Letter to the Editor

Effects of Maternal Linseed Oil Supplementation on Metabolic Parameters in Cafeteria Diet-induced Obese Rats*

BENAISSA Nawel[1], MERZOUK Hafida[1,#], MERZOUK Sid Ahmed[2], and NARCE Michel[3]

Because linseed oil may influence maternal and fetal metabolisms, we investigated its role in the modulation of lipid metabolism in cafeteria diet-induced obese rats and their offspring. Female Wistar rats were fed control or cafeteria food, which were either supplemented or not supplemented with linseed oil (5%) for 1 month before and during gestation. At parturition, serum and tissue lipids and enzyme activities were analyzed. Cafeteria diet induced adverse metabolic alterations in both mothers and offspring. Linseed oil improved metabolic status. In conclusion, linseed oil displayed health benefits by modulating tissue enzyme activities in both obese mothers and their newborns.

Adverse nutritional environment during early life has long-lasting consequences and may change some physiological parameters into adulthood. Our group has shown that both maternal obesity and nutritional state in the early postnatal phase program changes in adiposity, hepatic metabolism, glucose homeostasis, and lipid profile and are important in promoting obesity in offspring[1-2].

We have used a cafeteria diet in pregnant rats and showed that maternal obesity has long-term metabolic consequences in the offspring, including an increase in lipogenic capacity in adipose tissues, impaired glucose homeostasis, and altered body composition and liver metabolism[1-2].

Previous findings have documented that not only the quantity but also the fat type in the diet affects the rate of weight gain. N-3 polyunsaturated fatty acids (n-3 PUFA) generally reduce the rate of weight gain compared with other fatty acids and play an important role in the prevention of metabolic diseases[3]. N-3 PUFA lowers both plasma cholesterol and triglycerides and are useful in improving insulin sensitivity and treating dyslipidemia. Modification of dietary fat composition may influence metabolic disorders associated with obesity. However, although it is well documented that consumption of diets high in n-3 PUFA can improve metabolic alterations, their beneficial effects on maternal and neonate obesity have not yet been elucidated.

Linseed oil, an important source of dietary α-linolenic acid (ALA, 18:3 n-3), has several health benefits during pregnancy and induces epigenetic changes in both maternal and offspring livers[4-5]. During lactation, linseed oil reduced the body weight of mothers and offspring, decreased milk lipids, and apparently increased insulin sensitivity in this critical period of life[6]. To the best of our knowledge, there are no reports in the literature on the effect of linseed oil supplementation on metabolic status during maternal obesity and their repercussions on offspring.

As linseed oil may influence maternal and fetal metabolisms, our aim was to evaluate the effect of linseed oil supplementation in the diet before and during gestation on maternal and neonate disturbances induced by cafeteria diet.

Healthy adult Wistar rats (aged 2 months), weighing 100-150 g, were obtained from Pasteur Institute (Algiers, Algeria). The use of animals according to our experimental design was approved by the Regional Ethical Committee. The study was conducted in accordance with the national guidelines for the care and use of laboratory animals. Twenty male rats fed a control diet (standard laboratory chow; ONAB, Algeria) were used for mating. Forty female rats were randomly assigned into four feeding groups for 30 d prior to and during

doi: 10.3967/bes2015.041

*This work was supported by the French Foreign Office (International Research Extension Grant TASSILI 08MDU723); and by the Algerian Research Project (PNR, 2011).

1. Laboratory of Physiology and Biochemistry of Nutrition, Department of Biology, Faculty of Natural and Life Sciences, Earth and Universe, University Abou-Bekr Belkaïd, Tlemcen 13000, Algeria; 2. Department of technical Sciences, Faculty of Engineering, University Abou-Bekr Belkaïd, Tlemcen 13000, Algeria; 3. INSERM UMR 866, 'Lipids Nutrition Cancer', University of Burgundy, Faculty of Sciences, 6 Boulevard Gabriel, Dijon 21000, France

gestation. The group 1 (control; *n*=10 females) received the control diet (C). Group 2 was fed a control diet enriched with linseed oil at 5% (CL 5%, *n*=10). Group 3 was fed a cafeteria-style fat-rich hypercaloric diet (OB, *n*=10). Group 4 was fed a cafeteria diet enriched with linseed oil at 5% (OBL 5%, *n*=10). The composition of four diets is given in Table 1. At parturition and after overnight fasting, female rats and their offspring (average litter size per group was 60 pups) were sacrificed. Blood samples were assayed for biochemical parameters. Maternal abdominal white adipose tissue and maternal and neonate livers were removed, homogenized, and tested for biochemical parameters and enzyme activities as previously described[3-4].

The individual effects of cafeteria diet and oil supplementation were distinguished by two-way ANOVA and Fisher's least significant difference tests.

The cafeteria diet was associated with increased body weight in mothers and in their offspring compared to standard chow-fed ones (Table 2). Supplementation with linseed oil at 5% induced a reduction in body weight in obese mothers and their newborns.

The cafeteria diet significantly increased serum glucose, cholesterol, and triglyceride levels in both obese mothers and their newborns compared with controls (Table 2). Linseed oil supplementation significantly reduced serum glucose, cholesterol, and triglyceride levels in OB group but had no effects on the CL group. However, after linseed oil supplementation, serum cholesterol and triglyceride levels remained higher in the OBL group than that in the CL group.

OB rats and their newborns had significantly higher liver cholesterol and triglycerides than controls (Table 2). OB rats also had significantly higher adipose lipid levels than control mothers. The linseed oil significantly decreased liver and adipose lipid levels in the OB group.

Our result showed a significant reduction in hepatic acyl-CoA dehydrogenase activity and a significant increase in hepatic fatty acid synthase activity in OB rats compared with control rats (Figure 1). However, the activities of these enzymes were not affected in their newborns. Hepatic triglyceride lipase activity was not altered in OB mothers, but was significantly enhanced in their newborns compared to control newborns.

Linseed oil significantly raised maternal and neonate hepatic acyl-CoA dehydrogenase activity in both control and obese groups. However, this enzyme activity remained lower in OBL rats compared to CL rats. Hepatic fatty acid synthase activity was reduced by linseed oil in both control and obese mothers, but was unaffected in their newborns. Linseed oil had no effect on maternal hepatic triglyceride lipase activity, but reduced its activity in OBL newborns.

Table 1. Composition of Experimental Diets

Energy Sources	C	CL	OB	OBL
(% energy)				
Protein	20	20	16	16
Carbohydrate	60	60	24	24
Fat	10	10	50	50
Sunflower oil	10	5	10	5
Linseed oil	-	5	-	5
Vitamin E (mg/100 g)	5	5	5.5	5.5
Energy (Kcal/100 g)	386	386	523	523
(% fatty acids)				
SFA	29	23	44	37
C18:1 n-9	21	22	28	29
C18:2 n-6	46	38	27	23
C18:3 n-3	3	16	1	11
C20:4 n-6	1	1	0	0

Note. C: control diet; CL: control diet enriched with linseed oil at 5%; OB: cafeteria diet; OBL: cafeteria diet enriched with linseed oil at 5%; SFA: saturated fatty acids. The control and cafeteria diets for CL and OBL were given in powder form, and were supplemented with purified Linseed oil at 5%. Fatty acid composition was analyzed by gas liquid chromatography.

Table 2. Biochemical Parameters of the Study Rats

Parameters	C	OB	CL	OBL	*P* (ANOVA)
Mothers					
Body weight (g)	244.33±4.41	376.16±3.80$^{\$}$	216.66±4.90*	240.50±2.53$^{*\$}$	0.006
Glucose (g/L)	1.42±0.15	3.49±0.12$^{\$}$	1.42±0.11	1.50±0.10*	0.010
Cholesterol (g/L)	0.76±0.02	1.27±0.02$^{\$}$	0.75±0.01	0.85±0.01$^{*\$}$	0.004
Triglyceride (g/L)	1.15±0.01	1.85±0.03$^{\$}$	0.96±0.02	1.29±0.02$^{*\$}$	0.006
Liver-C (mg/g)	8.12±0.35	16.33±1.12$^{\$}$	3.57±0.30*	4.62±0.20*	0.004
Liver-TG (mg/g)	16.98±0.36	37.11±0.38$^{\$}$	13.18±1.38*	24.67±0.35$^{*\$}$	0.006
Adipose-C (mg/g)	6.34±0.22	14.68±0.53$^{\$}$	3.98±0.25*	4.35±0.22$^{\$}$	0.007
Adipose-TG (mg/g)	56.48±1.58	85.46±1.92$^{\$}$	46.61±1.14*	57.34±1.63$^{*\$}$	0.006
Newborns					
Body weight (g)	5.17±0.12	7.24±0.18$^{\$}$	5.10±0.18	5.78±0.17*	0.010
Glucose (g/L)	1.08±0.07	1.70±0.06$^{\$}$	0.81±0.05	0.77±0.04*	0.010
Cholesterol (g/L)	0.58±0.01	0.82±0.01$^{\$}$	0.57±0.01	0.60±0.01*	0.007
Triglyceride (g/L)	0.83±0.01	1.29±0.01$^{\$}$	0.85±0.01	0.98±0.01*	0.005
Liver-C (mg/g)	4.80±0.16	9.74±0.06$^{\$}$	4.01±0.13	4.21±0.14*	0.010
Liver-TG (mg/g)	10.97±0.60	25.33±1.05$^{\$}$	8.27±0.41*	15.27±0.48$^{*\$}$	0.004

Note. Values are presented as means±SEM. C: control diet; CL: control diet enriched with linseed oil at 5%; OB: cafeteria diet; OBL: cafeteria diet enriched with linseed oil at 5%. *Statistical difference for CL *vs.* C or OBL *vs.* OB (linseed oil effect). $^{\$}$Statistical difference for OB *vs.* C or OBL *vs.* CL (diet effect).

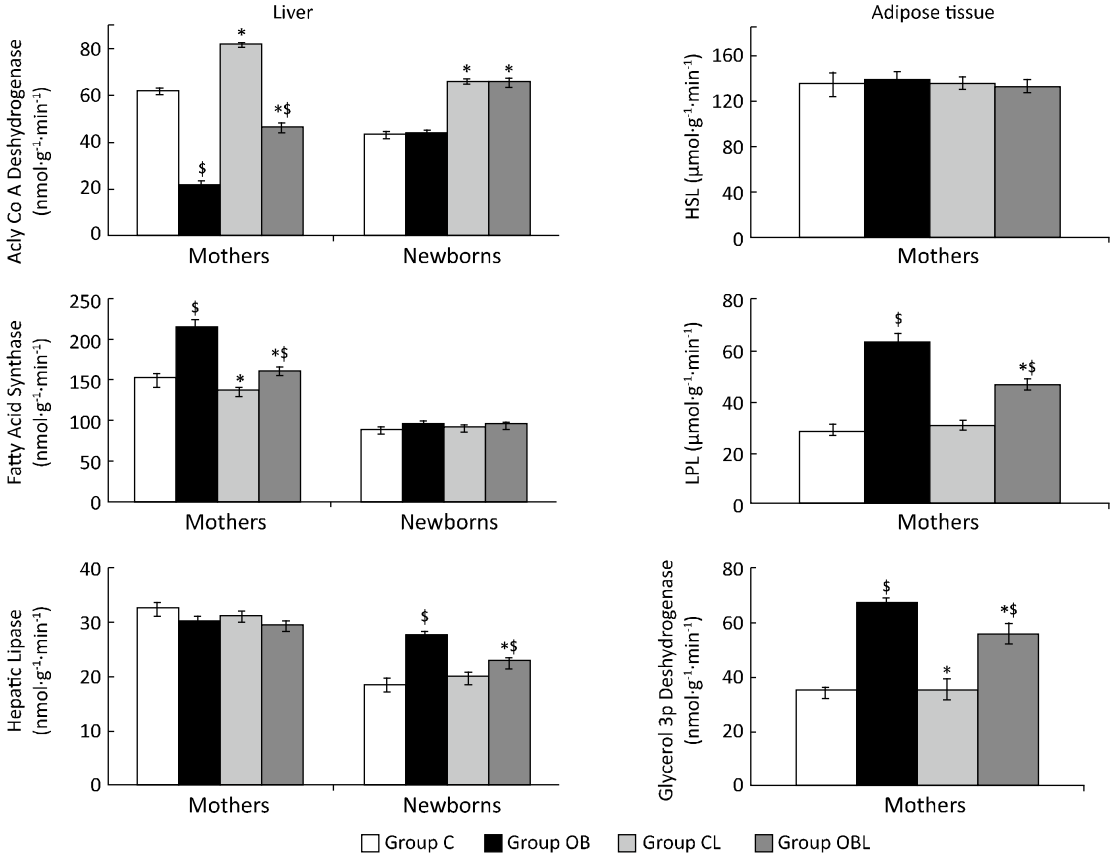

Figure 1. Hepatic and adipose tissue enzyme activities in mother rats and their newborns. Values are presented as means±SEM. C: control diet; CL: control diet enriched with linseed oil at 5%; OB: cafeteria diet; OBL: cafeteria diet enriched with linseed oil at 5%. *Statistical difference for CL *vs.* C or OBL *vs.* OB (linseed oil effect). $^{\$}$Statistical difference for OB *vs.* C or OBL *vs.* CL (diet effect).

The cafeteria diet induced a significant increase in the activity of adipose glycerol-3-phosphate dehydrogenase and lipoprotein lipase (LPL) in OB rats compared with control rats (Figure 1). However, adipose hormone-sensitive lipase (HSL) activity was unaffected by the cafeteria diet. Linseed oil supplementation had no effects on adipose glycerol-3-phosphate dehydrogenase and LPL activities in control rats, but induced a significant reduction of these activities in OBL rats. Adipose HSL activity was unaffected by linseed oil supplementation.

Maternal nutrition is an easily modifiable environmental factor that can affect fetal growth and development with potential long-term consequences. Our group has previously shown that maternal overnutrition and obesity program changes in neonate adiposity, leptin, glucose homeostasis, and lipid profile[1-2]. Disease prevention should take place throughout life, starting with the mother's diet during gestation. The search for functional foods, such as linseed oil, has become more common due to their beneficial effects and their role in the prevention of diseases. In the present study, we explored the metabolic effects of a cafeteria diet enriched with 5% linseed oil on pregnant rats and their newborns. The increase of body weight in cafeteria diet-fed pregnant rats was strongly associated with the increase in adipose lipid depots, confirming the obesogenic properties of the cafeteria diet. Offspring of these dams were heavier than offspring from dams fed a control diet, in agreement with our previous studies[1-2].

In our study, the pregnant rats fed the cafeteria diet presented an increase in serum glucose, cholesterol, and triglyceride levels compared to control pregnant rats. These findings correlated with an increase in hepatic and adipose triglyceride and cholesterol levels and are likely due to increased hepatic synthesis and secretion of lipoproteins as well as an increase in adipose lipid accumulation, as described in obesity[7]. Hepatic triglyceride lipase plays a major role in lipid metabolism as a lipolytic enzyme that hydrolyzes triglycerides and phospholipids in lipoproteins and also facilitates the uptake of lipids by the liver. LPL hydrolyses circulating triglyceride-rich lipoproteins and provides substrates for fatty acid uptake into adipose tissue. HSL is the principal regulator of non-esterified free fatty acid release from adipose tissue. Glycerol-3-phosphate dehydrogenase catalyzes the conversion of dihydroxyacetone phosphate derived from glucose into glycerol-3-phosphate, which is then acylated to form triglycerides. Acyl-CoA dehydrogenase catalyzes the β-oxidation of fatty acids. Cafeteria diet-fed pregnant rats showed an increase in the activities of hepatic fatty acid synthase, adipose glycerol-3-phosphate dehydrogenase, and LPL, in addition to a decrease in the activity of hepatic acyl-CoA dehydrogenase. However, the activities of hepatic triglyceride lipase and HSL remained unchanged in these rats. The combination of increased hepatic lipogenesis and reduced β-oxidation in obese pregnant rats was associated with liver steatosis, in agreement with previous studies[2]. Previous data indicated that increased de novo lipogenesis and decreased fatty acid oxidation in the liver could lead to lipid accumulation and hepatic steatosis in obesity[8]. Increased adipose tissue lipid content was related to an increase in the enzymatic activities involved in lipid storage, such as LPL and glycerol-3-phosphate dehydrogenase in obese rats. The associations between obesity, hyperlipidemia, hepatic hyperlipogenesis, lower fatty acid oxidation, and adipose lipid accumulation are well established, and contribute to the risk of coronary artery diseases[8].

We showed that offspring of cafeteria diet–fed dams had significantly higher glucose and lipid concentrations than offspring of control dams fed a normal diet, in agreement with previous studies[2]. However, enzymatic activity modifications were different from that in mothers since the activities of fatty acid synthase and acyl-coA dehydrogenase were unaffected, while hepatic lipase activity was increased in newborns of obese rats compared to newborns of control rats. High hepatic triglyceride lipase in these newborns might contribute to increased uptake of lipids by the liver.

Linseed oil supplementation modulated several liver and adipose parameters in both control and obese pregnant rats, with beneficial effects including lower body weight, lower lipid accumulation, and modulation of enzyme activities. Linseed oil appears to be especially effective in obese pregnant rats and their offspring in terms of metabolic improvements. Indeed, after linseed oil supplementation, the mean weight of the newborns in the obese group was similar to that in the control group, indicating prevention of neonate obesity. Linseed oil induced a significant reduction in glycemia and in lipidemia in both obese mothers and their newborns. These carbohydrate and lipid regulations were in agreement with previous reports and seemed to be

related to improvements in insulin sensitivity[4]. These improvements were accompanied by depletion in adipose tissue and liver triglyceride and cholesterol levels. Oil supplementation also decreased the activities of liver fatty acid synthase, adipose glycerol-3-phosphate dehydrogenase, and LPL, and increased the activity of liver acyl-coA dehydrogenase, which could explain the lipid depletion in the liver and adipose tissue in obese rats. Linseed oil supplementation reduced hepatic lipid accumulation by stimulating β-oxidation, suppressing fatty acid synthesis, and enhancing cholesterol secretion into bile, as previously reported[4]. Previous researchers have showed that a diet rich in linolenic acid decreases adipose LPL activity[9]. Decreased fatty acid synthesis and lipogenesis and increased fatty acid oxidation were also previously reported in rats fed a diet enriched in linseed oil[10]. In the present study, specific changes in enzymatic activity were observed in newborns after maternal linseed oil supplementation. Fatty acid synthase activity was unaffected, while hepatic triglyceride lipase activity was reduced by maternal oil supplementation. However, neonate hepatic acyl-CoA dehydrogenase activity was increased by linseed oil supplementation, which is similar to that seen in their mothers. Linseed oil has beneficial effects on lipid metabolism in obese mothers and their newborns. In our study, female rats were treated with linseed oil in a preventive approach while at the same time being fed a cafeteria diet. Further studies are currently underway in our laboratory on the effects of linseed oil supplementation when mothers are already obese.

In conclusion, our results clearly demonstrate that linseed oil displays remarkable health benefits for the prevention of obesity and associated metabolic disorders by decreasing plasma and tissue lipids and by modulating adipose tissue and liver enzyme activities in both mothers and newborns.

The authors declare that they have no conflicts of interest.

#Correspondence should be addressed to Professor MERZOUK H, E-mail: hafidamerzouk_2 @hotmail.com; Tel: 00-213-778303645

Biographical note of the first author: BENAISSA Nawel, born in 1986, master, majoring in nutrition, biochemistry, and physiology.

Received: December 9, 2014;

Accepted: March 19, 2015

REFERENCES

1. Bouanane S, Merzouk H, Benkalfat NC, et al. Hepatic and very low-density lipoprotein fatty acids in obese offspring of overfed dams. Metabolism Clin Exp, 2010; 59, 1701-9.
2. Benkalfat NC, Merzouk H, Bouanane S, et al. Altered adipose tissue metabolism in offspring of dietary obese dams. Clin Sci, 2011; 121, 19-28.
3. Breslow J. N-3 Fatty acids and cardiovascular disease. Am J Clin Nutr, 2006; 83, 1477-82.
4. Tripathi V, Abidi AB, Markerb S, et al. Linseed and linseed oil: health benefits- a review. Int J Pharm Biol Sci, 2013; 3, 434-42.
5. Niculescu MD, Lupu DS, Craciunescu CN. Perinatal manipulation of α-linolenic acid intake induces epigenetic changes in maternal and offspring livers. FASEB J, 2012; 27, 350-8.
6. 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 Toxicology, 2014; 69, 69-75.
7. Grundy SM, Hansen B, Smith SC, et al. Clinical management of metabolic Syndrome. Circulation, 2004; 109, 551-6.
8. Qureshi K, Abrams GA. Metabolic liver disease of obesity and role of adipose tissue in the pathogenesis of nonalcoholic fatty liver disease. World J Gastroent, 2007; 13, 3540-53.
9. Hwalla Baba N, Ghossoub Z, Habbal Z. Differential effects of dietary oils on plasma lipids, lipid peroxidation and adipose tissue lipoprotein lipase activity in rats. Nutr Res, 2000; 20, 1113-23.
10. Takeuchi H, Nakamoto T, Mori Y, et al. Comparative effects of dietary fat types on hepatic enzyme activities related to the synthesis and oxidation of fatty acid and to lipogenesis in rats. Biosci Biotech Biochem, 2001; 65, 1748-54.