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Perinatal consumption of flaxseed oil and flaxseed flour has beneficial effects on cardiac fibrosis of male offspring from rat dams with experimental diabetes

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ABSTRACT

Maternal diabetes during critical periods of development can affect the health of adult offspring. Nevertheless, studies suggest that the presence of functional foods, such as flaxseed, can reverse the effects of metabolic programming. This study evaluated the effect of maternal consumption of flaxseed flour and a flaxseed oil based diets in left ventricular histomorphometry and in blood pressure in adult male offspring of diabetic rats. Rats ($n = 24$) were induced into diabetes with a high-fat diet (60% lipid) and streptozotocin (35 $mg \cdot kg^{-1}$). After diabetes confirmation (glucose > 300 mg/dL), rats were sent to mating, and afterward pregnancy confirmed, they were allocated into four groups: control group (CG), high fat group (HG), flaxseed oil group (FOG) and flaxseed flour group (FFG). At weaning, six males of each group were separated and fed with a commercial diet chow until they reached 100 days of life, moment in which they were euthanized. The systolic blood pressure, the thickness of the left ventricular wall and collagen volume density were analyzed. Exposure to a hyperglycemic environment led to an increased systolic blood pressure, nevertheless FOG showed a significant reduction when compared to HG ($p < 0.001$). The left ventricular wall thickness was significantly lower in FFG ($p = 0.030$) and a minor amount of collagen in the left ventricle was found in FOG and FFG ($p < 0.001$). Those results suggest that a diet with flaxseed flour and flaxseed oil avoids the cardiac remodeling of offspring from diabetic dams.

1. Introduction

Cardiovascular diseases are a major public health problem and account for 17.3 million human deaths worldwide (WHO, 2013) The development of cardiovascular disease is not only determined by the risk factors already known, but can also be caused by early life, such as during pregnancy and/or lactation and may result in adverse effects, as well as by increased blood pressure and cardiac remodeling (Huyen et al., 2010). This fact can be explained by the term "programming", which is the process by which a particular factor that acts early in life, or a critical or sensitive period of development and growth, can have adverse effects on the health of the offspring in adulthood (Warner & Ozanne, 2010). There are several metabolic disorders that occur during pregnancy and lactation, which can lead to fetal programming, including maternal malnutrition and gestational diabetes.

The incidence of diabetes has increased in recent years affecting individuals of all ages, including young children and women in reproductive age (Min et al., 2014). According to Ragnarsdottir and Conroy (2010), 7% of pregnancies are affected by this disease, from which 90% are classified as gestational diabetes mellitus. Diabetes during pregnancy may influence the cardiac development of the offspring (Yessoufou & Moutairou, 2011), inducing cardiac malformation which will accompany up to adulthood (Dabelea & Crume, 2011).

However it has been observed that intrauterine and postnatal nutrition can alter the expression of the fetal genome, and have the long-term consequences on the physiological and metabolic responses of the fetus, which can influence the occurrence of some diseases in adulthood (Wu, Bazer, Cudd, Meininger, & Spencer, 2004). However, dietary management during this period can be a strategy to reverse the effects of metabolic programming either preventing or reducing the progression of cardiovascular disease in adulthood (Demicheva & Crispi, 2013).

Abbreviations: ALA, alpha-linolenic acid; AL, linoleic acid; CG, control group; FFG, flaxseed flour group; FOG, flaxseed oil group; HG, high-fat group; LV, left ventricle; STZ, streptozotocin; SBP, systolic blood pressure; Vv, volume density.

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An example is the fish oil, rich in n–3, that when consumed during pregnancy and lactation avoids the appearance of diseases related to the heart in adult offspring, in as much as the animals had reduced blood pressure and cardiac remodeling, with a smaller cardiac hypertrophy and interstitial fibrosis (Catta-Preta, Oliveira, Mandarim-de-Lacerda, & Aquila, 2006; Gregório et al., 2008). Concerning the n–3, one of the main plant sources is flaxseed, which contains 41% of the lipids, from which 50–55% are composed of alpha-linolenic acid (ALA; n–3) and 15–18% by linoleic acid (AL; n–6). Flaxseed has also high amounts of fiber, lignans and protein, contributing to the intake of these nutrients (Shim, Gui, Arnison, Wang, & Reaney, 2014).

The improving at lipid profile, decrement blood pressure and prevention of the remodeling of the aorta are some of the effects correlated to the intake of flaxseed flour, which can help in the decrease of cardiovascular disease (Brant, Cardozo, Velarde, & Boaventura, 2012; Al-Bishri, 2013; Cardozo et al., 2014). In addition there is no consistent data that shows the effect of the administration of this oleaginous during pregnancy and lactation in diabetic rats and its implication in the offspring. Based on these considerations, the aim of this study was to evaluate the effect of maternal use of flaxseed oil and flaxseed flour during pregnancy and lactation upon histomorphometry of the left ventricle and on the blood pressure of the adult male offspring of diabetic rats.

2. Materials and methods

2.1. Experimental design

This research project was approved by the Ethics Committees for the Use of Animals from the Center of Laboratory Animals at the Federal Fluminense University (UFF), Niterói, Brazil under n. 681/2015. All procedures followed the norms of the National Research Council (US) Institute for Laboratory Animal Research.

Twenty-four Wistar rats from the Center of Laboratory Animals (NAL) colony from UFF, with approximately three months old, were kept in collective cages, with constant temperature (21 ± 2 °C), controlled light–dark cycle (12/12 h), and water and food ad libitum.

2.1.1. Experimental diabetes induction

Experimental diabetes induction was accomplished through a diet of high-energy density in combination with streptozotocin (STZ). From the total amount of animals used, 18 female rats were fed with a high-fat diet (60% of the total energy intake), in an initial period of 3 weeks, and 6 were fed with a diet based on casein; both were ad libitum. After 3 weeks on the high-fat diet, the rats were given an intraperitoneal injection of streptozotocin (Sigma Chemicals, St Louis, MO, USA) in a low dose (35 mg kg^{-1}) diluted in vehicle (0.01 mol L^{-1} sodium citrate solution, pH 4.5) in accordance with Correia-Santos et al. (2012). The rats which were fed the control diet received only the vehicle (0.01 mol L^{-1} sodium citrate solution, pH 4.5) via intraperitoneal injection. After this procedure, the rats continued to receive the diets for one more week.

2.1.2. Diabetes follow-up: mating, pregnancy and lactation

After the confirmation of diabetes (in which the serum concentration of glucose was above 300 mg/dL), the rats were sent to mating in the proportion of two female rats to each male, and were given either a high-fat diet or one based in casein, both of which had 17% of protein (AIN-93G) (Reeves, Nielsen, & Fahey, 1993). When gestation was confirmed through the vaginal plug method, the animals were caged individually and divided into four groups (n = 6): the control group (CG) which received a diet based on casein; the high-fat group (HG) which received a high-fat diet (49% of the total calories coming from lipids); the flaxseed flour group (FFG) which received a high-fat diet with an addition of 25% flaxseed flour; and the flaxseed oil group (FOG) which received a high-fat diet with an addition of flaxseed oil, which replaced the soybean oil (Table 1). The soybean oil presented 54% linoleic acid (n–6) and 8% of α-linolenic acid (n–3), showing n–6/n–3 as 7:1, the flaxseed oil presented approximately 16% of linoleic acid (n–6) and 57% of α-linolenic acid (n–3), showing n–6/n-3 as 1:3, and the flaxseed flour presented 7% of n–6 and 23% of n–3, showing n–6/n–3 as 1:3.3.

After the weaning, 21 days of life, six male pups of each group were separated and began being fed with a commercial diet (Nuvital, Nuvilab, PR, Brazil) ad libitum until 100 days of age, when rats were euthanized. Body weight (BW) and food consumption were recorded three times during the week.

2.2. Measurement of systolic blood pressure

When the animals reached three months of age, the systolic blood pressure (SBP) was measured, using the non-invasive method plethysmography caudal (tail plethysmograph V1.10 — Insight). Systolic blood pressure in mmHg was determined.

After preconditioning the containment chamber, the animals were pre-heated to 35 ± 2 °C for 5 min. The official SBP from each animal was calculated by averaging three consecutive stable measurements (with a difference of about 1 minamong them).

2.3. Blood collection and leptin determination

At 100 days of age, the rats received a lethal dose of Thiopentax (sodium thiopental of 1 g, Cristália Produtos Químicos Farmacêuticos Ltda., Brazil) at 5% (0.15 mL/100 g of body weight) by intraperitoneal injection. The blood was taken via cardiac puncture and put into tubes without anticoagulant, centrifuged (Sigma centrifuge) for 15 min at 3500 rpm to separate the serum. Serum leptin was analyzed using Multiplex Biomarker Immunoassays for Luminex xMAP technology (Milipore, Billerica, MA, USA, cat. no. RADPK-81K).

Table 1
Nutritional composition of experimental diets during gestation and lactation.

Ingredient	Diets			
	Control (g)	High-fat (g)	High-fat + flaxseed oil (g)	High-fat + flaxseed flour (g)
Casein[a]	190	230	230	200
Flaxseed flour[b]	0	0	0	250
Corn starch[c]	539.486	299.486	299.486	229.486
Sucrose[d]	100	100	100	100
Soybean oil[e]	70	70	0	0
Flaxseed oil[f]	0	0	70	0
Lard[g]	0	200	200	170
Cellulose[h]	50	50	50	0
Vitamin mix[i]	10	10	10	10
Mineral mix[i]	35	35	35	35
Cystine[j]	3	3	3	3
Choline bitartrate[j]	2.5	2.5	2.5	2.5
tert-butylhydroquinone (BHT)	0.014	0.014	0.014	0.014
Total (g)	1000	1000	1000	1000
Carbohydrates (% of total kcal)	64	32	32	31.6
Proteins (% of total kcal)	19	19	19	19.3
Lipids (% of total kcal)	17	49	49	49
Energy (kcal/kg)	3950	4950	4950	4954.7

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[f] Giroil Agroindustria Ltda. (Santo Ângelo, RS, Brazil).
[g] Sadia Comercial Ltda.
[h] Microcel da Blanver Ltda. (Cotia, SP, Brazil).
[i] PragSoluções Comércio e Serviços Ltda-ME (Jáu, SP, São Paulo).
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