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BRIEF REPORT

Body composition in male rats subjected to early weaning and treated with diet containing flour or flaxseed oil after 21 days until 60 days

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The aim of this study was analyzed if the flour or flaxseed oil treatment contributes to body composition in male rats subjected to early weaning. Pups were weaned for separation from mother at 14 (early weaning, EW) and 21 days (control, C). At 21 days, part of the pups was evaluated (C21 *v.* EW21). After 21 days, control (C60) was fed with control diet. EW was divided in control (EWC60); flaxseed flour (EWFF60); flaxseed oil (EWFO60) diets until 60 days. Body mass, length and body composition by dual-energy X-ray absorptiometry were determined. EW21 (*v.* C21) and EWC60 (*v.* C60 and EWFF60) showed lower ($P < 0.05$) mass, length and body composition. EWFO60 (*v.* C60 and EWFF60) showed lower ($P < 0.05$) body mass and length, body and trunk lean mass, bone mineral density and content and bone area. Flaxseed flour, in comparison with flaxseed oil, contributes to recovery of body composition after early weaning.

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Key words: DXA, early weaning, flaxseed flour, flaxseed oil, rat

Introduction

Breastfeeding contributes to improved infant health outcomes in both industrialized and developing countries and it is recommended as the unequaled method for feeding infants.[1] Some studies have shown that duration and exclusivity of breastfeeding is protective in reducing and preventing chronic diseases, such as obesity and osteoporosis.[2,3] However, no more than 35% of infants worldwide are exclusively breastfed during the first months of life.[4]

Given the prevalence of precocious interruption of breastfeeding in humans, animal models that emulate this phenomenon might provide useful information regarding the deleterious effects of this procedure on development and health.[5] And it is well known that an adequate nutrient supply during early life is essential to establish the metabolic status.[6] In this context, bioactive component such as polyunsaturated fatty acids, have been used in the prevention of chronic diseases associated with higher adiposity,[7,8] and could act in the body development after early stages of life.

Alpha linolenic acid (ALA, 18: 3n-3) from plant sources, have been studied due to its potential beneficial effects in the body composition. Based in the previous wistar rat models, our group reported the effects of flaxseed (*Linum usitatissimum* L.), containing high concentrations of ALA, in the adequate offspring growth and prevention of overweight in adult life.[9,10] Taken together, these observations suggest that flaxseed could be a promoter for the healthy development after precocious interruption of lactation. Thus, in the present study, we evaluated the body composition in rats subjected to early weaning and subsequently treated with diet containing flour or flaxseed oil after 21 days until 60 days.

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 (protocol 597/2014). All procedures were in accordance with the provisions of Brazilian Society of Science and Laboratory Animals 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 Centre of Laboratory Animals of Fluminense Federal University were housed under a temperature-controlled room (23 ± 1°C), 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, because this procedure

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maximizes lactation performance.[11] During the lactation period, the dams were fed a control diet containing 7 g of soybean oil and 20 g of casein/100 g, in agreement with American Institute of Nutrition (AIN-93G) recommendations.[12] The pups from the control group (C, $n = 15$) were separated from the mothers on the 21st postnatal day. The pups in the early weaning group (EW, $n = 37$) were separated from their mothers on the 14th postnatal day. Due to procedure difficulties to check the non-maternal separated pups, the free access to drinking water and control diet was not evaluated in the C and EW pups until day 21. After the separation from their mothers, the rats from C and EW groups were maintained together in their original cage (up to six pups per cage).

At 21 days, six rats of control (C21, $n = 6$) and six rats of EW (EW21, $n = 6$) were randomly separated, and after 2 h of fasting, body mass and length (cm, measured as distance between tip nose to tip tail) were evaluated. They were then anesthetized with Thiopentax® (sodium thiopental, 0.1 mg/100 g) and subjected to dual-energy X-ray absorptiometry (DXA) using a Lunar DXA 200368 GE instrument (Lunar, with software encore 2008 version 12.20, GE Healthcare, Madison, Wisconsin). The evaluation was blind, since the DXA technician did not know the experimental protocol. Body fat (g and %) and lean mass (g), trunk fat (g and %) and lean (g) mass, and bone analysis [bone mineral density (BMD) total (g/cm^2); bone mineral content (BMC) total (g/cm^2), bone area (cm^2)] were measured for each rat.[10,13,14]

After 21 days, the remaining control animals (C60, $n = 9$) were fed with control diet containing 20 g of casein, 52.95 g of cornstarch, 7 g of soybean oil and 5 g of fiber/100 g. The remaining EW animals were divided into three groups: EW fed with control diet (EWC60, $n = 10$); EW fed with diet containing 25 g of flaxseed flour, 45.84 g of cornstarch and 15 g of casein/100 g (EWFF60, $n = 11$); and EW fed with a diet containing 7 g flaxseed oil, 52.95 g of cornstarch, 5 g of fiber and 20 g of casein/100 g (EWFO60, $n = 10$). The diets have same amounts of sucrose (20 g), mineral (3.5 g) and vitamin mix (1 g), L-cystine (0.3 g) and choline bitartrate (0.25 g), per 100 g. The flaxseed flour contains 17% of protein, 45% of carbohydrate and 26% of fat, while the flaxseed oil contains 3.66 g of α-linolenic acid and 0.86 g of linoleic acid for each 7 g. The 25 g/100 g of flaxseed flour aimed to meet the entire recommended fiber intake and it was not necessary to add oil because this seed is a source of this component.[15] After 39 days of treatment, at 60 days, the C60, EWC60, EWFF60 and EWFO60 groups were anesthetized and evaluated as described for the C21 and EW21 animals.

Statistical analyses were carried out using the 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 analysis of variance, followed by Newman–Keuls post-test and expressed as means ± S.E.M. with significance level of 0.05. In addition, the precision was expressed as coefficient of variation (CV, [standard deviation/measured mean concentration] × 100).

Results

At 21 days, the experimental group showed lower body mass ($P < 0.05$) and similar body length. With regard to body composition analyses by DXA, the EW21 showed lower ($P < 0.05$) mass and percentage of body fat; similar body lean mass; lower ($P < 0.05$) mass and percentage of trunk fat and lower ($P < 0.05$) trunk lean mass. BMD ($P < 0.05$) and BMC ($P < 0.05$) were lower in EW21 group. Bone area was not significantly different among the groups, even so the EW21 showed lower values (−24%) compared with the C21 (Table 1).

Table 1. *Body mass, length and body composition analyzed by DXA, at 21 days*

	C21			EW21		
	Mean	S.E.M.	CV	Mean	S.E.M.	CV
Body mass (g)	45.25^a	1.21	6.58	37.42^b	1.97	12.94
Body length (cm)	17.58	0.32	4.56	16.75	0.42	6.19
Body fat mass (g)	9.83^a	1.49	37.18	5.16^b	0.40	19.03
Body fat mass (%)	24.68^a	3.80	37.74	13.94^b	0.57	9.29
Body lean mass (g)	29.33	1.78	14.89	28.80	0.66	5.15
Trunk fat mass (g)	3.83^a	0.87	55.75	1.20^b	0.20	37.27
Trunk fat mass (%)	18.38^a	1.27	15.48	13.14^b	0.31	5.40
Trunk lean mass (g)	12.00^a	0.77	15.81	9.00^b	0.54	3.61
BMD (g/cm^2)	0.0571^a	0.0013	5.58	0.0468^b	0.0007	4.14
BMC (g)	0.6667^a	0.0714	26.27	0.4333^b	0.0333	18.84
Bone area (cm^2)	11.67	1.28	26.92	8.80	0.37	9.51

C, control; EW, early weaning; S.E.M., standard error of the mean; CV, coefficient of variation; DXA, dual-energy X-ray absorptiometry; BMD, bone mineral density; BMC, bone mineral content.

[a,b]Values with different superscripts are significantly different ($P < 0.05$, Student's *t*-test).

C21 ($n = 6$), control group weaning at 21 days. EW21 ($n = 6$), experimental group early weaning at 14 days.

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At 60 days, the EWC60 group showed lower ($P < 0.05$) body mass and length, body and trunk fat and lean mass, BMD, BMC and bone area compared with C60 and EWFF60. C60 and EWFF60 showed similar results. EWFO60 group showed lower ($P < 0.05$) body mass and length, body and trunk lean mass, BMD, BMC and bone area compared with C60 and EWFF60. Body and trunk fat mass of EWFO60 were similar when compared with C60 and EWFF60 (Table 2).

Discussion

Optimal nutrition during childhood is critical to support the growth and development during the first 12 months following birth.[16] The current study evaluated rat deprived of breast milk at 14 days, when it begins the consumption of solid food. At this age, pups still breastfeed, but they can survive independently of their mothers.[15,17] However, the lack of calories, macro and micronutrients derived from milk were not offset, justifying the lower body parameters observed in the EW21 rats.

The period of 21 until 60 days was characterized as recovery phase with the purpose to stimulate the catch-up effect, considered a physiological adaptation that allows man and animals to return to their genetically programed growth trajectory after a period of growth retardation. The catch-up is dependent on the amount of food intake, efficiency of the utilization of energy and the type of dietary fat composition.[18,19] The EWC60 group corroborated with Ozanne *et al.*,[20] which showed that animals that were growth deprived during the lactation period remained permanently smaller. In fact, babies in lower socioeconomic groups have sustained degrees of prenatal nutritional deprivation, usually followed by continuous malnutrition until early adolescence.[21] EW groups showed similar food intake,[15] nevertheless body parameters remained smaller in the EWC60, which may be related to the diet containing fat provide by soybean oil, while the EWFF60 and EWFO60 were fed with diet containing fat of flaxseed.

Animal studies evidence that the intake of a high LA/ALA ratio during postnatal life promotes early adipose tissue growth, whereas ALA has anti-adipogenic properties, inducing the fatty acid oxidation genes through PPARα (peroxisome proliferator-actived receptor alpha) and the suppression of lipogenic genes through SREBP-1c (sterol regulatory element-binding protein).[10,13] In addition, ALA alters gene expression in skeletal muscle upregulate anabolic pathways, resulting in greater lean tissue mass.[22] With regard to bone structure, Ribeiro *et al.*,[10] Costa *et al.*[13] and Farina *et al.*[23] reported that the combination of ALA intake and the smaller amounts of LA were associated with protective effect to bone formation by osteoblast activity. To our knowledge, this is the first study to address the effect of flour or flaxseed oil in body composition of rats subjected to early weaning. When compared with EWC60 and EWFO60 groups, the flaxseed flour treatment contributes to recovery of body composition. The flaxseed flour contains lipids (50–55% as ALA and 15–18% as LA), fibers, protein,

Table 2. *Body mass, length and body composition analyzed by DXA, at 60 days*

	C60			EWC60			EWFF60			EWFO60		
	Mean	S.E.M.	CV	Mean	S.E.M.	CV	Mean	S.E.M.	CV	Mean	S.E.M.	CV
Body mass (g)	308.10[a]	5.29	8.88	258.00[b]	10.11	12.37	314.70[a]	4.31	4.55	271.20[b]	8.62	11.75
Body length (cm)	39.75[a]	0.21	2.04	38.22[b]	0.40	3.14	39.95[a]	0.32	2.71	37.80[b]	0.46	3.90
Body fat mass (g)	63.00[a]	5.57	26.55	44.40[b]	2.60	18.52	66.36[a]	2.32	11.64	60.90[a]	4.68	24.32
Body fat mass (%)	22.28[a]	1.49	20.10	18.48[b]	0.60	10.33	22.74[a]	0.81	11.85	23.72[a]	1.12	14.94
Body lean mass (g)	216.20[a]	4.58	6.37	184.80[b]	11.37	19.46	225.70[a]	4.43	6.52	196.60[b]	5.86	9.44
Trunk fat mass (g)	46.11[a]	4.37	28.45	30.30[b]	1.86	19.43	46.64[a]	2.82	20.05	39.90[a]	3.64	28.90
Trunk fat mass (%)	21.31[a]	1.43	20.25	17.25[b]	0.60	11.85	21.32[a]	0.85	13.26	22.11[a]	1.16	16.69
Trunk lean mass (g)	164.80[a]	8.19	14.92	142.00[b]	5.80	12.93	167.70[a]	4.47	8.84	134.30[b]	5.82	13.71
BMD (g/cm^2)	0.1149[a]	0.0023	6.07	0.1047[b]	0.0014	4.44	0.1139[a]	0.0011	3.25	0.1078[b]	0.0022	6.67
BMC (g)	6.089[a]	0.239	11.80	4.950[b]	0.185	11.83	6.127[a]	0.112	6.07	5.310[b]	0.246	14.68
Bone area (cm^2)	52.67[a]	1.26	7.23	47.20[b]	1.26	8.46	53.64[a]	0.69	4.27	49.10[b]	1.29	8.34

C, control; EW, early weaning; FF, flaxseed flour; FO, flaxseed oil; S.E.M., standard error of the mean; CV, coefficient of variation; DXA, dual-energy X-ray absorptiometry; BMD, bone mineral density; BMC, bone mineral content.

[a,b]Values with different superscripts are significantly different ($P < 0.05$, one-way analysis of variance, followed by Newman–Keuls post-test).

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 until 60 days.

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minerals and carbohydrates distributed among phenolic acids, sugars, lignin and hemicelluloses.[9,10,15] Probably, the composition of flaxseed flour contributes to a greater biological utilization of nutrients and to recovery of fat and lean mass, and of bone structure.

Although the EWFO60 group showed similar body and trunk fat mass, when compared with the EWFF60 and C60 groups, we consider that EWFO60 showed high development of body fat mass, because the lean mass and bone content are reduced. The flaxseed oil contains high concentration of ALA, however, to our surprise a phenomenon of 'catch-up fat', with sparing energy was observed.[20,23] Further studies are necessary to investigate whether changes in energy expenditure are involved.

The present data lend support to the hypothesis that flaxseed flour, in comparison with flaxseed oil, contributes to recovery of body composition after EW. Thus, flaxseed flour intake seems to be a viable approach for the body growth after precocious interruption of lactation.

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Conflicts of Interest

None.

Ethical Standards

The protocol used to deal with experimental animals was approved by Ethics Committee on Animal Research of Fluminense Federal University, Niteroi-RJ, Brazil (protocol 597/2014). All procedures are in accordance with the provisions of Brazilian Society of Science and Laboratory's Animals and the Guide for the Care and Use of Laboratory Animals.

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