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EFFECTS OF FLOUR OR FLAXSEED OIL UPON INTRA-ABDOMINAL ADIPOSITY IN MALE RATS SUBJECTED TO EARLY WEANING

B. Ferolla da Camara Boueri[1], C. Ribeiro Pessanha[1], A. D'Avila Pereira[1], D. Cavalcante Ribeiro[1], A. de Sousa dos Santos[2], C.C. Alves do Nascimento-Saba[2], C.A. Soares da Costa[1], G. Teles Boaventura[1]

Abstract: The present study was designed to evaluate intra-abdominal adiposity in rats subjected to early weaning and subsequently treated with diet containing flour or flaxseed oil until young life. Pups were weaned for separation from mothers at 14 days (early weaning, EW), and 21 days (control, C). After 21 days, control (C60) was fed with control diet. EW was divided into control (EWC60); flaxseed flour (EWFF60); flaxseed oil (EWFO60) diets until 60 days. At 21-60 day, intra-abdominal fat mass were evaluated. At 60 days, retroperitoneal adipocyte area was determined. At 21 days, EW group displayed lower ($P<0.05$) intra-abdominal fat mass. At 60 days, EWC60 group displayed lower ($P<0.05$) intra-abdominal fat mass. Adipocytes area were lower ($P<0.05$) in experimental groups. EWC60 displayed lower ($P < 0.05$) adipocytes area (vs. EWFF60 and EWFO60). Flour and flaxseed oil diets contribute to recovery of intra-abdominal adiposity after precocious interruption of lactation..

Key words: lactation, adipocytes, morphometry, development.

Introduction

Overweight and obesity are a public health problem in both developed countries and those in development.1 Studies suggest the importance of the first few years of life in establishing healthy patterns of growth and that breastfeeding may be a protective factor childhood obesity (1-3). According to the World Health Organization, exclusive breastfeeding is recommended for the first six months of life, followed by breastfeeding in combination with the introduction of complementary foods until at least 12 months of age. However, no more than 35% of infants worldwide are exclusively breastfed during the first months of life (4, 5).

In 1958 British cohort study, Parsons et al. (6) identified a J-shape curve in which restricted nutrition and low weights in early life is correlated with subsequent obesity at age thirty-three. Given the prevalence of precocious interruption of breastfeeding in humans, animal models emulating this phenomenon might provide useful information regarding deleterious effects of this procedure (7) on higher adiposity development. Previously, in experimental models, Maia et al. (8) and Boueri et al. (9) reported that early weaning decreases body adipogenesis at 21 days. Compared to adult life in human (10), Nobre et al. (11) related that early weaning programmed for higher visceral fat mass in rat at 180 days, corroborating the J-shape curve identified by Parsons et al. (6)

Lactation is a critical period for the programming of obesity. Indeed, hiperlipidic diet immediately after growth retardation induces a catch-up growth and leads to the programming of obesity at adulthood (12). However, normolipidic diet containing flaxseed (Linum usitatissimum) has potential to promote adipocyte hypertrophy down-regulation (13, 14). And considering that adult obesity prevention trials have largely focused on schoolchildren or adolescents (15), the present study was designed to evaluate intra-abdominal adiposity in rats subjected to early weaning and subsequently treated with diet containing flour or flaxseed oil until young life.

Materials and methods

The protocol used to deal with experimental animals was approved by Ethics Committee on Animal Research of Fluminense Federal University, Niteroi-RJ, Brazil

1. Laboratory of Experimental Nutrition, Departament of Nutrition and Dietetics, Fluminense Federal University, Niterói, RJ, Brazil; 2. Physiological Sciences, Institute of Biology Roberto Alcantara Gomes, State University of Rio de Janeiro, Rio de Janeiro, RJ, Brazil.

Corresponding Author: Carlos Alberto Soares da Costa. Laboratory of Experimental Nutrition, College of Nutrition, Fluminense Federal University. Rua Mário Santos Braga, 30, Niterói, RJ, 24015-110, Brazil. E-mail: nutcarlos@hotmail.com Telephone and fax number: +55 21 26299860

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Table 1
Intra-abdominal adiposity at 60 days

	C60	EWC60	EWFF60	EWFO60
Intra-abdominal fat mass (g)	10.570 ± 1.050[a]	6.521 ± 0.572[b]	10.720 ± 0.454[a]	9.226 ± 0.974[a]
Intra-abdominal fat mass (g/100g)	3.530 ± 0.353[a]	2.500 ± 0.162[b]	3.406 ± 0.135[a]	3.267 ± 0.250[a]
Gonadal fat mass (g)	4.469 ± 0.409[a]	3.109 ± 0.278[b]	4.517 ± 0.148[a]	4.027 ± 0.279[a]
Retroperitoneal fat mass (g)	3.499 ± 0.488[a]	1.740 ± 0.232[b]	3.552 ± 0.231[a]	3.148 ± 0.358[a]
Mesenteric fat mass (g)	2.606 ± 0.222[a]	1.672 ± 0.112[b]	2.656 ± 0.180[a]	2.557 ± 0.281[a]

C60 (n=9), control group weaning at 21 days. EW, experimental groups early weaning at 14 days and treated with control (EWC60, n=10), flaxseed flour (EWFF60, n=11) or flaxseed oil (EWFO60, n=10) diet, respectively during period of 21 to 60 days. a,bValues displaying different superscripts are significantly different (p<0.05, one-way variance analysis, followed by Newman-Keuls post-test). C, control; EW, early weaning; FF, flaxseed flour; FO, flaxseed oil.

(protocol 597/2014). All procedures were in accordance with the Brazilian Science and Laboratory Animals Society provisions and the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication N 85-23, revised in 1996).

Wistar rats from the Laboratory Animals Center of the Fluminense Federal University were housed in a temperature-controlled room (23 ± 1 ºC), humidity (60 ± 10%) with an artificial dark-light cycle (lights on from 7 am to 7 pm). Virgin female rats (3 months old) were caged with male rats, and, after mating, each female was placed in an individual cage with free access to water and standard laboratory food (Nuvilab®, Paraná, Brazil).

Within 24 h of birth, excess pups were removed, and only six male pups were kept per dam, a procedure maximizing lactation performance (16). During lactation period dams were fed a control diet containing 7 g soybean oil and 20 g casein/100g, in agreement with American Institute of Nutrition (AIN-93G) recommendations (17). Control group pups (C, n=9) were separated from their mothers on the 21st postnatal day. Pups in early weaning group (EW, n=31) were separated from their mothers on the 14th postnatal day (9, 18). Due to procedure difficulties for checking the non-maternal separated pups, free access to drinking water and control diet was not evaluated for C and EW pups until day 21. After separation from their mothers, rats from C and EW groups were held together in their original cage (up to six pups per cage).

At 21 days, body mass of six rats of control (C21, n=6) and six rats of EW (EW21, n=6) group were evaluated. They were then anesthetized with Thiopentax® (Sodium thiopental, 0.1mg/100g) and euthanized by exsanguination. Intra-abdominal fat mass were dissected and weighed. Masses were expressed as absolute (g) and relative (g/100g) mass (adjusted to body mass).

After 21 days, control animals (C60, n=9) were fed with control diet containing 20 g casein, 52.95g cornstarch, 7 g soybean oil and 5 g fiber/ 100g. EW animals were divided into three groups: EW fed with control diet (EWC60, n=10); EW fed with diet containing 25 g flaxseed flour, 45.84 of cornstarch and 15 g casein/ 100g (EWFF60, n=11); and EW fed with a diet containing 7 g flaxseed oil, 52.95 g cornstarch, 5 g fiber and 20 g casein/ 100g (EWFO60, n=10). Diets have same amounts of sucrose (20g), mineral (3.5g) and vitamin mix (1g), L-cystine (0.3g) and choline bitartrate (0.25g), per 100g. Flaxseed flour contains 17% protein, 45% carbohydrate and 26% fat, while the flaxseed oil contain 3.66g α-linolenic acid and 0.86g linoleic acid for each 7g. The 25g/100g flaxseed flour aimed to meet entire recommended fiber intake, and oil addition not being necessary to as this seed is a source of this component (18).

At 60 days, C60, EWC60, EWFF60 and EWFO60 groups were euthanized by exsanguination as described for C21 and EW21 animals. Intra-abdominal fat mass (Gonadal, retroperitoneal and mesenteric) were dissected and weighed. Retroperitoneal fat samples were collected and fixed in buffered formaldehyde, for morphological analyses. Tissue was embedded in paraffin, cut into 5-mm sections, and stained with hematoxylin-eosin (HE). Profiles with at least 100 adipocytes were randomly selected and captured for each animal, for morphometric analyses. Sectional area of adipocytes (mm2) was determined on digital images (TIFF format, 36 bit color, 1360 X 1024 pixels) acquired with an Optronics CCD video camera system and Olympus BX40 light microscope and analysed with the U.S. National Institutes of Health IMAGE-J software http://rsbweb.nih.gov/ij/(NIH, USA) (13, 14).

Statistical analyses were carried out using Graph Pad Prism statistical package version 5.0, 2007 (San Diego, CA, USA). The results at 21 days were analyzed by Student's t-test. The remaining results were analyzed using one-way variance analysis, followed by Newman-Keuls post-test and expressed as means ± S.E.M. with significance level of 0.05.

Results

At 21 days, experimental group shown lower absolute ($P < 0.05$, EW21: 0.199 ± 0.038 v. C21: 0.442 ± 0.064 g) and relative ($P < 0.05$, EW21: 0.544 ± 0.081 v. C21: 0.966 ± 0.127 g/100g) intra-abdominal fat mass.

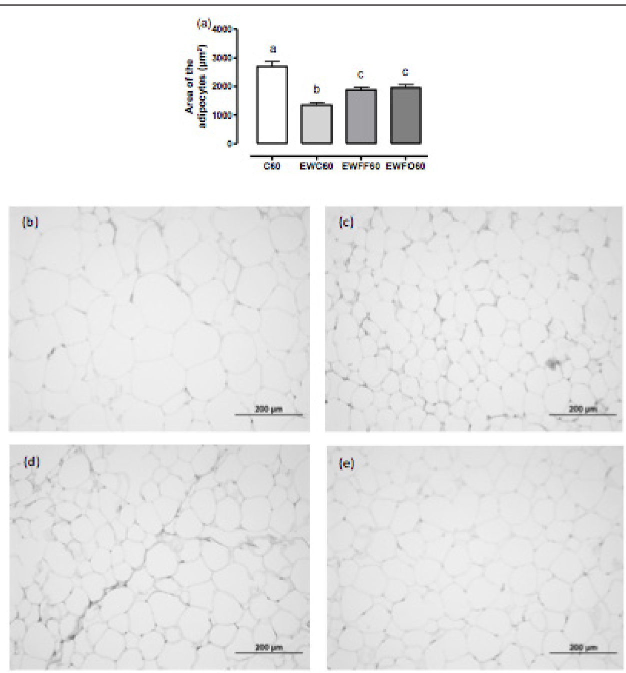
At 60 days, EWC60 group shown lower ($P < 0.05$)

intra-abdominal (absolute and relative), gonadal, retroperitoneal and mesenteric fat mass when compared to the other groups (Table 1).

Adipocytes area were lower ($P < 0.05$) in experimental groups (EWC60: 1349 ± 95.27; EWFF60: 1877 ± 94.09 and EWFO60: 1953 ± 112.20 μm2) compared to control group (2695 ± 182.80 μm2). EWC60 shown lower ($P < 0.05$) adipocytes area compared to EWFF60 and EWFO60 groups (Figure 1).

Figure 1

Area of the adipocytes (a) and photomicrographs of the adipocytes, staining with HE, original magnification (200X). C60 (n=9, b) and EWC60 (n=10, c) groups treated with control diet. EWFF60 (n=11, d) and EWFO60 (n=10, e) treated with diet containing 25/100g flaxseed flour or 7g/100g flaxseed oil, respectively, and analyzed at 60 days. a,b,cValues displaying different superscripts are significantly different (p<0.05, one-way variance analysis, followed by Newman-Keuls post-test). C, control; EW, early weaning; FF, flaxseed flour; FO, flaxseed oil; HE, hematoxylin-eosin



Discussion

Infancy is a critical period of growth and physiological development, in which breast milk plays important role for infant nutrition. Deviations in growth in early life are associated with increased risk of disease in the short and long term (4, 19). We have considered period of 14-21 days corresponds to second 6 months of life in humans because at 14 days of life, rats begin consumption of solid food, at this age, pups still breastfeed (18, 20). At birth, body fat accounts for ~15% of weight, and this increases to ~25% at six months, peaking at ~30% at 12 months.4 However, precocious interruption of lactation was associated with lower body mass and intra-abdominal fat mass development at 21 days.

Babies in lower socioeconomic groups have sustained degrees of nutritional deprivation, usually followed by continuous malnutrition until early adolescence (21). In the present study, food intake was similar between groups (18), even so EWC60 group showed lower body mass and intra-abdominal fat development, corroborating with Costa et al. (22) that observed, after maternal malnutrition during lactation, lower body development and permanent changes in Wistar male rat metabolism and structure at 60 days.

One of the strategies for the treatment of malnutrition is to increase energy density of foods by increasing the lipid content. However, high fat diet did not contribute to recovery from maternal malnutrition during lactation (20). Contrary, EWFF60 and EWFO60 groups, treated with normo -caloric and -fat diets containing flour or flaxseed oil, respectively, showed recovery of intra-abdominal fat mass. The composition may be more relevant than high energy density in the dietary treatment of malnutrition.

Flaxseed (Linum usitatissimum) has been described as an excellent alpha- linolenic acid (ALA, 18: 3n-3) source, presenting an average of 30% lipids in its composition, with 51% to 55% corresponding to ALA (23, 24). Regarding adiposity depots, Costa et al. (13) and Ribeiro et al. (14) reported lower intra-abdominal adipocyte area in rats fed flaxseed diet, in early and adult life, respectively. ALA induces the fatty acid oxidation genes through peroxisome proliferator-actived receptor alpha (PPARα) and the suppression of lipogenic genes through sterol regulatory element-binding protein (SREBP-1C), decreasing the size of adipocytes (25, 26). Although the present study did not displayed blood parameters regarding to obesity and or metabolism, these pathways help to explain the decrease in adipocyte area in EWFF60 and EWFO60 groups.

However, Boueri et al.9 after evaluation of body composition by dual-energy X-ray absorptiometry, observed a phenomenon of "catch-up fat" in EWFO60 group, because rats showed lower lean and bone mass, and fat mass similar to control group. Findings support an adverse effect of flaxseed oil on adiposity, with likely implications for obesity in adult life. Contrary, flaxseed flour was associated with adequate adiposity recovery regardless of body mass. Probably the composition of flour contributed to the outcomes, because in addition to ALA, flaxseed flour contains high-quality protein, carbohydrates and minerals distributed among phenolic acids, lignin and hemicelluloses (13, 14, 18).

The potential implications of the present findings for the treatment of malnutrition in early period of life are of great interest and importance. Flour and flaxseed oil diets contributes to recovery of intra-abdominal adiposity in young life. Nevertheless, further studies are necessary to clarify if flour and flaxseed oil treatment, after precocious interruption of lactation, contributes to obesity prevention in adult life.

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Conflict of interest: The authors declare that there no conflict of interest.

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