Nutrición Hospitalaria

Nutr Hosp. 2014;30(4):831-836
ISSN 0212-1611 • CODEN NUHOEQ
S.V.R. 318

Original / *Investigación animal*

The use of flaxseed flour during pregnancy and lactation reverses lower birth weight in offspring from diabetic mothers but averts the development during lactation

André Manoel Correia-Santos[1], Gabriela Câmara Vicente[1], Akemi Suzuki[1], Aline D'Ávila Pereira[1], Juliana Saraiva dos Anjos[1], Kátia Calvi Lenzi de Almeida[2] and Gilson Teles Boaventura[1]

[1]Laboratory of Experimental Nutrition. Federal Fluminense University, Rio de Janeiro, Brazil. [2]Medicine School, Rio de Janeiro Federal University – Macaé Campus, Rio de Janeiro, Brazil.

Abstract

Diabetes is a complication which occurring during gestation might substantially influence the development of offspring during fetal life and postnatally. Flaxseed is a source of omega-3, that the appropriate supply during gestation and lactation are determinant for a suitable perinatal growth and development. The present study aimed to assess beneficial effects of the use of flaxseed flour during pregnancy and lactation on body development from birth to weaning of offspring from diabetic mothers.

***Methods:* twelve rats from a total of eighteen were induced to diabetes by high-fat diet during four weeks, also receiving one lower dose of streptozotocin. After confirmation of diabetes (glucose>300mg/dL), they were mated and when pregnancy was confirmed, they were divided in 3 groups: high-fat group (HFG), high-fat flaxseed flour group (HFFFG) and control group (CG), receiving high-fat diet, high-fat diet added flaxseed flour and control diet, respectively. They were fed this way during whole gestation and lactation. The body development of offspring was measured weekly since the first day after birth until weaning.**

***Results:* At birth, the average body mass of offspring from diabetics mothers who received only high-fat diet was 23,6% lighter than body mass of offspring from non-diabetics mothers (p<0,05), while the animals from diabetic mothers who consumed flaxseed flour during pregnancy and lactation showed the same body mass than the control group. During all experiment HFFFG group showed decreased body mass (about 20%, p<0,05) in comparison with control group.**

***Conclusion:* The treatment with flaxseed flour was capable of avoiding lower birth weight in offspring from diabetic mothers. However, the consumption of flaxseed**

Correspondence: André Manoel Correia-Santos.
Laboratório de Nutrição Experimental.
Rua Mario Santos Braga, 30, 5ºAndar, Valonguinho,
Centro, Niterói, RJ, Brasil. CEP: 24020-140.
Federal Fluminense University (UFF).
E-mail: andremcorreia@gmail.com

Recibido: 23-V-2014.
Aceptado: 23-VII-2014.

EL USO DE LA HARINA DE LINAZA DURANTE EL EMBARAZO Y LA LACTANCIA REVIERTE MENOR PESO AL NACER EN LOS HIJOS DE MADRES DIABÉTICAS, PERO EVITA EL DESARROLLO DURANTE LA LACTANCIA

Resumen

La diabetes es una complicación que ocurre durante la gestación puede influir sustancialmente el desarrollo de las crías durante la vida fetal y postnatal. La linaza es una fuente de ácidos grasos omega-3, que la oferta apropiado durante la gestación y lactancia son determinantes para un adecuados crecimiento y desarrollo perinatal. Este estudio tuvo como objetivo evaluar los efectos beneficiosos del uso de la harina de linaza durante el embarazo y la lactancia en el desarrollo corporal desde el nacimiento hasta el destete de las crías de madres diabéticas.

***Métodos:* Los doce ratas, de un total de dieciocho fueron inducidas a la diabetes con dieta alta en grasas durante cuatro semanas también recibir una dosis reducida de estreptozotocina. Después de la confirmación de la diabetes (glucosa> 300mg/dL), que fueron apareadas y cuando se confirmó el embarazo, fueron divididos en 3 grupos: grupo de alto contenido de grasa (HFG), grupo de alto contenido de grasa con harina de linaza (HFFFG) y grupo control (GC), recibiendo la dieta alta en grasas, dieta alta en grasa añadida harina de linaza y dieta control, respectivamente. Fueron alimentados de esta manera durante toda la gestación y la lactancia. El el desarrollo corporal de las crías se midió semanalmente desde el primer día después de su nacimiento hasta el destete.**

***Resultados:* En el nacimiento, la masa corporal medio de las crías de madres diabéticas que recibieron sólo la dieta rica en grasas era 23,6% más ligero que la masa corporal de las crías de los no diabéticos madres (p <0,05), mientras que los animales de la diabetes madres que consumieron la harina linaza durante el embarazo y la lactancia mostraron la misma masa corporal que el grupo control. Durante todo el grupo HFFFG experimento mostró masa corporal disminuido (20%, p <0,05) en comparación con el grupo control.**

***Conclusión:* El tratamiento con harina linaza fue capaz de evitar bajo peso al nacer en los hijos de madres diabéticas. Sin embargo, el consumo de harina de linaza**

flour by mothers during lactation yielded decrease offspring weight at weaning.

(*Nutr Hosp*. 2014;30:831-836)

DOI:10.3305/nh.2014.30.4.7637

Key words: *Flaxseed, rats, diabetes mellitus, experimental, body weight.*

por las madres durante la lactancia cedió disminuir el peso crías al destete.

(*Nutr Hosp*. 2014;30:831-836)

DOI:10.3305/nh.2014.30.4.7637

Palabras clave: *linaza, las ratas, la diabetes mellitus, experimental, el peso corporal.*

List abbreviation

CG – Control Group
DHA - docosahexaenoic acid
HFG - High-Fat Group
HFFFG - High-fat Flaxseed Flour group
LabNE - Experimental Nutrition Laboratory
SDG - secoisolariciresinol diglycoside
STZ - streptozotocin
UFF - Federal Fluminense University

Introduction

Compelling evidence exists suggesting that an early exposure to an adverse fetal and / or postnatal environment may increase susceptibility to a number of chronic diseases in later life of the offspring. Diabetes is a complication which occurring during gestation might substantially influence the development of offspring during fetal life and postnatally[1]. Sons of diabetic mothers during gestation are more prone to adverse and secondary effects in the growth. Studies with animal models of diabetes during gestation showed that low hyperglycemia intrauterine exposure (due to a deficient insulin secretion) is associated with newborns of normal weight or macrossomic, which become adults with glucose intolerance[2]. Opposed, newborns from mothers with severe hyperglycemia are microsomic[2–7].

The incidence of diabetes still remains increasingly, affecting individuals in all cities and ages, including women in childbearing age, which are in high risk to present this disease in gestation[8,9]. Since organogenesis can be affected by hyperglycemia[2], a rigorous control should be done before conception in patients with pre-existing diabetes. The intensive control of glucose during all pregnancy and lactation can contribute for the decreasing of this adversity. In this way, the scientific community suggests that the appropriate supply of omega-3 fat acids during gestation and lactation are determinant for a suitable perinatal growth and development, protecting the child from adverse consequences in postnatal life[10]. Among foods from vegetal origins, flaxseed contains the largest concentration of alfa linolenic fatty acid (omega-3)[11]. The seed has about 57% of omega-3, 16% of omega-6, 18% of monounsaturated fatty acids and only 9% saturated fatty acids[11–13]. The goal of this study was to assess the effect of the use of flaxseed flour during the gestation and lactation upon the weight, from birth to weaning of offspring from *wistar* rats who were induced to experimental diabetes in the pre gestational period.

Material and Methods

Ethical aspects

This study was performed in accordance with the guidelines of the "Care and Use of Laboratory Animals" (US National Institutes of Health 85-23, revised 1996) and the handling and experimental protocols were approved by the Animal Ethics Committee of the Fluminense Federal University, Niterói, Rio de Janeiro, Brazil (035/2011).

Animals

Eighteen female *Wistar* rats (12 weeks old) were obtained from colonies kept in the Laboratory of Experimental Nutrition (LabNE) from the College of Nutrition of Federal Fluminense University (UFF) and were maintained under controlled conditions (21 ± 2ºC, humidity 60 ± 10%, and 12-h dark/12-h light cycle), with unrestricted access to food and water.

Experimental protocol

Diabetes induction

Nondiabetic female wistar rats (n=12) were fed a high-fat diet (60 % of energy from lipid, 14% of energy from protein and 26 % of energy from carbohydrate) for an initial period of three weeks, and other six rats were fed a control diet based on casein (10 % of energy from lipid, 14% of energy from protein and 76 % of energy from carbohydrate), both *ad libitum*, thus forming two groups: High-Fat Group (HFG) (n=12) and Control Group (CG) (n=6). After three weeks of high-fat diet, the rats of HFG received intraperitoneal injection of streptozotocin (STZ) (*Sigma* Chemical, St. Louis, MO, EUA) at a lower dose (35 $mg.kg^{-1}$) dissolved in vehicle (Sodium Citrate Buffer 0,01M, pH = 4,5)[14,15]. The rats that consumed the control diet received intraperitoneally the vehicle solution only. Diabetes confirmation was established from a plasma glucose concentration greater than 300 mg/dL[15].

Period of pregnancy and lactation

All rats were mated overnight with nondiabetic male wistar rats at a ratio of 2 females to 1 male. The mornings when spermatozoa were detected on vaginal swabs were designated as day zero of pregnancy. The mating procedure was continued for 15 consecutive days, which comprises approximately three estrous cycles. Thus, with the confirmation of pregnancy, the rats were placed in individual cages and divided into 3 groups (n=6): High-fat Flaxseed Flour group (HFFFG), High-fat Group (HFG) e Control Group (CG). During the gestation and lactation period, the groups HFFFG and HFG received high-fat diets (49% of total calories coming from lipids) and the control group received control diet (based on casein) (Table 1). The diet supplied to HFFFG had a concentration of 25% of flaxseed, with the aiming to supply all recommendation of fiber[16]. The brown flaxseed was crushed in a blender to obtain the flour, which was later weighed and immediately used in the manufacture of chow. The percentage of lipids decreased from 60% to 49%, due to gestation and lactation phase because it is necessary a higher intake of protein (from 14% to 19%). The content of vitamins and minerals followed the recommendations of AIN-93G[16]. The control of food intake groups was performed daily. After spontaneous birth, diabetic females continued to receiving the high-fat diet specific to their group, and control group, control diet. At birth, we analyzed the number of pups by litter and the mass of the litter, and then the sex of offspring was identified from the anal-genital distance, defining the proportion male / female of the litter. The litter was then randomly reduced to six pups (with a 1:1 gender ratio, as much as possible) to ensure a standard plane of nutrition. From birth until weaning the body mass of male and female was measured together weekly using a digital balance (Filizola®, accurate to 0.5 g).

Statistical analyses

Data are shown as mean and standard error of the mean. The normal distribution of values was analyzed using the *Kolmogorov-Smirnov* test. Differences among groups were tested with one-way analysis of variance

Table I
Nutritional composition of experimental diets used during pregnancy and lactation.

Nutrients (g/Kg)	*Diets*		
	Control	*High-fat*	*High-Fat + Flaxseed flour*
Casein(1)	190	230	200
Flaxseed flour(2)	0	0	250
Corn starch(1)	539,486	299,486	229, 486
Sucrose(3)	100	100	100
Fat (soybean oil)(4)	0	0	0
Fat (Lard)(5)	0	200	170
Fiber (Cellulose)(6)	50	50	0
Vitamin mix(7)	10	10	10
Mineral mix (AIN-93G)(7)	35	35	35
Cystina(8)	3	3	3
Choline(8)	2,5	2,5	2,5
antioxidant	0,014	0,014	0,014
Total	1000	1000	1000
Carbohydrates (% of total Kcal)	64	32	31,6
Protein (% of total Kcal)	19	19	19,3
Fat (% of total Kcal)	17	49	49
Energy (kcal/kg)	3950	4950	4954,7

AIN-93G = American Institute of Nutrition-93G
(1)Comércio e Indústria Farmos Ltda. (Rio de Janeiro RJ, Brasil).
(6)Maizena da Unilever Bestfoods Brasil Ltda. (Mogi Guaçu, SP, Brasil)
(3)União (Rio de Janeiro, RJ, Brasil)
(1)Liza da Cargill Agricultura Ltda. (Mairinque, SP, Brasil).
(6)Microcel da Blanver Ltda. (Cotia, SP, Brasil).
(5)Sadia Comercial *Ltda*
(7)PragSoluções Comércio e Serviços Ltda-ME (Jáu, SP, São Paulo)
(8)M. Cassab Comércio e Indústria Ltda. (São Paulo, SP, Brasil)
(2)Mãe terra (São Paulo, SP, Brasil).
(4)Giroil Agroindustria LTDA (Santo Ângelo, RS, Brasil).

and post hoc Tukey test. A *P*-value < 0.05 was considered statistically significant (GraphPad Prism v. 5.04 for Windows, GraphPad Software, San Diego, CA, USA).

Results

During gestation and lactation, we can see at table 2 that high-fat groups and high-fat flaxseed flour group showed average concentrations of glucose superior than control group (p<0.0001) and hyperglycemia was observed, which affected the diet consumption, where hyperglycemic rats consumed more diet than rats from CG (p=0.0013). At birth, the litter size (p=0.0653) and the ratio of male and female offspring were unaffected by hyperglycemia, when compared with control group (table 2). However, the average of body mass of the offspring from diabetic mothers that received only high-fat diet was 23.6% lighter than average of body mass of offspring from mothers that received control diet (p<0.0001) while the animals from diabetic mothers who consumed flaxseed flour during pregnancy and lactation showed the same body mass than the control group (Figure 1).

On the seventh day of life, the average body mass of the offspring from diabetic mothers were lower than the control group (Figure 1). The high-fat group was 16.9% lighter than control group (p=0.0018). On the fourteenth day, the average body mass of the offspring from diabetic mothers in both groups were lower than the control group (p <0.0001), with the high-fat group being 31.3% lighter than the control group and the high-fat flaxseed flour group being 23,5% lighter when compared to control group (Figure 1) .

At weaning, twenty-first day, again, all offspring from diabetic mothers were lighter than control group (High-fat, 24.6% and high-fat flaxseed flour group, 16,1%) (p=0.0003).

Discussion

Analyzing the data related to consumption of diets of the rats, it can be perceived that diabetic rats showed higher food intake when compared to control group, corroborating with Yamada et al. (2002)[17] that using *wistar* rats observed hyperphagia in diabetic animals induced by STZ. The average of body mass of offspring from diabetic mothers that received only high-fat diet was lower than the average of body mass of offspring from mothers that received control diet, these results corroborate with the findings of Fetita et al. (2006)[2], Poston *et al* (2010)[18] and Yessoufou & Moutairou (2011)[1]. The low weight of high-fat group can be explained by the fact that STZ, when administered, induces diabetes by the direct toxic effects on pancreatic β-islet cells. The fetus is confronted with severe intrauterine hyperglycemia which induces fetal islet hypertrophy and β-cell hyperactivity and may result in early hyperinsulinemia. This overstimulation of fetal β cells limits their adaptation, and they become depleted of insulin granules, and incapable of secreting insulin. β-cell exhaustion results in fetal hypoinsulinemia. Hypoinsulinemia and a reduced number of insulin receptors in target cells lead to a reduction in fetal glucose uptake. The growth of fetal protein mass is suppressed, and fetal protein synthesis is consistently low, leading to fetal microsomia. Postnatal development is retarded, and these offspring remain small at adulthood[1,19]. We can observe that the offer of a diet with 25% flaxseed flour exerted a beneficial effect on birth weight of offspring from diabetic mothers as it showed

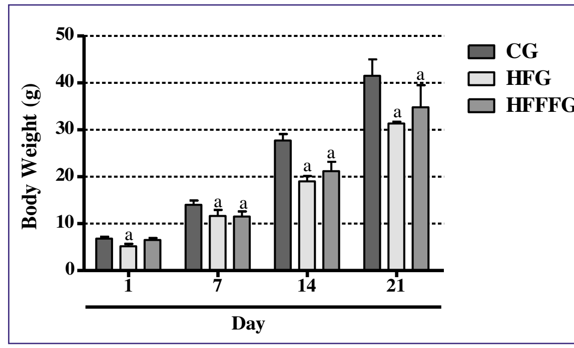

Fig. 1.—Evolution of offspring body weight throughout the experiment. The letter [a] represents statistical difference when compared to the CG, p<0,05, One-way ANOVA and post hoc test of Tukey. Groups: CG, control group; HFG, high-fat group and HFFFG, high-fat flaxseed flour group.

Table II
Food intake, glucose concentration during pregnancy and lactation, litter size and the offspring ratio male: female

Data	*CG*	*HFG*	*HFFFG*
Food intake (g)	689 ± 31,7[a]	930,4 ± 43,1[b]	893,9 ± 43,6[b]
Glucose (mg/dL)	90,5 ± 5,4[a]	438,5 ± 3,5[b]	422,2 ±24,3[b]
Litter size (n)	10,5 ± 2,6[a]	5,8 ± 3,9[a]	8,1 ± 2,3[a]
Ratio male : female	1:1	1:1	1:1

The data were expressed as mean ± SEM
Values not sharing similar letter in the same line are different (p<0,05) in the One-way ANOVA and post hoc test of Tukey.
Groups: CG, control group; HFG, high-fat group and HFFFG, high-fat flaxseed flour group.

the same birth weight as compared to the control group. Some mechanisms try to explain this benefit, among them, the vasodilator property of docosahexaenoic acid (DHA), derived from omega-3, increase placental flow intrauterine[20] and consequently the avidity in the supply of nutrients and oxygen to the fetus, which will ensure an increase on birth weight.

In regard to litter size, this did not differ among groups, although, we observe that high-fat group presented, on average, fewer litter size. This fact, although not significant, was influenced by glycemia because we can observe that higher glycemia meant lower offspring number. These results are derived from the combined effects of insulin deficiency and hyperglycemia that bewilder the physiological functioning of various metabolic signals involved in the regulation of the reproductive system [21].

It is well known that at specific periods in the reproductive cycle, such as pregnancy, the sensitivity to hormones is increased. Therefore, the administration of lignans should be done with caution because of its phytoestrogen properties, due to the fact that these substances may be related to infertility and hyperestrogenism in some species of animals[22].

At weaning, twenty-first day, all offspring from diabetic mothers showed lighter than control group. Regarding the high-fat flaxseed flour group, similar results were found by Cardozo *et al* (2010)[11] and Collins *et al* (2003)[23]. Both of which showed that maternal consumption of a diet based on the flaxseed and defatted flaxseed by nondiabetic rats promoted less weight gain during lactation and lower offspring's body weight at weaning, respectively. Guard et al. (2014)[24] offered a high-fat diet with flaxseed oil to wistar rats during lactation and also observed a low weight of male and female offspring at weaning compared to pups of mothers who consumed diet with normal amount of lipid showing that even though the flaxseed is a source of n-3, it was not capable to maintain the body mass of the offspring during lactation.

Flaxseed is a rich source of secoisolariciresinol diglycoside (SDG), a plant lignin and precursor of mammal lignans, which acts positively on the human body to prevent and treat cancer and other diseases[22]. SDG is a phytoestrogen, and it is well known that estrogen directly inhibits fat deposition by reducing lipogenesis, mainly through the reduction of lipoprotein lipase activity (LPL), which is the enzyme that regulates the reuptake of lipids in the adipocyte[25,26]. Thus, less weight can be explained partly by the action of estrogen in adipocytes. Another factor would be that the streptozotocin used to induce diabetes in rats leads to a reduced ability to synthesize fatty acids in the mammary gland, while, in human, the milk from diabetic mothers contains lower amounts of long-chain polyunsatured fatty acids when compared with nondiabetic mothers. Because of this, changes in milk composition may have contributed to the growth retardation observed in the offspring after birth of all diabetic experimental groups[3].

It is noteworthy that literature lacks experimental studies similar to the design of this research, in which we used a seed, functional vegetal food, during pregnancy and lactation of diabetic mothers, making it difficult to better discuss the results. In conclusion, these results show that the model of *in utero* exposure to maternal diabetes led to low birth weight and that this effect was attenuated with the use of flaxseed flour. Findings also suggest that transference of constituents of flaxseed by milk during lactation program the offspring to a lower weight at weaning, indicating that flaxseed was unable to reverse the effects of diabetes in during lactation.

Acknowledgments

This research was supported by grants from the Brazilian agencies CNPq (Conselho Nacional de Ciência e Tecnologia) and FAPERJ (Fundação para o Amparo a Pesquisa do Estado do Rio de Janeiro).

Conflicts of interest

The authors declare no conflict of interest.

References

1. Yessoufou A, Moutairou K. Maternal diabetes in pregnancy: early and long-term outcomes on the offspring and the concept of "metabolic memory". *Exp Diabetes Res* 2011; 218598.
2. Fetita L-S, Sobngwi E, Serradas P et al. Consequences of fetal exposure to maternal diabetes in offspring. *J Clin Endocrinol Metab* 2006; 91: 3718–3724.
3. Blondeau B, Joly B, Perret C et al. Exposure in utero to maternal diabetes leads to glucose intolerance and high blood pressure with no major effects on lipid metabolism. *Diabetes Metab* 2011; 37: 245–351.
4. Holemans K, Aerts L, Van Assche FA. Lifetime consequences of abnormal fetal pancreatic development. *J Physiol (Lond.)* 2003; 547: 11–20.
5. Kervran A, Guillaume M, Jost A. The endocrine pancreas of the fetus from diabetic pregnant rat. *Diabetologia* 1978; 15: 387–393.
6. Kiss AC, Lima PH, Sinzato YK et al. Animal models for clinical and gestational diabetes: maternal and fetal outcomes. *Diabetol Metab Syndr* 2009; 1: 21.
7. Song Y, Li J, Zhao Y et al. Severe Maternal Hyperglycemia Exacerbates the Development of Insulin Resistance and Fatty Liver in the Offspring on High Fat Diet. *Exp Diabetes Res* 2012; 2012: 1–8.
8. Hunt KJ, Schuller KL. The increasing prevalence of diabetes in pregnancy. *Obstet Gynecol Clin North Am* 2007; 34: 173–199.
9. Nolan CJ, Damm P, Prentki M. Type 2 diabetes across generations: from pathophysiology to prevention and management. *Lancet* 2011; 378: 169–181.
10. Sattar N, Berry C, Greer I.A. Essential fatty acids in relation to pregnancy complications and fetal development. *Br J Obstet Gynaecol* 1998; 105: 1248–1255.
11. Cardozo LFMF, Soares LL, Chagas MA et al. Maternal consumption of flaxseed during lactation affects weight and hemoglobin level of offspring in rats. *J Pediatr (Rio J)* 2010; 86: 126–130.
12. De Almeida KCL, Fernandes FS, Boaventura GT et al. Efecto de la semilla de linaza (Linum Usitatissimum) en el crecimiento de ratas wistar. *Rev Chil Nutr* 2008; 35: 443–451.

13. Wiesenfeld PW, Babu US, Collins TFX et al. Flaxseed increased alpha-linolenic and eicosapentaenoic acid and decreased arachidonic acid in serum and tissues of rat dams and offspring. *Food Chem Toxicol* 2003; 41: 841–855.
14. Correia-Santos AM, Suzuki A, Anjos JS et al. Induction of Type 2 Diabetes by low dose of streptozotocin and high-fat diet-fed in wistar rats. *Medicina (Ribeirão Preto)* 2012; 45: 432–440.
15. Srinivasan K, Viswanad B, Asrat L et al. Combination of high-fat diet-fed and low-dose streptozotocin-treated rat: a model for type 2 diabetes and pharmacological screening. *Pharmacol Res* 2005; 52: 313–320.
16. Reeves PG, Nielsen, FH, Fahey Jr GC. AIN-93 purified diets for laboratory rodents: final report of the American Institute of Nutrition ad hoc writing committee on the reformulation of the AIN-76A rodent diet. *J Nutr* 1993; 123: 1939–1951.
17. Yamada R, Griggio MA. Luz J. Energy balance of pregnant diabetic rats. *Br J Nutr* 2002; 87: 509–515.
18. Poston L. Developmental programming and diabetes - The human experience and insight from animal models. *Best Pract Res Clin Endocrinol Metab* 2010; 24: 541–52.
19. Saito FH, Damasceno DC, Kempinas WG et al. Repercussions of mild diabetes on pregnancy in Wistar rats and on the fetal development. *Diabetol Metab Syndr* 2010; 2: 26.
20. Rogers I, Emmett P, Ness A et al. Maternal fish intake in late pregnancy and the frequency of low birth weight and intrauterine growth retardation in a cohort of British infants. J Epidemiol Community Health 2004; 58: 486–92.
21. Codner E, Merino PM, Tena-Sempere M. Female reproduction and type 1 diabetes: from mechanisms to clinical findings. *Hum Reprod Update* 2012; 18: 568–585.
22. Leite CDFC, Vicente GC, Suzuki A et al. Effects of flaxseed on rat milk creamatocrit and its contribution to offspring body growth. *J Pediatr (Rio J)* 2012; 88: 74–78.
23. Collins TF, Sprando RL, Black TN Effects of flaxseed and defatted flaxseed meal on reproduction and development in rats. *Food Chem Toxicol* 2003; 41: 819–834.
24. Guarda DS, Lisboa PC, de Oliveira E et al. Flaxseed oil during lactation changes milk and body composition in male and female suckling pups rats. *Food Chem Toxicol* 2014; 69C: 69-75.
25. Hamosh M, Hamosh P. The effect of estrogen on the lipoprotein lipase activity of rat adipose tissue. *J Clin Invest* 1975, 55: 1132–1135.
26. Figueiredo MS, de Moura EG, Lisboa PC. Flaxseed supplementation of rats during lactation changes the adiposity and glucose homeostasis of their offspring. *Life Sci* 2009; 85: 365–371.