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Original Article

LINSEED OIL SUPPLEMENTATION IMPROVES LECITHIN CHOLESTEROL ACYLTRANSFERASE AND TISSUE LIPASE ACTIVITIES IN PREGNANT OBESE RATS AND THEIR OFFSPRING

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ABSTRACT

Objective: The aim of the present work was to study how maternal dietary linseed oil modulates lecithin cholesterol acyltransferase (LCAT) and adipose tissue lipase responses to cafeteria diet in rats during pregnancy and lactation and their offspring at weaning and adulthood.

Methods: The dams were fed a control (C) or a Cafeteria (CAF) diet enriched or non-enriched with linseed oil at 2, 5%. Changes in serum glucose, total cholesterol (TC) and triglyceride (TG) levels, liver and adipose tissue lipids, LCAT, adipose tissue lipoprotein lipase (LPL) and hormone-sensitive lipase (HSL) activities were determined at the end of the experiment.

Results: The cafeteria diet led to higher energy intake, body weight, hyperglycemia and hyperlipidemia, liver and adipose TC and TG contents and LPL activity in dams and their pups. Pregnant and lactating mothers showed lower HSL activity with any changes in serum LCAT activity. In contrast, their offspring at day 90 had a significant increase in HSL activity and a decrease in LCAT activity. Linseed oil (LO) supplementation modulates liver and adipose tissue TC and TG contents in both control and obese dams and their offspring, with beneficial effects resulting in lower body weight, decreased in TC,TG, low density lipoprotein-high density lipoprotein1(LDL-HDL1-C), increased in HDL2,3-C,serum LCAT activity and up-regulated lipolytic enzyme activities.

Conclusion: The supplement of linseed oil in the diet of pregnant and lactating dams is effective in amelioration of lipid profile and modulation of enzyme activities in these dams and their offspring which might contribute to prevent obesity and dyslipidemia.

Keywords: Linseed oil, Cafeteria diet, Lipid, Enzymes, Pregnancy, Offspring

INTRODUCTION

After the industrial revolution, the human diet has changed from unrefined whole grains and vegetables with high fiber to refined grains with low fiber content and more animal products. These changes besides the reduced physical activity of humans due to mechanization caused an increase in the risk of some chronic diseases related to diet and lifestyle like obesity, diabetes, cardiovascular and heart-related diseases [1].

Epidemiologic, clinical and experimental studies suggested that maternal obesity during pregnancy is an important risk factor for fetal overnutrition leading to fetal obesity [2]. Maternal obesity gives rise to an offspring phenotype predisposed to the development of adulthood obesity and diabetes [3-5]. Obesity is associated with glucose and lipid metabolism abnormalities and increased cardiovascular risk [6, 7].

An important consequence of insulin resistance is enhanced adipose tissue lipolysis and reduced free fatty acid (FFA) uptake and esterification, leading to an increased flux of FFA into non-adipose tissues such as liver and muscle. Increased FFA flux has been suggested to increase the intracellular availability of TG and to stimulate indirectly the assembly and secretion of very low-density lipoprotein (VLDL) particles [2]. Many studies have focused on improving lipid profiles (as one of the most important risk factors of chronic diseases) by planning a better diet or introducing herbal treatments. Thus, many plants were proposed to have health benefits in reducing blood lipid profile.

Linseed oil (LO) is the main component of the (*Linum Usitatissimum L.)* and one of the world's most important vegetable sources of α-linolenic acid (LNA, 18:3n-3). As a nutritionally essential polyunsaturated fatty acid (PUFA), LNA can act as the precursor of longer chain n-3 PUFA Eicosapentaenoic acid (EPA) and Docosahexaenoic acid (DHA) or compete with linoleic acid or direct interaction with ion channels and nuclear receptors, and thus may exert various biological functions in the human body, such as accelerating brain growth in preterm and neonates and, antiarrhythmic functions and neuroprotective functions [8]. In addition, LNA is also reported to have beneficial effects on blood lipid profiles [1, 9].

Studies in animals and humans [10, 11] have shown a reduction of total cholesterol, low-density lipoprotein cholesterol (LDL-C) and triglycerides after the consumption of linseed oil. Positive effects were also observed on glucose metabolism, with reductions in blood glucose and insulin, leading to increased insulin sensitivity [12, 13].

However, serum lipids are not sufficient indicators of lipoprotein metabolism. In addition, serum lipoprotein concentrations and compositions are determined by many factors. The action of enzymes, such as lecithin cholesterol acyltransferase (LCAT), on lipoproteins and movements of lipids in plasma, controls the lipoprotein levels [14]. Then, Dietary fatty acids are known to play an important role in the development as well as prevention of dyslipidemia [14].

There are no studies in the literature on the effect of linseed oil supplementation on enzyme activities and metabolic profiles of mothers during gestation and lactation and their repercussions on their offspring at weaning and adulthood.

The purpose of this study was to evaluate the effects of linseed oil-enriched diet on lipoprotein compositions as well as on LCAT, lipoprotein lipase (LPL) and hormone-sensitive lipase (HSL) activities in obese rats and their offspring by determining the time course of changes in serum lipoprotein (very low, low and high density lipoprotein (VLDL, LDL-HDL1, HDL)) lipids, LCAT, LPL and HSL activities. The following study should demonstrate the beneficial effects of n-3 PUFA content in linseed oil on the offspring of obese mothers, with the aim of reducing the development of obesity and its complications in this offspring.

MATERIALS AND METHODS

Experimental protocol

Adult Wistar rats were obtained from Animal Resource Centre (Algeria). After mating, the first day of gestation was estimated by the presence of spermatozoids in vaginal smears. Pregnant rats weighing 180 to 200 g were housed individually in wood-chip–bedded plastic cages at a constant temperature (25 °C) and humidity (60%±5%) with a 12-hour light/dark cycle. The rats had free access to water and were randomly assigned to one of 4 experimental diets. The control group (control, C, n=10) was fed standard laboratory chow (ONAB, Algeria). The cafeteria group 2 (diet-induced obese, CAF, n=10) was fed a palatable rich-fat diet. In group 3 (control linseed, CL, n=10), rats were on standard chow supplemented with linseed oil (2.5 % linseed oil; g per 100 g diet). In group 4 (diet-induced obese linseed, CAFL, n=10), rats were on cafeteria diet supplemented with linseed oil (2.5 % linseed oil). The control diet (386 kcal/100 g) was composed 20% of energy as protein, 20% of energy as lipids and 60 % of energy as carbohydrates. The components of the cafeteria diet were paté, cheese, bacon, chips, cookies, and chocolate (in a proportion of 2:2:2:1:1:1, by weight); and control diet (mix/control diet, W/W) was given to each rat daily. The composition of the cafeteria diet (523 kcal/100 g) was 16% of energy as protein, 24% of energy as carbohydrates and 60% of energy as lipids. The composition of the four diets is listed in table 1. Linseed oil was obtained from INRA (INRA, Algeria). Fresh food was given daily, and body weights were recorded. The dams were fed the same diet continuously for the entire gestation and lactation periods.

A total of 320 pups from all groups of dams were delivered spontaneously and weighed within 12 h. The postnatal litter size was adjusted at 8 pups/dam to maintain a similar postnatal nutritional intake during the suckling period. Weaning occurred on day 30 of lactation.

Offspring were weaned on the control commercial diet or the cafeteria diet enrich or non-enriched with linseed oil. Male rats were housed separately and were followed into adulthood (12 w). Four groups were then formed (C; n=10, CL; n=10, CAF, n=10, CAFL n=10). Food intake and body weights of rats were recorded daily.

The study was conducted in accordance with the national guidelines for the care and use of laboratory animals. All the experimental protocols were approved by the Regional Ethical Committee.

Table 1: Nutritional components of diets

Properties	Levels (%)			
	Control Diet (C)	Cafeteria Diet (CAF)	Control Linseed oil (CL) 2,5%	Cafeteria Linseed oil (CAFL) 2,5%
		(% energy)		
Protein	20	16	20	16
Total fat	17.5	57.5	17.5	57.5
Total carbohydrate	60	24	60	24
Sunflower oil	2.5	2.5	-	-
Linseed oil	-	-	2.5	2.5
Vitamin E(mg/100g)	5	5.5	3	3.50
Energy (Kcal/100 g)	386	523	386	523
Crude fiber	04	02	04.5	01.5
Humidity	07.5	09	07.5	08.5
		(% fatty acids)		
SFA	27	42	20	30
MUFA	24	30	18	24
C18: 2n-6	45	27	36	20
C18: 3n-3	03	01	25	26
C20: 4n-6	01	-	01	-

The control and cafeteria diets, in powder form, were supplemented with the purified oil as indicated. SFA: saturated fatty acids. MUFA: monounsaturated fatty acids. Fatty acid composition was analyzed by gas liquid chromatography, INSERM UMR 866, "Lipids Nutrition Cancer", University of Burgundy, France.

Blood and tissue sample

At day 21 and day 51 for dams, at day 30 and day 90 for pups, ten rats of each group were anesthetized with intraperitoneal injection of sodium pentobarbital (60 mg/kg body weight) and then bled from the abdominal aorta. Serum was obtained by low-speed centrifugation (1000 g, 15 min). Liver and visceral adipose tissue (AT) were removed, washed with cold saline, quickly blotted and prepared for lipolytic activities determination.

Isolation of lipoprotein fractions

Serum lipoproteins of density<1.21 kg/l were isolated by single ultracentrifugation flotation (model L8-55 ultracentrifuge, 50 Ti rotor, Beckman instruments, Palo Alto, CA, USA), according to Havel *et al.* [15]. The three lipoprotein fractions (VLDL, LDL-HDL 1, HDL) were isolated from total lipoproteins by a single-spin discontinuous gradient according to the method of Redgrave *et al.* [16] as modified By Meghelli-Bouchenak *et al.* [17]. These fractions were dialyzed against 0.15 mol/l NaCl and 1 mmol/l disodium EDTA (pH 7.4) at 4 °C in spectra/por-2 dialysis tubing (Spectrum Medical Industries, Los Angeles, CA).

Chemical analysis

Plasma glucose, triglycerides and total cholesterol (TC) were determined using enzymatic colorimetric assays (Sigma Diagnostics Inc., St. Louis, MO).

Tissue and lipoprotein fractions TG and TC contents levels were determined using enzymatic colorimetric assays (Sigma, St. Louis, MO).

Assay for LCAT activity

LCAT (EC 2.3.1.43) activity was assayed by conversion of unesterified [3 H] cholesterol to esterified [3 H] cholesterol, according to the method of Glomset and Wright [18], as previously described by Merzouk *et al.* [19]. Serum LCAT activity was expressed as nmol of esterified cholesterol/h/ml of serum.

Assay for lipases activities

To estimate HSL (EC 3.1.1.3) activity, a spectrophotometric esterase assay based on the hydrolysis of p-nitrophenyl butyrate (PNPB) was used as described by Kabbaj *et al.* [20], with an interassay CV of 6.5 %. Adipose tissue homogenates were incubated with PNPB and buffer (0.1 M NaH2 PO4/Na2HPO4, pH 7.25, 0.9 % NaCl and 1 mM dithiothreitol) at 37 °C for 10 min. The reaction was stopped by the addition of 3.25 ml of methanol/chloroform/heptane (10:9:7, by vol.). After centrifugation at 800 g for 20 min, solutions were incubated for 3 min at 42 °C, and the absorbance of the supernatant was measured at 400 nm in a UV spectrophotometer. The enzymatic

activity is expressed as μmol of p-nitrophenol released · min−¹ of protein. For the LPL (EC 3.1.1.34) assay, tissue homogenate (the enzyme source) was incubated at 37 ºC for1h with [3H] triolein (trioleoylglycerol) emulsion substrate [final concentrations: 1.42 mmol/l triolein, 0.1 mmol/l lysophosphatidylcholines, 0.2 %(w/v) albumin, 5 % (v/v) heat-inactivated serum (providing apolipoprotein C-II, an activator of LPL), 0.1 mmol/l Tris/HCl, pH 8.0 and 0.15 mol/l NaCl], as described by Nilsson–Ehle and Ekman [21], with an interassay CV of 5.5 %. At the end of the incubation period, the fatty acids released were extracted with chloroform/ methanol/heptane (1.25:1.41:1, by vol), followed by 0.1 mol/l potassium carbonate/borate buffer, pH 10.5. 3H radioactivity in 1.5 ml aliquots of the methanol/water upper phase was measured in 10 ml of scintillation liquid (Ready Solv HP/6; Beckman) in a 7500 LS liquid scintillation counter (Beckman). Enzyme activity is expressed as nmol of fatty acids released · min −¹· mg-¹ of protein.

Statistical analysis

Results are expressed as means±standard deviation (SD). The results were tested for normal distribution using the Shapiro–Wilk test. Data not normally distributed were logarithmically transformed. Significant differences among the groups were analyzed statistically by a one-way analysis of variance (ANOVA). When significant changes were observed in ANOVA tests, Fisher least significant difference tests were applied to locate the source of the significant difference. The significance level was set at P<0.05. These calculations were performed using STATISTICA version 4.1 (STATSOFT, Tulsa, OK).

RESULTS

Effect of linseed oil on body, liver and adipose tissue weight

Body weight was significantly increased in dams fed cafeteria diet at day 21 and day 51 compared with controls. Furthermore, the adipose tissue weight was also statistically significant in these rats compared with the control group (35, 68 %; 44, 23 %) respectively. At day 21 and 51, LO administration decreased the body weight gain in obese CAFL compared with CAF rats. At day21, statistically significant reduction of adipose tissue weight was observed in CAFL group as was compared with CAF group, however, the cafeteria diet, and LO had no effect on liver weight gain at day 21. At day 51, LO administration increased liver weight gain in CAFL compared with CAF rats. Food and energy intake was significantly increased in pregnant dams (day21) fed the cafeteria diet compared with controls. LO had no effect on food and energy intake in these rats. Lactating dams (day51) fed the cafeteria diet had a significant increase in energy intake compared with control-fed rats with no changes in food intake. LO administration decreased food and energy intake in CAFL-fed dams compared with CAF-fed rats. An increase of energy intake was seen in CL-fed rats compared with control rats (table 2).

Table 2: Effect of linseed oil on body, liver and adipose tissue weights in obese and control dams.

Group	Control	Cafeteria	Control linseed oil	Cafeteria linseed oil
		Day (21)		
Body weight(g)	257±4,01^{b}	326,75±10,2^{a}	215±8,98^{c}	253,2±8,64^{b}
Food intake (g/d/rat)	24.3±1.08^{b}	29.11±1.14^{a}	24.95±2.5^{b}	28.45±2.49^{a}
Energy intake (kcal/d/rat)	93.79±4.16^{b}	152.24±4.4^{a}	96.32±9.65^{b}	148.79±32.43^{a}
Liver weight(g)	9,74±0,17^{a}	9,58±0,2^{a}	9,90±0,9^{a}	9,38±1,41^{a}
A. T weight(g)	2,65±0,22^{b}	4,12±0,21^{a}	4,12±0,21^{a}	3,57±0,48^{b}
		Day (51)		
Body weight(g)	230±7,5^{b}	334±5,02^{a}	248,4±5,02^{b}	232,2±16,85^{b}
Food intake (g/d/rat)	38.04±2.37^{a}	38.14±2.22^{a}	32.25±2.78^{b}	33.7±2.34^{b}
Energy intake (kcal/d/rat	90.15±9.14^{d}	199.47±15.01^{a}	124.48±10.73^{c}	176.25±12.23^{b}
Liver weight(g)	10,29±0.93^{b}	9,13±0,63^{b}	10,96±2,066^{b}	12,38±1,57^{a}
A. T weight(g)	2,35±0,20^{b}	4,22±0,06^{a}	1,68±0,108^{b}	3,82±0,30^{a}

Values are presented as means±standard deviations (SD). C (n=10): rats fed control diet. CAF (n=10): rats fed cafeteria diet. CL (n=10): rats fed control linseed diet. CAFL (n=10): rats fed cafeteria linseed diet. Values with different superscript letters (a, b, c, d) are significantly different (P<0.05)

Both at weaning (day30) and at adulthood (day 90 of age), body weight gain, liver and adipose tissue weight of obese pups from cafeteria fed dams had significantly increased as compared with normal basal diet, L. O administration reduces the Body weight gain, liver and adipose tissue weight in CAFL rats compared with CAF rats. However, no statistically significant changes in liver and adipose tissue weight were noted in CL rats in comparison with rats feeding control diet. Food and energy intake was significantly increased in obese adult offspring compared with control rats. Feeding linseed oil reduced food and energy intake significantly in CAFL-fed rats compared with CAF-fed rats with no changes in CL group compared with controls (table 3).

Table 3: Effect of linseed oil on body, liver and adipose tissue weights in obese and control dams offspring

Group	Control	Cafeteria	Control linseed oil	Cafeteria linseed oil
		Day(30)		
Body weight (g)	52,14±4,06^{b}	95,63±4,41^{a}	62,18±4,62^{b}	62,5±2,73^{b}
Liver weight (g)	2,84±0,25^{b}	5,23±0,48^{a}	3,09±0,41^{b}	2,88±0,45^{b}
A. T weight (g)	0,85±0,056^{b}	2,63±0,16^{a}	0,72±0,24^{b}	0,69±0,23^{b}
		Day(90)		
Body weight (g)	325,75±12,36^{b}	460,25±17,53^{a}	271,25±33,26^{c}	317,75±14,38^{b}
Food intake (g/d/rat)	23.45±2.53^{b}	32±3 ^{a}	21.06±1.90^{b}	29.36±2.91^{a}
Energy intake (kcal/d/rat)	90.67±9.79^{b}	167.36±15.39^{a}	81.29±7.33^{b}	153.55±15.21^{a}
Liver weight (g)	06,70±0,30^{b}	8,72±0,56^{a}	6,70±1,48^{c}	08,01±0,50^{b}
A. T weight (g)	2,73±0,069^{b}	7,72±0,179^{a}	3,31±0,92^{b}	3,08±0,81^{b}

Values are presented as means±standard deviations (SD). C (n=10): offspring of dams fed control diet. CAF (n=10): offspring of dams fed cafeteria diet. CL (n=10): offspring of dams fed linseed control diet. CAFL (n=10): offspring of dams fed cafeteria linseed diet. Values with different superscript letters (a, b, c, d) are significantly different (P<0.05).

Effect of linseed oil on metabolic parameters in dams

Pregnant and lactating mothers fed the cafeteria diet had higher serum glucose concentration than control rats. Linseed oil consistently lowered the concentration of plasma glucose in CAFL-fed dams at day 21 and 51 compared with CAF-fed rats. No significant changes in plasma glucose concentration were observed between CL rats and control rats (table 4).

Both at day 21 and at day 51, mothers fed the cafeteria diet had a significant increase in serum TC and TG levels than control rats. Linseed oil administration had significantly decreased serum TC and TG in CAFL-fed rats compared with CAF-fed rats. The levels of LDL-HDL1-C were significantly increased in cafeteria-fed rats compared to control rats both at day 21 and day 51. However, in CAFL rates the levels of LDL-HDL1-C were significantly decreased as was compared with CAF-fed rats, with no changes in HDL2,3-C levels. Liver TC and TG contents were significantly increased in cafeteria-fed dams at day 21 and day 51 as was compared with control rats.

Feeding LO diet to rats induced a significant decrease in hepatic TC and TG contents in CAFL-fed dams at day 21 (-24, 32%;-12, 37%) and day 51 by (-15, 36%;-11, 2%) in comparison with CAF-fed rats.

Adipose tissue TG contents were significantly increased in obese dams at day 21 and 51 compared with control rats. Cafeteria diet enriched or non-enriched with linseed oil, significantly increased adipose tissue TG contents in comparison with their controls (CL, C) respectively. LO diet induced a significant decrease in adipose tissue TG contents in CAFL-fed mothers at day 21 and at day 51 compared with CAF-fed rats, and increased in CL-fed dams compared with C-fed dams.

On the other hand, our results revealed that there were no changes in serum LCAT activity in all groups at day 21 and 51 (table 4). In contrast, adipose tissue enzyme activities showed some changes. HSL activity was significantly lower in dams feeding cafeteria diet at day 21 and 51 compared with control-fed rats. LO administration, induced a significant increase in HSL activity in both obese and control mothers at day 51 (+43, 35%;+39, 3%) respectively compared with their controls.

Also, adipose tissue LPL activity was significantly increased in pregnant and lactating dams feeding cafeteria-diet compared with control rats. LO diet reduced adipose tissue LPL activity significantly in CAFL-fed dams at day 21 (-29, 25%) and day 51 (-6, 50%) compared with CAF-fed rats (table 4).

Table 4: Effect of Linseed oil on lipid parameters in obese and control pregnant and lactating dams

Parameters	Control	Cafeteria	Control linseed oil	Cafeteria linseed oil
		Day 21		
Glucose (g/l)	1.30±0.18^{c}	2.02±0.10^{a}	1.43±0.14^{c}	1.78±0.16^{b}
Serum TC (g/l)	0.95±0.08^{b}	1.67±0.08^{a}	0.79±0.21^{b}	0.83±0.30^{b}
LDL-HDL1-C (g/l)	0.34±0.05^{c}	0.58±0.03^{a}	0.27±0.09^{c}	0.49±0.10^{b}
HDL2-HDL3-C (g/l)	0.45±0.04^{a}	0.48±0.05^{a}	0.44±0.13^{a}	0.42±0.09 ^{a}
Serum TG (g/l)	0.83±0.08^{b}	1.27±0.063^{a}	0.93±0.19^{b}	0.94±0.08^{b}
Liver TC (mg/g liver)	5.41±0.98^{b}	32.66±0.8^{a}	5.77±0.60^{b}	8.34±2.01^{b}
Liver TG (mg/g liver)	23.6±4.89^{c}	63.57±4.59^{a}	28.82±4.73^{c}	51.20±4.11^{b}
Adipose tissue TG (mg/g AT)	53.86±1.3^{d}	159.27±7.27^{a}	74.97±3.83^{c}	139.77±8.49^{b}
LCAT activity (nmol/l/min)	533.4±117.7^{a}	666±158.83^{a}	699.2±173.28^{a}	650±176.91^{a}
HSL activity (µmol/g/min)	66.25±2.21^{a}	38.75±5.37^{b}	66.25±5.31^{a}	50.25±8.18^{b}
AT-LPL activity (nmol/mg/min)	40.12±1.48^{c}	79.2±5.73 ^{a}	37±1.73^{c}	50±3.46^{b}
		Day 51		
Glucose (g/l)	1.26±0.07^{c}	1.66±0.08^{a}	1.16±0.12^{c}	1.41±0.12^{b}
Serum TC (g/l)	1.16±0.10^{b}	1.69±0.09^{a}	0.97±0.25 ^{b}	0.78±0.26^{b}
LDL-HDL1-C (g/l)	0.32±0.03^{b}	0.64±0.03^{a}	0.38±0.11 ^{b}	0.33±0.13^{b}
HDL2-HDL3-C (g/l)	0.44±0.05^{a}	0.46±0.04^{a}	0.42±0.13^{a}	0.332±0.1^{a}
Serum TG (g/l)	0.92±0.07^{b}	1.33±0.08^{a}	1.25±0.12^{a}	1.02±0.26^{a}
Liver TC (mg/g liver)	8.53±2.22 ^{c}	32.97±4.93^{a}	9.67±1.16^{c}	17.61±2.01^{b}
Liver TG (mg/g liver)	31.75±4.75^{c}	61.86±5.83^{a}	35.77±4.91^{c}	50.66±1.66^{b}
Adipose tissue TG (mg/g AT)	67.77±2.27^{d}	153.22±9.28^{a}	97.72±15.14^{c}	135.63±10.39^{b}
LCAT activity (nmol/l/min)	717.8±187.36 ^{a}	630±251.1^{a}	412.02±99.66^{a}	410.96±44.38^{a}
HSL activity (µmol/g/min)	47.2±5.01^{c}	28.4±4.97^{d}	86.5±4.93^{a}	71.75±6.13^{b}
AT-LPL activity (nmol/mg/min)	28.5±3.41^{c}	77.8±2.17 ^{a}	34±2.82 ^{c}	71.3±4^{b}

Values are presented as means±standard deviations (SD). C (n=10): rats fed control diet. CAF (n=10): rats fed cafeteria diet. CL (n=10): rats fed control linseed diet. CAFL (n=10): rats fed cafeteria linseed diet. Values with different superscript letters (a, b, c, d) are significantly different ($P<0.05$).

Effect of linseed oil on metabolic parameters in offspring

Offspring at day 30 and day 90 had higher serum glucose concentration than control rats. Linseed oil lowered the concentration of plasma glucose in CAFL group at day 90 compared with CAF-fed rats. However, CAFL pups at day 30 had similar plasma glucose levels to pups from CAF-fed dams. No significant changes in plasma glucose concentration were observed between CL rats and control rats (table 5)

At weaning, Serum TC and TG levels in offspring of cafeteria-fed dams were significantly higher than those in control rats. However, pups from cafeteria enrich with linseed oil-fed dams had lower levels of TC and TG as compared with pups from cafeteria-fed dams. No statistically significant changes were noted between CL and control pups (table 5). At day 90, serum TC and TG concentrations were significantly increased in obese pups compared with their controls. LO administration reduced TC but not TG in CAFL group compared with CAF group, whereas TC levels did not vary between CL and control group. In contrast, LO increased TG levels in CL-fed rats compared with control rats (table 5).

At weaning, pups from cafeteria-fed dams showed a significant increase in LDL-HDL1-C and a significant decrease in HDL2, 3–C compared with pups from control-fed dams. Pups from CAFL-fed dams had higher HDL2, 3-C than pups from CAF-fed dams (table 5).

At adulthood, rats fed the cafeteria diet showed a significant increase in LDL-HDL1-C and a significant decrease in HDL2, 3-C compared with the control group. The levels of LDL-HDL1-C were significantly decreased (-20%) in CAFL with a significant increase in HDL2, 3-C (+19%) when compared to cafeteria rats. No significant changes were seen between control and CL rats (table 5).

On the other hand, liver TC and TG contents were significantly increased in cafeteria pups at day 90 and only TC at day 30 as was

compared with control rats. Feeding LO diet to rats induced a significant decrease in hepatic TC and TG contents in CAFL-fed rats at day 90 (-20, 87%;-9, 58%) respectively and a slight decrease at day 30 (-3, 76% of TC) in comparison with CAF-fed rats (table 5). Also, adipose tissue TG contents were significantly increased in obese offspring at day 30 and 90 compared with control rats. Cafeteria diet, enriched or non-enriched with linseed oil significantly increased adipose tissue TG contents in comparison with their controls (CL, C) respectively. LO diet induced a significant decreased in adipose tissue TG contents in CAFL rats at day 30 (-13, 26%) and at day 90(-24.41%) compared with CAF-fed rats. Concerning serum LCAT activity, any changes were noted in all groups at day 30. In contrast adult rats from cafeteria-fed dams (day 90), showed a significant reduction of serum LCAT activity compared to control rats. Linseed oil diet induced a slight increase (+6, 35%) in serum LCAT activity in CAFL-fed rats compared with CAF fed rats (table 5).

At weaning, no changes in adipose tissue lipase activities (HSL and LPL) were noted in the offspring of CAF-fed dams compared with control rats. In contrast, adult rats feeding cafeteria diet had a significant increase in HSL and LPL activities compared with control rats. Dietary supplementation with linseed oil induced a significant increase in HSL activity in CAFL and CL groups at day 30 compared with their controls (CAF, C) respectively. However, no changes were seen between CAFL and CAF-fed rats at adulthood. Also, L O diet reduced adipose tissue LPL activity significantly in CAFL group of pups at day 30 (-39, 77%) and day 90 (-38, 45%) compared with CAF-fed rats (table 5).

Table 5: Effect of Linseed oil on lipid parameters in obese and control offspring

Parameters	Control	Cafeteria	Control Linseed oil	Cafeteria Linseed oil
		Day 30		
Glucose (g/l)	1±0.034^{b}	1.39±0.05^{a}	1.16±0.088^{b}	1.44±0.05^{a}
Serum TC (g/l)	1.1±0.041^{b}	1.63±0.078^{a}	1.10±1.040^{b}	1.12±0.53^{b}
LDL-HDL1-C (g/l)	0.30±0.03 ^{b}	0.58±0.036^{a}	0.38±0.105^{b}	0.54±0.19^{b}
HDL2-HDL3-C (g/l)	0.42±0.03^{a}	0.26±0.03^{b}	0.35±0.07^{a}	0.36±0.09^{a}
Serum TG (g/l)	0.62±0.02^{b}	0.88±0.04^{a}	0.58±0.12^{b}	0.62±0.11^{b}
Liver TC (mg/g liver)	8.77±1.03^{b}	10.99±1.02^{a}	05.08±0.77^{b}	07.23±0.71^{b}
Liver TG (mg/g liver)	11.97±1.54 ^{a}	14.01±2.65^{a}	10.21±1.63^{a}	12.41±1.49^{a}
Adipose tissue TG (mg/g AT)	24.54±2.38^{c}	57.07±3.54^{a}	25.69±4.95 ^{c}	43.81±6.40^{b}
LCAT activity (nmol/l/min)	460.42±72.80^{a}	322.62±94.32^{a}	603.78±160.72^{a}	579.85±184.4^{a}
HSL activity (µmol/g/min)	53.6±11.08^{b}	69.9±29.87^{b}	65.16±5.77^{c}	90±8.48^{a}
AT-LPL activity (nmol/mg/min)	81.6±13.6^{a}	85.5±19.9^{a}	43±1.41 ^{b}	45.6±11.1^{b}
		Day 90		
Glucose (g/l)	1.16±0.04^{c}	2.52±0.15^{a}	1.15±0.03^{c}	2.26±0.08^{b}
Serum TC (g/l)	1.02±0.08 ^{b}	1.62±0.083^{a}	0.827±0.14^{b}	0.899±0.02^{b}
LDL-HDL1-C (g/l)	0.35±0.02 ^{b}	0.54±0.03^{a}	0.36±0.29 ^{b}	0.34±0.15^{b}
HDL2-HDL3-C (g/l)	0.52±0.02^{a}	0.31±0.039 ^{b}	0.51±0.12^{a}	0.5±0.06^{a}
Serum TG (g/l)	0.87±0.08^{b}	1.28±0.02^{a}	1.018±0.15^{a}	1.05±0.22^{a}
Liver TC (mg/g liver)	12.27±1.32^{b}	28.77±1.23^{a}	7.26±1.08 ^{b}	7.9±0.97^{b}
Liver TG (mg/g liver)	24.05±1.32^{c}	47.12±2.03^{a}	12.8±0.95^{d}	37.54±3.48^{b}
Adipose tissue TG (mg/g AT)	23.65±6.58^{c}	60.91±2.55^{a}	18.38±5.08^{c}	36.5±3.35^{b}
LCAT activity (nmol/l/min)	698.37±144.6^{a}	365.6±61.03^{b}	533.3±138.44^{a}	429±109.47^{a}
HSL activity (µmol/g/min)	32±6.16^{b}	46.2±9.85^{a}	67±6.24 ^{a}	60.5±2.88^{a}
AT-LPL activity (nmol/mg/min)	45.5±8.66^{b}	83.25±8.95^{a}	45.8±15.31^{b}	44.8±11.69^{b}

Values are presented as means±standard deviations (SD). C (n=10): offspring of dams fed control diet. CAF (n=10): offspring of dams fed cafeteria diet. CL (n=10): offspring of dams fed linseed control diet. CAFL (n=10): offspring of dams fed cafeteria linseed diet. Values with different superscript letters (a, b, c, d) are significantly different (P<0.05).

DISCUSSION

Nutrition during early development is associated with the offspring's growth, organ development, body composition and body functions. It also exerts long-term effects on health, morbidity and mortality risks in adulthood, as well as on the development of neural functions and behavior, a phenomenon called 'metabolic programming' [22, 23].

The present study showed that in the rat, maternal nutrition in pregnancy and lactation has a long-term programming effect on lipid metabolism in the offspring. Rats given cafeteria diet during pregnancy and lactation had offspring that in adult life had significant increases in lipoprotein and liver lipids compared with those of controls. In addition, the effects of LO on the alterations of lipid and lipoprotein profile in cafeteria-fed rats were investigated. In the present study, cafeteria diet was given to dams to induce dietary obesity. Cafeteria diet feeding induced an increase in total energy intake that may explain the higher body and adipose depot weights. In addition, the offspring of these dams had higher energy intake and they were heavier than offspring from dams fed control standard diet, and they remained obese throughout adulthood, in agreement with previous studies [24-26]. Rasmy *et al.* [11] have reported that the rats fed with high saturated fat showed a significant increase in body weight, adipose tissue and liver weight, which leads to secondary complications clinically. In our study, body, adipose tissue and liver weight gain in CAF rats were decreased significantly upon treatment with LO. The hypolipidemic effects may be responsible for the beneficial action of LO on body weight gain, adipose tissue and liver weights [11].

We showed that offspring of cafeteria-fed dams had similar glucose concentrations to those of control rats. In contrast, mothers at day 21 and 51 receiving cafeteria diet and their offspring at 90 d old had significantly higher levels of serum glucose than rats receiving a control diet. Cafeteria diet-induced obesity characterized by hyperglycemia and hyperlipidemia. It is well known that feeding a high-fat diet to rodents causes insulin resistance, hyperglycemia and hyperlipidemia [2]. Moreover, linseed oil supplementation was also associated to a reduction in serum glucose in agreement with previous studies [27, 28]. Linseed oil is a source rich in LNA (18:3n-3), LNA can act as the precursor of n-3 long chain polyunsaturated fatty acids (n-3 LC-PUFA). In rodents *in vivo*, n-3 LC-PUFAs have a protective effect against high fat diet induced insulin resistance. Indeed, the protective effect of n-3 LC-PUFA results from the prevention of the decrease of phosphatidylinositol 3' kinase (PI3 kinase) activity and of the depletion of the glucose transporter protein GLUT4 in the muscle and the prevention of the decreased expression of GLUT4 in adipose tissue. In addition, n-3 LC-PUFAs inhibit both the activity and expression of liver glucose-6-phosphatase which could explain the protective effect with respect

to the excessive hepatic glucose output induced by a high fat diet [27]. Then, diet with high content of PUFA such as linseed oil is very effective in improving cell membrane lipid structure especially fatty acids and phospholipids which have an important role in enhancing insulin sensitivity and decreasing blood glucose in diabetic rats [28].

This study shows that mothers feeding cafeteria diet and their offspring at day 30 and 90 had high TC, TG and LDL-HDL1-C levels, which was correlated with an increase in hepatic TG contents only at day 90. These findings were due probably to the accretion of dietary fatty acids, and to increased synthesis and secretion in lipoprotein observed during pregnancy as a consequence of hyperglycemia [29].

Overproduction of VLDL and the rise in hepatic lipids, a common feature of obesity, are direct consequences of hepatic hyper lipogenesis [2]. In addition, the rise in cholesterol in liver and/or plasma may be due to increased uptake of exogenous cholesterol and subsequent deposition and decreased cholesterol catabolism as evidenced by a reduction in bile acid production and turnover of bile acids [11]. On the other hand, linseed contains bioactive nutrients with high levels of essential n _ 3 fatty acids, lignans and fiber that have been associated with a reduction of cholesterol and triglyceride levels [11, 30, 31]. Similar findings are reported in the present study in CAFL group. Linseed oil decreased the plasma TC, TG and LDL-HDL1-C levels, and contributed to their beneficial changes. ALA rich LO results in a higher cholesterol secretion into bile, leading to a depletion of the intra-hepatic pool of cholesterol and thus to an increase in cholesterol synthesis and turnover [11, 32]. Moreover, ALA rich diet reduces hepatic lipid accumulation both by stimulating ß-oxidation, and by suppressing the expression and activities of many hepatic fatty acid synthesis such as fatty acid synthase (FAS), malic enzyme and glucose 6-phosphate dehydrogenase [33-35], and hence decreases fatty acid synthesis in liver. In addition, LO has been shown to have a hypocholesterolaemic effect, which might result from increases of hepatic LDL-receptor expression and cholesterol catabolism/output [9]. Supplementation with ALA significantly inhibited the hepatic triacylglycerol accumulation and fatty liver formation [35]. Ide *et al.* [36] reported that LO could have exerted its protective effect probably as a better substrate for mitochondrial and peroxisomal ß-oxidation. All these mechanisms may account for the better regulation of hepatic lipid metabolism by LO [36].

In the present study, offspring (day 30) from CAF-fed dams had lower levels of serum TC,TG and liver TC content with no significant changes in liver TG content compared with control rats. Troina *et al.* [10] showed that lactose and total cholesterol contents in milk of linseed-fed mothers were lower without changes in the protein and triglyceride contents on the 4th day of lactation. At the end of lactation, total cholesterol content was still lower. As the offspring, the mothers showed lower serum cholesterol at 21 d old, it is probable that, besides the lower cholesterol transfer through the milk, the components of linseed, which have cholesterol-lowering properties, are also being transferred into the milk. In fact, lignans that are associated with lowering cholesterol have been shown to be transferred to the pups through mother's milk [37].

On the other hand, in offspring of CAF-fed dams, HDL2, 3-C was decreased significantly. HDL cholesterol levels are frequently reduced in obesity [38]. These findings were associated with a significant reduction in serum LCAT activity in this offspring at day 90. A significant increase in TC/HDL-C and LDL-C: HDL-C ratios were observed in rats fed high saturated fat. LO may cause inhibition of the apolipoprotein B synthesis or increase its catabolism which explains the reduction of these ratios in rats feeding high saturated fat enrich with linseed oil [11]. ALA-rich diet resulted in the significant decrease of a number of atherogenic factors, such as TC, LDL-C and LDL-C/HDL-C [32]. In fact, our data shows that LO induced a significant increase of HDL2, 3-C in offspring of CAF-fed dams at 30 and 90 d old, however, no alterations were observed in HDL2, 3-C levels in their mothers. These findings were associated with an increase in serum LCAT activity in CAFL-fed rats at day 90. LO may increase LCAT enzyme mass by increasing its synthesis in these rats. This increase was probably due to the effects of maternal diet and duration of treatment with linseed oil. Previous researches have showed that an increase in LCAT activity and cholesteryl ester (CE) levels in the plasma were observed in PUFA-fed obese rats [14].

Indeed, in our present study, obese pregnant and lactating dams and their offspring at 30 and 90 d old presented significant increase in adipose tissue TG content with alteration in adipose tissue enzymes, such as an increase in adipose tissue LPL activity. The enhanced activity of adipose tissue LPL activity is significantly associated with enhanced TG uptake by adipose tissue [39]. In offspring of cafeteria dams, increased adipose tissue weight and TG contents are concomitant with the increase in enzyme activities involved in lipid storage such as LPL [40, 41]. This could be due to high insulin levels in these obese rats at weaning and at adulthood [26]. In contrast, our data shows that administration of LO induced a significant decrease in adipose tissue TG content in CAFL-fed mothers and pups at day 30 and day 90. This reduction was probably due to the extended period of consumption of LO. These findings were associated with a significant decrease in adipose tissue LPL activity in these rats. In fact, serum triglycerides, adipose LPL activity and ratio of LPL: HSL were significantly reduced when the diet contained high levels of PUFA [42, 43]. In addition, Hwalla Baba *et al.* [42] reported that diet rich in LNA reduces adipose LPL activity. However, HSL activity in CAF-fed dams at day 21 and 51 was lower than these in control rats, which was probably due to the reduction of HSL expression. An impaired catecholamine-induced lipolysis and a reduced HSL expression in preadipocyte and differentiated adipocytes is observed in obesity [44]. Jocken *et al.* [45] indicated that insulin resistant state rather than fat mass per se causes the decrease in adipose tissue, adipose triglyceride lipase and HSL protein levels. In contrast, adipose HSL activity was normal at day 30 but high at day 90 in obese rats [26] which corresponded to our findings in pups of cafeteria-fed dams. Indeed, postnatal cafeteria feeding induced an increase in adipose HSL activity in both obese and control rats [26]. LO increased HSL activity in lactating CAFL-fed dams and offspring at day30. In agreement with previous reports [42], polyunsaturated fatty acids caused an increase in HSL activity.

CONCLUSION

In conclusion, maternal over nutrition induces permanently programmed adipose tissue metabolism that contributes to the maintenance of obesity in the offspring. The supplement of Linseed oil in the diet of pregnant and lactating dams is effective in amelioration of metabolic profiles and modulation of enzyme activities in these dams and their offspring, which might contribute to prevent obesity, atherogenesis, and dyslipidemia. These effects are more pronounced after two months.

CONFLICT OF INTERESTS

Declare none

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