flour was associated with high body adiposity.

1. Introduction

Adipose tissue, primarily composed of adipocytes as well as pre-adipocytes, is not only a passive fuel reservoir, but also an endocrine organ that produces a variety of factors, such as leptin, which regulate systemic metabolic homeostasis via effects on energy storage (Luo & Liu, 2016). The obesity is characterized by an increase in the mass of adipose tissue, and it can arise by increasing cell size (hypertrophy), cell number (hyperplasia or adipogenesis) or both (Feng, Reuss, & Wang, 2016; Forest, Joffin, Jaubert, & Noirez, 2016; Howell & Powell, 2017).

Overweight and obesity are a public health problem in both developed countries and those under development (Marie et al., 2013). It usually begins early in life, persists into adulthood and significantly increases the risk for morbidity, such as dyslipidemia, type-2 diabetes mellitus and coronary heart disease (Eknoyan, 2006; Fagot-Campagna, 2000; James, Leach, Kalamara, & Shayeghi, 2001). Modification of lifestyle and dietary pattern is an important strategy to obesity prevention (Subhan & Chan, 2016).

Experimental and clinical studies displayed a relation between dietary intake and adipose tissue (Feng et al., 2016; Forest et al., 2016; Howell & Powell, 2017); traditionally, fat in the diet is the first factor to be avoided in prevention of obesity. However, the type of fat consumed could be more decisive than the total amount of fat in the diet related to body composition and distribution of adipose tissue (Paniagua, 2016).

Regarding essential fatty acids, alpha-linolenic acid (ALA, 18: 3n-3) is associated with reduced obesity risk because it exerts numerous effects on adipose tissue, for example, inducing fatty acid oxidation genes through peroxisome proliferator-actived receptor alpha and by suppression of lipogenic genes through sterol regulatory element-binding protein (Hsu & Huang, 2006; Huang et al., 2016; Massiera et al., 2003). In this context, flaxseed (*Linum usitatissimum*) can contribute to the prevention of obesity, since the seed is considered an excellent ALA source (Goyal, Sharma, Upadhyay, Gill, & Sihag, 2014).

This study aimed at examining whether diet that contains ALA, provided by flaxseed (*Linum usitatissimum*) flour is associated with low body adiposity in adult male rats.

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2. Materials and methods

2.1. Ethics statement and experimental design

Ethics Committee on Animal Research of Federal Fluminense University, Niteroi – RJ, Brazil (protocol 209/2012), approved the protocol used when dealing with experimental animals. All the procedures complied with the Brazilian Science and Laboratory Animals Society and the Guide for Care and Use of Laboratory Animals provisions published by US National Institutes of Health (NIH Publication N 85-23, revised in 1996).

We maintained Wistar rats in a temperature-controlled (23 ± 1 °C) and humidity-controlled (60 ± 10%) room, with artificial dark-light cycle (lights on from 7 am to 7 pm). Virgin female rats (3 months old, respectively) were caged with male rats. After mating, we placed each female in an individual cage with free access to water and standard food (Nuvilab®, Paraná, Brazil).

Within 24 h of birth, we adjusted the animals to six male pups per dam, which maximized lactation performance (Fishbeck & Rasmussen, 1987) During the lactation period, we randomly assigned pups to the following groups: control (C, n = 12 pups), whose dams were fed with control diet containing 20 out of 100 g casein; experimental (FF, n = 12 pups), whose mothers were fed with diet containing 25 out of 100 g flaxseed flour. We manufactured and stored the diets as pellets at 4 °C in agreement with American Institute of Nutrition (AIN-93G) recommendations for rodent diets (Reeves, 1997) The amount of flaxseed flour included – 25 out of 100 g – aimed at meeting all fiber intake recommendations, not being necessary adding oil because flaxseed seed comprises a source of this component (Table 1).

At 21 days of age, the pups weaned, and they continued to receive the same feed until completing 180 days of age. We evaluated food (g) and energy (kcal) intake, body mass (g) and length (cm, measured as distance between nose tip to tail tip) in a weekly basis (Costa et al., 2016).

2.2. Oral glucose tolerance test

The oral glucose tolerance test (OGTT) was administered to rats in the penultimate week of experiment. After 6 h overnight fast, glucose (1 g kg^{-1}) was given orally and, at 0, 15, 30, 60 and 120 min, blood samples were collected from the distal end of the tail and analysed immediately with a glucometer (mg dL^{-1}) and the corresponding test strips (Accu-Check Active®; Roche, São Paulo, Brazil) (Asht et al., 2014).

Table 1
Composition of experimental diet.

Ingredient (g per 100 g)	C	FF
Casein	20.00	15.00
Flaxseed flour	–	25.00
Cornstarch	52.95	45.84
Sucrose	10.00	10.00
Soybean oil	7.00	–
Fiber	5.00	–
AIN-93M Mineral Mix	3.50	3.50
AIN-93 Vitamin Mix	1.00	1.00
L-Cystine	0.30	0.30
Choline Bitartrate	0.25	0.25
Tert-butylhydroquinone (mg)	14.0	14.0
Protein	17.00	17.00
Lipid	7.00	7.00
Carbohydrate	54.06	49.00
Energy (kcal)	347.20	327.00

C, control diet; FF experimental diet containing 25 g per 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 flour: Armazen® with 17% of protein, 45% of carbohydrate and 26% of fat. Formulated on recommendations of American Institute of Nutrition (AIN-93G) for rodent diets.

2.3. Body composition

At 180 days, after overnight fast, were anesthetized with Tiopentax (Sodium thiopental, 0.1 mg out of 100 g) and subjected to dual-energy X-ray absorptiometry (DXA) using a Lunar DXA 200368 GE instrument (Lunar, with specific software encore 2008 version 12.20; GE Health care, Madison, WI, USA. The evaluation was carried out in a blinded manner, as the DXA technician did not know the experimental protocol. Total lean and fat mass (g) and trunk fat mass (g) were measured for each rat (Costa et al., 2012).

2.4. Serum glucose, insulin and leptin

Blood was collected by cardiac puncture following DXA procedures. Samples were centrifuged, and the serum was stored at −80 °C for later analysis. Glucose (mg dl^{-1}) was measured by an enzymatic-colorimetric method (Bioclin BS-120, Belo Horizonte, MG, Brazil). Insulin and leptin (ng ml^{-1}, respectively) were measured using multiplex assay kits (Millipore rat bone panel RBN1MAG-31K-03, Billerica, MA, USA). Based on serum analyses of glucose and insulin, the homeostasis model assessment-insulin resistance (HOMA-IR) index was calculated (Costa, Carlos, Santos, Moura, & Nascimento-Saba, 2013).

2.5. Morphometry

Intra-abdominal adipose tissue (retroperitoneal, mesenteric and epididymal) was dissected and weighted (g). Retroperitoneal fat was collected and fixed in buffered formaldehyde (Santana et al., 2011). Tissues were embedded in paraffin, cut into 5 µm sections, and stained with hematoxylin-eosin (HE). Profiles with at least 100 adipocytes were randomly selected and captured for morphometric analyses. A sectional adipocyte area (μm^2) was determined and digital images were acquired with an Optronics CCD video camera system and Olympus BX51 light microscope, analysed with U.S. National Institutes of Health IMAGE-J software http://rsbweb.nih.gov/ij/ (Costa et al., 2016).

2.6. Statistical analysis

Statistical analyses were carried out using the Graph Pad Prism statistical package (version 5.0, 2007 San Diego, CA, USA). Food and energy intake, body mass, length and OGTT were analysed using two-way ANOVA, followed by post hoc Bonferroni post-test. The remaining results were analysed using Student's *t*-test. All results were expressed as means ± standard mean error (SEM) with a 0.05 significance level.

3. Results

During experimental period, food and energy intake and body length were similar between FF and C groups. Regarding body mass, FF group showed after 35 day until 180 day, higher mass than control ($P < .001$) (Fig. 1).

OGTT showed lower ($P < .001$) concentrations of glucose in the FF group at 0, 15, 30, 60 and 120 min. Regarding serum analyses, the FF group showed lower (−15%, $P < .001$, FF: 105.50 ± 4.21 vs. C: 124.30 ± 3.42 mg dl^{-1}) glucose levels. Insulin (FF: 2.10 ± 0.35 vs. C: 2.02 ± 0.29 ng ml^{-1}) and HOMA-IR (FF: 11.70 ± 2.07 vs. C: 12.98 ± 2.14) were similar between groups. Leptin was lower (−56%, $P < .001$, FF: 10.40 ± 2.67 vs. C: 24.15 ± 3.03 ng ml^{-1}) in FF group (Fig. 2).

Body composition analysis by DXA shows that total lean mass was similar between groups. Total fat mass (+38%, $P < .05$) and trunk fat mass (+35%, $P < .05$) were higher in the FF group. Intra-abdominal (+52%, $P < .05$), mesenteric (+36%, $P < .05$) and retroperitoneal (+42%, $P < .05$) fat mass were higher in FF group. Epididymal fat