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Linum usitatissimum (Flaxseed) Oil during Postpartum Period Contributes to Lean Mass and Healthy Serum Lipid Profile in Rats

Bianca Ferolla da Camara Boueri[1,*], Carolina Ribeiro Pessanha[1], Aline D'Avila Pereira[1], Danielle Cavalcante Ribeiro[1], Fernanda Carvalho de Santana[2], Jorge Mancini-Filho[2], Carlos Alberto Soares da Costa[1], Gilson Teles Boaventura[2]

[1]Department of Nutrition and Dietetics, Fluminense Federal University, Niterói, Brazil
[2]Department of Food Science and Experimental Nutrition, University of São Paulo, São Paulo, Brazil
*Corresponding author: biancaferolla@hotmail.com

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Abstract There is a dearth of efficient strategies to support postpartum among women in order to protect them from chronic diseases in later life. Flaxseed oil (FO) is a food derived oil that has functional ingredients as alpha-linolenic acid (ALA). The objective of this study was to investigate the influence of FO during postpartum period in body parameters and lipid profile in rats. After the delivery of their pups, rats were randomly divided into two groups: control – diet with soybean oil as fat source- or FO – diet with FO as a fat source. After 51 days offering experimental diets, each group was evaluated on body composition, intra-abdominal fat, serum lipid profile and polyunsaturated fatty acids. The diet based on FO recorded high serum levels of ALA ($P<0.0001$) and eicosapentaenoic fatty acids ($P<0.05$). The diet also recorded a decrease in gamma-linolenic ($P<0.05$), dihomo-gamma-linolenic ($P<0.05$) and arachidonic fatty acids ($P<0.0001$). These aforementioned results lead to the activation of metabolic and physiologic pathways that provided higher lean mass ($P<0.05$), lower results on total cholesterol ($P<0.05$) and low density cholesterol ($P<0.05$). Hence, consumption of FO during postpartum can promote lean mass and healthy body composition, better lipid profile and contribute to chronic disease prevention.

Keywords: *flaxseed oil, alpha-linolenic acid, body composition, postpartum period, rats*

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1. Introduction

Chronic noncommunicable diseases (CND) such as cardiovascular diseases, diabetes and cancer are among the main causes of deaths worldwide [1]. Certain Life stages' of women such as pregnancy and postpartum, require special attention in order to prevent changes in metabolism and physiology. Those changes could lead to future health disturbances related to CND [2,3]. Accumulation of body mass in high fat, variation in triglycerides and cholesterol levels are some of the changes that happen in the maternal body in order to support the new-life and lactation [4,5,6]. However, physiological and structural changes return to normal during postpartum period. Nevertheless, in some cases they may persist and compromise the mother's health in later life [2,5,6,7].

As a potential nutritional strategy, the flaxseed (*Linum usitatissimum*), has a unique composition of nutritional and functional ingredients. Flaxseed is rich in polyunsaturated fatty acids (PUFA), mainly alpha-linolenic acid (ALA) a small chain form of omega-3 (n-3) [8,9]. Polyunsaturated fatty acids are represented for n-3 and omega-6 (n-6) and they are essential in mammalian diets. Alpha-linolenic acid is obtained from diets and can be converted into a long chain eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). However, the n-6 small chain, called linoleic acid (LNA), gets converted into long chain arachidonic acid (ARA) [8]. An increased production of eicosanoids from ARA's metabolism can be associated with effects that could lead to CND such as cardiovascular disease, diabetes and obesity [10]. However, the addition of dietetic sources rich in n-3 fatty acids such as flaxseed in a diet, have provided positive results, such as in body mass, adiposity and lipid profile [11-16]. Flaxseed oil (FO) is an important source of n-3 fatty acids that can be consumed as a supplement or food ingredient. The advantages of FO compared to fish oils are: it is more sustainable, presents less chances of mercury toxicity and it can be introduced in a vegetarian diet [17].

Animal studies are useful to simulate stages periods of human life, such as postpartum. In order to understand

how nutrients can affect health parameters and help mothers in establishing health promoting changes to prevent CND in later life. Flaxseed oil is rich in ALA and when it is added to a diet might help mothers achieve a better body composition and lipid profile. The objective of this study was to investigate the influence of FO during postpartum period in female rats for studying body parameters and lipid profile.

2. Materials and Methods

The present study was approved by the Fluminense Federal University Ethical Committee on Animal Research (887/2017). All procedures were in accordance with the Brazilian Science and Laboratory Animals Society and the Guide for Care and Use of Laboratory. During the study animals were placed in biotery with controlled temperature (23 ± 1°C), humidity (60 ± 10%) and an artificial system of light that was on from 7:00 to 19:00 hours.

Twelve female *Wistar* rats, ninety-day-old and nulliparous were subjected to mate and thereafter they were put in individual cages. Female rats received free standard diet (Nuvilab-CR1, Paraná, Brazil) and *ad libitum* water until the birth of pups. In first 24 hours after birth, pups were adjusted by six pups per mother in order to maximize lactation performance [18]. The mother rats were randomly assigned to either control C, (n=6) or FO (n=6) group and were offered two different semi-purified diets based on American Institute of Nutrition (AIN-93) recommendations (Table 1) [19]. The difference between C and FO groups was the fat source offered through diet: soybean oil or FO, respectively.

Table 1. Composition of experimental diets

Ingredients (g/100g)	AIN-93G		AIN-93M	
	C	FO	C	FO
Casein	20	20	14	14
Cornstarch	52.95	52.95	62.07	62.07
Sucrose	10	10	10	10
Soybean oil	7	-	4	-
Flaxseed oil	-	7	-	4
Alpha-linolenic acid	0.49	3.66	0.28	2.09
Linoleic acid	3.57	0.86	2.04	0.49
Cellulose	5	5	5	5
Mineral mix (AIN-93M)	-	-	3.5	3.5
Mineral mix (AIN-93G)	3.5	3.5	-	-
Vitamin mix	1	1	1	1
L-Cystine	0.3	0.3	0.18	0.18
Choline bitartrate	0.25	0.25	0.25	0.25
Tert-Butylhydroquinone	0.014	0.014	0.008	0.008

C, control; FO, flaxseed oil. Diets were formulated based on the American Institute of Nutrition AIN-93G and AIN-93M recommendation for rodent diets. C and FO, fed a diet containing casein, mineral and vitamin mix, L-cystine, choline bitartrate, Pragsolucões®; cornstarch, cellulose, FARMOS®; soyabean oil, Liza®; sucrose, União®; flaxseed oil: Giroil Agroindustria Ltda.

Flaxseed oil (Giroil Agroindustria LTDA, Santo Ângelo, RS, Brazil) was derived from brown flaxseed bought at a local market. The FO used in this study differs from soybean oil in fatty acid composition. Flaxseed oil has an interesting fatty acid composition. It is richer in PUFA (73%) than saturated (9%) and monounsaturated fatty acids (18%) [11]. Flaxseed oil presents 3.66g of ALA and 0.86g of LNA in 7 mL (Table 1). In the first 21 days of experimental period diets, there was an increase in calories to support lactation period (AIN-93G). After 21 days of lactation, rodent pups were separated from their mothers. Dams remained in experimental protocol and diets were adjusted for maintenance period (AIN-93M) until 51 postpartum. Postpartum protocol studies have suggested the period of 30 days after weaning as a potential moment to implement nutritional strategies using flaxseed upon adiposity and health parameters [12,13]. The study incorporated post-weaning period, 21 days of lactation period and 30 days of post-lactation. During experimental period, food and water continued to be *ad libitum* and food intake was measured (g) weekly for each group.

At the 51st day postpartum female rats were fasted for 8 hours and were weighted (g). The animals were anesthetized with Thiopentax® (Sodium Tiopental, 0.1mg/100g) and submitted for body composition analysis (fat mass, lean mass and trunk fat mass in g) by Dual-energy X-ray absorptiometry (DXA) using densitometer Lunar IDXA 200368 GE (Lunar, Wisconsin, USA) and using a software for small animals (encore 2008 Version 12.20 GE Healthcare). After body composition, animals were submitted to euthanasia through total exsanguination, in order to collect blood via cardiac puncture. The rodents had their compartments of intra-abdominal fat dissected and weighted (g): mesenteric, retroperitoneal and gonadal fat.

Samples of blood were centrifuged to obtain serum. Part of the serum samples were put in a biochemical automatic analyzer by colorimetric method (Bioclin BS-120, Belo Horizonte, MG, Brazil) to obtain: total cholesterol, high density lipoprotein cholesterol (HDL-c) and triglycerides (mg/dL). Low density lipoprotein cholesterol (LDL-c) and very low density lipoprotein cholesterol (VLDL-c) were calculated using Friedewald et al. (1972) and by Norbet (1995) formula, respectively [20,21]. The other part of serum samples were analyzed by gas chromatography. The gas chromatography (Shimadzu GC 17A) was equipped with a flame ionization detector (FID), COA-20 automatic injector and a GC10 class workstation. The separation of fatty acids was performed using a SP-2560 fused silica column (bis-cianopropilo polissiloxano, 100m x 0,25mm x 0,2mm, Supelco, Bellefonte, USA). The methodology was described in AOAC Official Methods 996.06 (2002) and with some considerations based on the study by Costa et al. (2016) [15,22]. Quantification of LNA, gamma-linolenic acid (GLA), ALA, eicosadienoic acid, dihomo-gamma-linolenic acid (DGLA), ARA, EPA, docosapentaenoic acid, DHA and total PUFA were determined (μmol/mL).

The data was analyzed by Student's t test by Graph Pad Prism (San Diego, CA, EUA) program. The results were expressed in mean ± standard error of mean (EPM) considering significance level $P<0.05$.

3. Results and Discussion

In the present study FO group presented lower serum n-6: GLA ($P<0.05$), DGLA ($P<0.05$), ARA ($P<0.0001$) and also a higher serum n-3: ALA ($P<0.0001$) and EPA ($P<0.05$) vs. C group (Table 2).

Table 2. Polyunsaturated fatty acid composition by gas chromatography at 51-day postpartum

Fatty Acid (µmol/mL)	C	FO	
	Mean ± SEM	Mean ± SEM	P-value
18:2n-6 - Linoleic	1269.00 ± 104.6	1213.00 ± 60.06	0.6578
18:3n-6 - Gamma-linolenic	30.73 ± 3.74	17.66 ± 1.09*	0.0283
18:3n-3 - Alpha-linolenic	34.67± 5.32	325.00 ± 28.32***	0.0001
20:2n-6 - Eicosadienoic	54.62 ± 19.30	28.13 ± 11.20	0.2801
20:3n-6 - Dihomo-gamma-linolenic	270. 50 ± 74.01	32.93 ± 5.28*	0.0186
20:4n-6 - Arachidonic	2134.00 ± 48.79	956.00 ± 117.00***	0.0001
20:5n-3 - Eicosapentaenoic	244.30 ± 74.06	461.10 ± 30.09*	0.0350
22:5n-3 - Docosapentaenoic	151.50 ± 43.30	109.90 ± 43.50	0.5234
22:6n-3 - Docosahexaenoic	171.50 ± 23.17	170.70 ± 11.78	0.9760
Total polyunsaturated fatty acids	4353.00 ± 241.50	3310.00 ± 223.30*	0.0193

Control (C, n = 6) group, treated with a control diet, and flaxseed oil (FO, n = 6) group, treated with diet containing flaxseed oil at 51-day postpartum; SEM standard error of the mean; significantly different to the control group (Student's t test, *P<0.05; ***P<0.0001; no significance P<0.05).

Table 3. Food intake, body mass, body composition by DXA, intra-abdominal fat mass and lipid profile at 51-day postpartum

	C	FO	
	Mean ± SEM	Mean ± SEM	P-value
Food intake (g)	60.81 ± 6.12	62.90 ± 6.36	0.82
Body mass (g)	265.60 ± 6.10	285.10 ± 4.63 *	0.02
Fat mass (g)	67.29 ± 2.64	74.29 ± 5.71	0.28
Lean mass (g)	174.50 ± 4.84	192.30 ± 2.87 *	0.01
Trunk fat mass (g)	50.14 ± 3.41	51.27 ± 5.02	0.81
Retroperitoneal fat (g)	4.16 ± 0.16	4.43 ± 0.60	0.69
Mesenteric fat(g)	3.49 ± 0.26	3.53 ± 0.25	0.90
Gonadal fat (g)	6.55 ± 0.55	6.05 ± 0.52	0.52
Intra-abdominal fat (g)	13.90 ± 1.03	13.64 ± 1.07	0.86
Total cholesterol (mg/dL)	70.43 ± 3.55	58.00 ± 3.48 *	0.03
VLDL-c (mg/dL)	11.88 ± 1.09	12.37 ± 1.82	0.83
LDL-c (mg/dL)	30.72 ± 3.97	16.97 ± 2.82 *	0.02
HDL-c (mg/dL)	28.86 ± 1.01	29.00 ± 1.80	0.95
Triglycerides (mg/dL)	59.40 ± 5.45	61.83 ± 9.09	0.83

Control (C, n = 6) group, treated with a control diet, and flaxseed oil (FO, n = 6) group, treated with diet containing flaxseed oil at 51-day postpartum; SEM = standard error of the mean; *significantly different from the control group (Student's t test, P<0.05; no significance P<0.05).

Similar results were found for Ribeiro et al. (2017) and Pereira et al. (2016) have found an increased serum of ALA and EPA and decreased ARA offering diets containing flaxseed flour and oil, respectively [23,24]. Small chain PUFA ALA and LNA could be transformed to long chain PUFA by the organism. Nevertheless, there is a competition between ALA and LNA for the same elongation and desaturation enzymes [25]. The higher concentration of ALA in FO fatty acid composition contributed to the lower ratio of n-6/n-3. The aforementioned results contributed to diminish the competition between n-6 and n-3 short chain PUFA for desaturation and elongation enzymes, leading to increased levels of EPA as observed in the results from the study.

Rats treated with control and FO diets during 51 days of postpartum had no difference in food intake (Table 3). However, the FO group presented an increase in body mass (P<0.05) vs. C group. Similar results were found in Pereira et al. (2016) and Abreu et al. (2018) with diets containing FO in puppies over lactation period or flaxseed flour during 180 days in male rats, respectively [24,26]. Ribeiro et al. (2017) studied the influence of a diet with flaxseed flour during postpartum period and found no difference in body mass [23]. In this study, it is possible to ascribe the increase in body mass by a significantly high lean mass (P<0.05) in FO group vs. C group. However, it is possible to associate postpartum period with lean mass loss in humans as it depends on factors, such as exercise, lactation and diet [27,28]. A low amount of lean mass can contribute to an increase in body fat and obesity due to the decrease in energy metabolism. Besides obesity, other CND can be associated with decreased lean mass, demonstrating the importance of alleviating loss of lean mass [29].

In this study, FO - rich in ALA – has shown to increase levels of lean mass, which positively effects the body composition in postpartum period. Galmiche et al. (2016) presented that a high-fat diet, rich in ALA or long-chain n-3, could preserve muscle mass during weight loss in adult rats [30]. That occurred by enriching sarcolemma with long-chain n-3 PUFA which provides membrane fluidity and insulin sensitivity in muscle together with the downregulation of genes involved in proteolysis. Other experimental studies suggest an anabolic answer from long-chain PUFA due a greater activation of AKT, mTOR and /or p70s6K signaling pathways in skeletal muscle [31,32]. Costa et al. (2016) suggests that increase in lean mass in rats treated with ALA-rich diet occurred due to differentiation in mesenchymal stem cells in skeletal and smooth muscles [15]. In the present study, a similar physiological mechanism may have occurred in the FO group. The ALA-rich diet contributed to an increased lean

mass and significantly enhanced body composition in postpartum rats.

Fat mass gain, mainly visceral fat, can be associated with postpartum period and parity [27,33]. The accumulation of visceral fat is recognized as a marker of metabolic disorders such as hypertension, changes in lipid metabolism, insulin resistance and thrombotic prevalence [34]. Changes in body fat components during postpartum can be a risk for CND [33,35]. Polyunsaturated fatty acids n-3 is described in scientific literature as a possible nutrient to reduce adiposity through several pathways: reducing triglycerides deposition in fat tissue; increasing mitochondrial biogenesis and beta-oxidation; suppressing lipogenic enzymes; decreasing proliferation and differentiation of pre-adipocytes to mature adipocytes; reducing size and number of adipocytes; inducing pre-adipocyte apoptosis [25,36]. In this study, no change in fat mass or intra-abdominal fat mass (Table 3) was observed between FO and C groups. Diverse results on fat mass were observed based on the dietary source, the ratio of n-6/n-3 PUFA and length of the study [12,24,26,37]. However, a 51-day postpartum study is insufficient to observe the effect of FO in body and intra-abdominal fat mass.

Besides modification in adipose tissue, parity can also be associated with abnormalities in lipid metabolism that could lead to cardiovascular diseases [38,39]. During pregnancy, there is an increase in total cholesterol due to changes in hormonal profile, adipose tissue accumulation and hepatic activity [40]. Changes in cholesterol metabolism are common during pregnancy and they are expected to go back to normal levels during post lactation period, although it depends on the mother's physiological state [41]. In this study, FO group presented a significant decrease in total cholesterol ($P<0.05$) and LDL-c serum levels ($P<0.05$) vs. C group (Table 3). Low total cholesterol has been observed by Pereira et al. (2016) in male pups treated with FO [24]. Similar results were reported by Tzang et al. (2009) and Vijaimohan et al. (2006) with male hamsters and rats respectively, fed with a high fat diet rich in FO [42,43]. Fukumitsu et al. (2013) observed that ALA from FO is associated with suppression of genes involved in cholesterol and triglycerides biosynthesis pathways and also decreased expression of sterol regulatory element binding proteins and fatty acid synthase [44]. These observations predicted lowered serum cholesterol. Bile acid synthesis is the chief mechanism for cholesterol degradation. Alpha-linolenic acid diet increases 7α-hydroxylase hepatic enzyme - responsible for a higher secretion of cholesterol in bile and a subsequently cholesterol synthesis and turnover [45].

4. Conclusions

The results showed that FO provides high levels of ALA and EPA in the serum that activates metabolic and physiological pathways to provide a better body composition and lipid profile. Flaxseed oil has an appealing nutritional composition that when add to a diet at postpartum improves health parameters. The results from this study can be a stepping stone for further investigations into clinical research on dietary components for health and wellness.

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Statement of Competing Interests

The authors have declared no conflict of interest.

List of Abbreviations

AIN - American Institute of Nutrition; ALA - alpha-linolenic acid (C18:3); ARA - arachidonic acid (C20:4); C – control; CND - chronic noncommunicable diseases; DGLA - dihomo-gamma-linolenic acid (C20:3); DHA - docosahexaenoic acid (C22:6); DXA - dual-energy X-ray absorptiometry; EPA - eicosapentaenoic acid (C20:5); EPM - standard error of mean; FO - flaxseed oil; GLA - gamma-linolenic acid (C18:3); HDL-c - high density lipoprotein cholesterol; LDL-c - low density lipoprotein cholesterol; LNA - Linoleic acid (C18:2); n-3 - omega-3; n-6 - omega-6; PUFA - polyunsaturated fatty acid(s); VLDL-c - very low lipoprotein cholesterol

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