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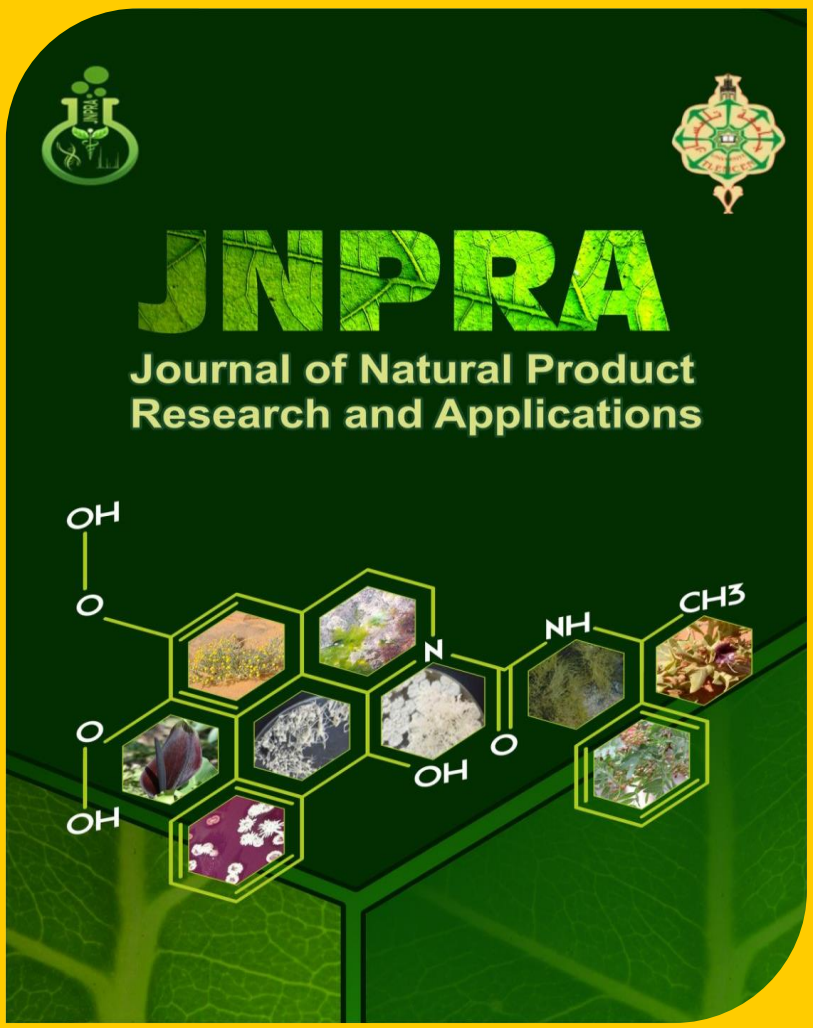

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Effect of Linseed Oil Supplementation on Lipid Peroxidation and Antioxidant Capacity in Pregnant Overfed Obese Rats and Their Offspring

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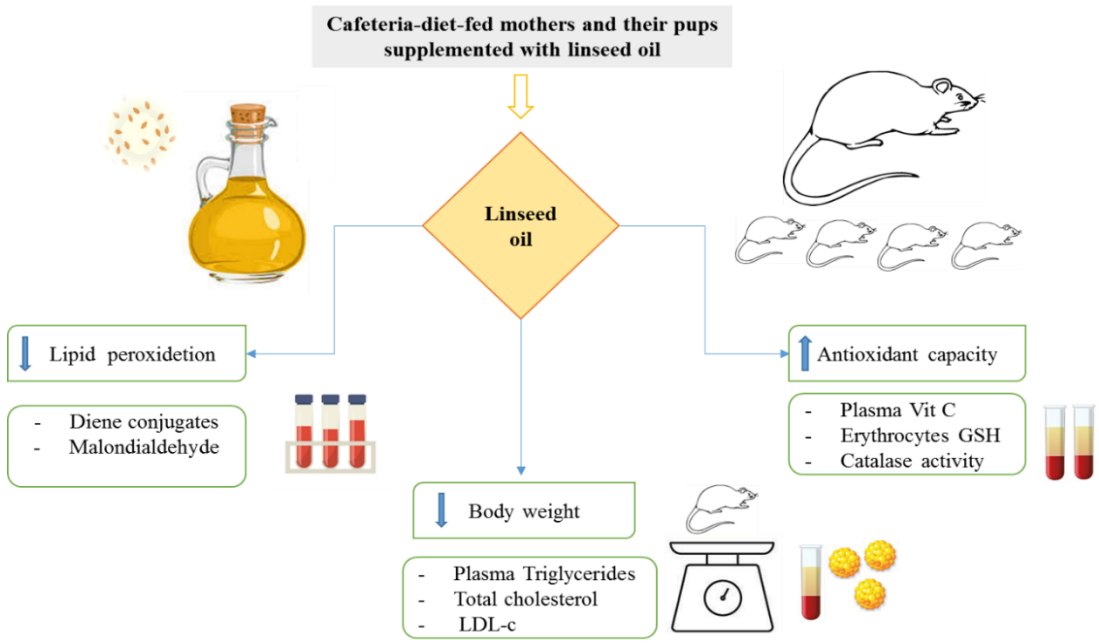
Highlights

- Protective effect of linseed oil on lipid peroxidation and antioxidant activity was evaluated in pregnant and lacing overfed obese rats and their offspring
- Linseed oil supplementation contribute to reduce body weight and lipid peroxidation in obese rats
- Linseed oil supplementation significantly improves antioxidant status in diet-induced obese rats and their offspring.
- It is suggested that Linseed oil could present a new strategy to treat obesity during pregnancy.

Graphical Abstract



Abstract
The aim of the present study was to evaluate the protective effects of linseed oil on lipid peroxidation, antioxidative capacity, as well as serum glucose, total cholesterol (TC), LDL-C and TG levels, in cafeteria-diet-fed dams during gestation and lactation, and in their offspring throughout adulthood. Food and energy intakes were also evaluated. The cafeteria diet led to higher energy intake, body weight, hyperglycemia and hyperlipidemia (higher TC, LDL-C and TG) in dams' rats and their pups.
Plasma vitamin C, Erythrocyte GSH levels and catalase activity were lower, whereas plasma diene conjugates (DC), Malondialdehyde and protein carbonyl levels (PC) in plasma and erythrocytes were higher in cafeteria-diet-fed mothers and their pups compared to controls.
Supplement of linseed oil significantly enhanced plasma antioxidant defense capacities, as evaluated by the marked increase in the levels of plasma vitamin C and erythrocyte GSH as well as the activities of CAT and the significant reduction in lipid peroxidation (lower DC and MDA) and PC in CAFL-diet-fed mothers and their offspring. Simultaneous intake of linseed oil also reduced body weight, plasma TG, TC and LDL-C contents in obese pregnant and lactating dams and their pups at day 30 and day 90.
The flaxseed oil supplementation may prevent lipid peroxidation and metabolic disorders which might be helpful in preventing obesity complications in mothers during pregnancy and lactation and in their offspring.

Keywords: linseed oil; cafeteria diet; lipid peroxidation; pregnancy; offspring.

Abbreviations
ALA: α-linolenic acid
C: control rats fed standard chow
CAF: rats fed cafeteria diet
CAFL: rats fed linseed oil supplemented cafeteria diet
CAT: catalase
CL: control rats fed the linseed oil supplemented control diet
DC: diene conjugates
GSH: reduced glutathione
LO: linseed oil
MDA: malondialdehyde
PC: protein carbonyls
ROS: reactive oxygen species

1. Introduction
Appropriate maternal nutrition during gestation and lactation plays important role for offspring to perform normal metabolic function, while maternal malnutrition may lead to metabolism related disorders in offspring including obesity (Falcone et al., 2018). Several reports suggest that both maternal obesity and nutritional state in the early postnatal phase are important in promoting obesity in offspring (Benkalfat et al., 2011; Catalano and Shankar, 2017).
Obesity is a pathological condition in which excess body fat has accumulated to the extent that it may have an adverse effect on health, leading to reduced life expectancy and/or increased health problems (Simko et al., 2019). It is a serious nutritional problem, as it increases the risk of morbidity from several pathologies, including hypertension, dyslipidemia, coronary heart disease (Savini et al., 2013), eclampsia, gestational diabetes and macrosomia (Galtier-Dereure et al., 2000). It has been shown that obesity in the nonpregnant and pregnant state is associated with inflammation and oxidative stress. Recently, studies

reported that expression of genes linked to oxidative stress is markedly elevated in placenta of obese women (Sen and Simmons, 2010). Also, in infants of obese mothers, obesity is associated with glucose and lipid metabolism abnormalities and oxidative stress (Whitaker, 2004).

Oxidative stress occurs when accumulation of reactive oxygen species damages DNA, proteins and lipids, an outcome normally limited by antioxidant defenses (Jones et al., 2013). It has been implicated in many diseases such as atherosclerosis, diabetes and obesity (Khan et al., 2006). Thus, maternal obesity in pregnancy creates a very abnormal milieu in which the embryo and fetus develop. Further, a normal redox state is critical for embryonic stem cell differentiation (Yanes et al., 2010).

Many studies have shown that obesity is associated with increased oxidative stress and lipid peroxidation (Vincent and Taylor, 2006), and that excessive maternal fat and energy intake can play an important role in the development of metabolic disorders observed in their offspring, and that maternal oxidative stress can be singled out as the factor involved. Fetal oxidative stress may represent an additional confounding influence and probably contributes to further disorders, aggravating features of the metabolic syndrome (Bouanane et al., 2009).

Long chain polyunsaturated fatty acids are essential nutrients for a healthy diet. The different kinds consumed by the mother during gestation and lactation may influence pregnancy, fetal and also neonatal outcome. The amount of fatty acids transferred from mother to fetus depends not only on maternal metabolism but also on placental function, metabolism and then transfer of fatty acids to the fetus.

Dietary supplementation with omega-3 polyunsaturated fatty acids (n-3 PUFAs) may limit oxidative stress by increasing antioxidant capacity (Cetin et al., 2009).

Linseed oil is a particularly rich source of α- linolenic acid (LNA) with concentrations ranging from approximately 40% to 60% (Xu et al., 2013). As a nutritionally essential polyunsaturated fatty acid (PUFA), LNA can act as the precursor of longer chain n-3 PUFA (EPA and DHA) or compete with linoleic acid to reduce arachidonic acid content or direct interaction with ion channels and nuclear receptors, and thus may exert numerous beneficial effects in the human body, such as antiarrhythmic, anti-inflammatory and neuroprotective functions as well as accelerating brain growth in preterm and neonates (Xu et al., 2013).

Although, many studies have reported that linseed oil is effective in amelioration of oxidative stress and lipid profile of plasma in young and aging rats fed a high-fat diet (Xu et al., 2012; Xu et al., 2013; Laissouf et al., 2013).

Other studies suggested that maternal dietary supplementation with n-3 PUFAs may be beneficial to pregnancy outcome. For example, increased maternal dietary intake of n-3 PUFAs in human pregnancy may reduce the risk of pregnancy complications (Oken et al., 2007). Potentially, such effects could be mediated via a reduced oxidative burden within the placenta, since n-3 PUFAs reduce ROS generation *in vitro* (Ambrozova et al., 2010), and dietary n-3 PUFA supplementation reduces plasma markers of *in vivo* oxidative stress (Yessoufou et al, 2006).

In contrast, n-3 PUFA supplementation has been shown to enhance free radical production and associated cellular damage, most notably in the uterus and liver (Song et al., 2000). As such, it is important to ascertain the potential benefits and risks associated with n-3 PUFA supplementation during pregnancy, as many women currently increase n-3 PUFA intake during gestation, and the majority of them will have uncomplicated pregnancies (Jones et al., 2013). However, studies regarding the effect of linseed oil on lipid peroxidation and on antioxidant status in obese dams during pregnancy or lactation and their offspring are limited.

The aim of the present study was to test the hypothesis that linseed oil can reduce oxidative stress in obese mothers and also in their offspring throughout adulthood.

2. Materials and Methods

2.1. 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). In group 2 (control linseed, CL, n=10), rats were on standard chow supplemented with linseed oil (2.5% linseed oil; g per 100g diet). The cafeteria group 3 (diet induced obese, CAF, n=10) was fed a palatable rich-fat 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/100g) was composed of 20% of energy as protein, 20% of energy as lipids and 60 % of energy as carbohydrates. The components of the cafeteria diet were carned meat, 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.

Pure 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 hours. 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.

Table 1. Nutritional components of diets (Bouanane et al., 2009; Laissouf et al., 2013).

Properties	**Levels (%)**			
	Control	CAF	CL	CAFL
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.

Offspring were weaned on to the control commercial diet or the cafeteria diet enriched or non-enriched with linseed oil. Male rats were housed separately and were followed into adulthood (12 weeks). 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.

2.2. Blood samples

At day 21 and day 51 for dams, at day 30 and day 90 for pups, ten rats of each group were anaesthetized with intraperitoneal injection of sodium pentobarbital (60 mg/kg body weight) and then bled from abdominal aorta. Serum was obtained by low speed centrifugation (1000 g, 15 min). Plasma was immediately used for biochemical parameters, diene conjugates, malondialdehyde, protein carbonyl and vitamin C determinations. After removal of plasma, erythrocytes were washed three times with two volumes of isotonic saline solution. Erythrocytes were lysed with cold distilled water (1/4), stored in the refrigerator at -4°C for 15 min and the cell debris were removed by centrifugation (2000 g for 15 min). Erythrocyte lysate was assayed for catalase (antioxidant enzyme) activity, MDA, PC and GSH levels.

2.3. 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. (1955). 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. (1975) as modified By Meghelli-Bouchenak et al. (1989). The three 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).

2.4. Chemical analysis

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

Lipoprotein fraction total cholesterol (TC) contents levels were determined using colorimetric enzymatic assays (Sigma, St. Louis, MO).

2.5. Assay of blood lipid peroxidation

The intensity of lipid peroxidation in the blood and liver were investigated by spectrophotometry for the levels of the products of lipid peroxidation diene conjugates (DC) (Yagi, 1987). The malondialdehyde levels were determined in plasma and erythrocytes by the procedure of Ohkawa et al. (1979) based on the reaction of MDA with thiobarbituric acid at 95C°.

2.6. Determination of protein carbonyls

Plasma and erythrocyte carbonyl proteins (markers of protein oxidation) were assayed by 2,4 dinitrophenylhydrazine reaction as described previously (Levine et al., 1990).

2.7. Determination of Plasmatic Levels of Vitamin C

Vitamin C levels were determined in plasma using the method of Roe and Kuether (1943).

2.8. Determination of GSH levels

Erythrocyte reduced glutathione (GSH) levels were measured using a Bioxytech GSH-400 kit (OXIS International, Inc., Portland, OR, USA).

2.9. Determination of catalase activity

CAT (EC 1.11.1.6) activity was measured by spectrophotometric analysis of the rate of H_2O_2 decomposition at 240 nm (Aebi, 1974).

2.10. 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 significant difference. The significance level was set at $P < 0.05$. These calculations were performed using STATISTICA version 4.1 (STATSOFT, Tulsa, OK).

3. Results

3.1. Effect of linseed oil on body weight and metabolic parameters

Body weight was significantly increased in dams fed cafeteria diet at day 21 and day 51 compared with controls. LO administration decreased the body weight in these rats. LO had no effect on food and energy intake in these rats. Lactating dams fed 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). Both at day21 and at day 51, mothers fed cafeteria diet had a significant increase in serum TC, LDL-C and TG levels than control rats. Linseed oil administration had significantly decreased serum TC, LDL-C and TG in CAFL-fed rats compared with CAF-fed rats (Table 2). Both at weaning and at day 90 of age, body weight of obese pups from cafeteria fed dams had significantly increased as compared with normal basal diet, LO administration reduced the body weight gain in CAFL rats compared with CAF rats (Table 3). Food and energy intake were significantly increased in adult obese offspring compared with control rats. Feeding linseed oil reduced significantly food and energy intake in CAFL-fed rats compared with CAF-fed rats with no changes in CL group compared with controls (Table 3).

At day 90; serum TC, LDL-C and TG concentrations were significantly increased in obese pups compared with their controls. LO administration reduced TC and LDL-C but not TG in CAFL group compared with CAF group, whereas TC and LDL-C levels did not vary between CL and control group. In contrast, linseed oil increased TG levels in CL-fed rats compared with control rats (Table 3). Both at day 21 and at day 51, mothers fed cafeteria diet and their offspring at day 30 and day 90 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 51and their offspring 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 (dams and offspring) and control rats (Tables 2 and 3).

Table 2. Effect of linseed oil on body weight and metabolic parameters in obese and control dams.

Group	**C**	**CAF**	**CL**	**CAFL**
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/day/rat)	24.3±1.08^{b}	29.11±1.14^{a}	24.95±2.5^{b}	28.45±2.49^{a}
Energy intake (kcal/day/rat)	93.79±4.16^{b}	152.24±4.4^{a}	96.32±9.65^{b}	148.79±32.43^{a}
Glucose (g/l)	1.30±0.18^{c}	2.02±0.1^{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-C (g/l)	0.34±0.05^{c}	0.58±0.03^{a}	0.27±0.09^{c}	0.49±0.10^{b}
HDL-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}
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/day/rat)	38.04 ±2.37^{a}	8.14±2.22^{a}	332.25±2.78^{b}	33.7±2.34^{b}
Energy intake (kcal/day/rat)	90.15±9.14^{d}	199.47±15.01^{a}	124.48±10.73^{c}	176.25±12.23^{b}
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-C (g/l)	0.32±0.03^{b}	0.64±0.03^{a}	0.38±0.11 ^{b}	0.33± 0.13^{b}
HDL-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}

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

Table 3. Effect of linseed oil on body weight and metabolic parameters in obese and control dam's offspring.

Group	**C**	**CAF**	**CL**	**CAFL**
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}
Glucose (g/l)	1±0.03 ^{b}	1.39±0.05 ^{a}	1.16±0.08 ^{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-C (g/l)	0.30±0.03 ^{b}	0.58±0.036^{a}	0.38±0.105^{b}	0.54±0.19^{b}
HDL-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}
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/day/rat)	23.45 ± 2.53^{b}	32 ± 3 ^{a}	21.06 ± 1.90^{b}	29.36 ± 2.91^{a}
Energy intake (kcal/day/rat)	90.67 ± 9.79^{b}	167.36 ±15.39^{a}	81.29± 7.33^{b}	153.55±15.21^{a}
Glucose (g/l)	1.16±0.04 ^{c}	2.52 ±0.15 ^{a}	1.15± 0.15 ^{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-C (g/l)	0.35±0.02 ^{b}	0.54±0.03^{a}	0.36±0.29 ^{b}	0.34±0.15^{b}
HDL-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}

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

3.2. Effect of linseed oil on lipid and protein oxidation

3.2.1. Mothers

A significant increase in the MDA (measure of lipid peroxidation) and carbonyl proteins (PC) levels was found in the plasma and erythrocyte of cafeteria induced obese rats when compared to control mothers at day 21 and day 51. Then, linseed oil supplementation decreased MDA and PC in plasma and erythrocyte of CAFL-fed mothers, at day 21 and day 51, compared to cafeteria- fed rats. No significant changes were noted in MDA and PC levels between CL and control groups of mothers.

The dams of $CAF_{21, 51}$ groups showed significant increase in plasma diene conjugates (DC) compared to control groups. Linseed oil decreased plasma DC in $CAFL_{21, 51}$ groups compared with $CAF_{21, 51}$ groups and in CL 51 group but not in CL 21 when compared with their controls. Then, DC was lower in CAFL-fed pregnant dams compared with CL group. In contrast, DC levels were enhanced in CAFL-fed lactating dams compared to CL-fed dams (Table 4).

Table 4. Effect of linseed oil on lipid and protein oxidation in obese and control pregnant and lactating dams.

Group	C	CAF	CL	CAFL
Day 21				
DC (µmol/l)	47±1.7 ^{b}	51.44±3.22 ^{a}	47.7±1.55 ^{a}	38.14±1.28 ^{c}
MDA plasma (µmol/l)	1.6±0.04 ^{b}	3.2±0.04 ^{a}	1.3±0.28 ^{c}	1.75±0.24 ^{b}
MDA Erythrocyte (µmol/l)	1.03±0.22 ^{b}	2.83±0.32 ^{a}	0.97±0.22 ^{b}	0.94±0.22 ^{b}
Carbonyl proteins plasma (µmol/L)	0.8±0.03 ^{b}	1.23±0.01 ^{a}	0.69±0.17 ^{b}	0.79±0.16 ^{b}
Carbonyl proteins Erythrocyte (µmol/L)	0.92±0.18 ^{c}	2.77±0.18 ^{a}	1.13±0.14 ^{c}	1.57±0.13 ^{b}
Day 51				
DC (µmol/l)	46.29±1.66 ^{b}	53.83±3.37 ^{a}	35.92±0.79 ^{d}	38.5±1.1 ^{c}
MDA plasma (µmol/l)	1.48 ± 0.14 ^{c}	4.12±0.13 ^{a}	1.63 ± 0.08 ^{c}	1.98±0.17 ^{b}
MDA Erythrocyte (µmol/l)	1.67±0.16 ^{b}	2.75±0.31 ^{a}	1.57±0.31 ^{b}	1.75±0.31 ^{b}
Carbonyl proteins plasma (µmol/L)	0.76±0.1 ^{b}	1.34±0.02 ^{a}	0.86±0.06 ^{b}	0.8±0.11 ^{b}
Carbonyl proteins Erythrocyte (µmol/L)	1.07±0.1 ^{c}	2.9±0.22 ^{a}	1.23±0.11 ^{c}	1.88±0.15 ^{b}

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

3.2.2. Offspring

Both at weaning and at adulthood, offspring of cafeteria-fed mothers showed a significant increase in plasma DC, plasma and erythrocyte MDA and PC levels, when compared to control offspring.

Diet enrich with linseed oil had decreased plasma DC, plasma and erythrocyte levels of MDA and PC in CAFL groups of pups compared to CAF groups (Table 5). Also, a significant decrease in erythrocyte MDA was shown in CL groups of pups compared to control groups at day 30 and day 90. Whereas, linseed oil did not affect plasma CD, MDA, PC and erythrocyte

PC levels in CL group of pups when compared to control groups at day 30 and day 90. No significant changes were observed in plasma DC, PC levels between CAFL-fed pups and CL_{30} and CL_{90} groups (Table 5).

Table 5. Effect of linseed oil on lipid and protein oxidation in obese and control offspring (day 30 and day 90).

Group	C	CAF	CL	CAFL
Day 30				
DC (µmol/l)	37.57±2.14 ^{b}	49.04±1.33 ^{a}	38.32±1.6 ^{b}	41.79±3.97 ^{b}
MDA plasma (µmol/l)	1.23±0.13 ^{c}	2.63±0.21 ^{a}	1.19±0.15 ^{c}	2.09 ±0.15 ^{b}
MDA Erythrocyte (µmol/l)	1.14±0.32 ^{b}	3.39±0.42 ^{a}	0.82±0.09 ^{c}	1.38±0.34^{b}
Carbonyl proteins plasma (µmol/L)	0.56±0.05 ^{b}	1.38±0.1 ^{a}	0.65±0.09 ^{b}	0.61±0.02 ^{b}
Carbonyl proteins Erythrocyte (µmol/L)	0.83±0.07 ^{c}	1.66±0.32 ^{a}	0.9±0.088 ^{c}	1.04±0.13 ^{b}
Day 90				
DC (µmol/l)	43.57±1.67 ^{b}	58.92±2.02 ^{a}	41.12±1.09 ^{b}	40.38±3.2 ^{b}
MDA plasma (µmol/l)	1.12±0.3 ^{c}	3.05±0.1 ^{a}	0.82±0.09 ^{c}	1.38± 0.34 ^{b}
MDA Erythrocyte (µmol/l)	1.16±0.28 ^{b}	2.46±0.29 ^{a}	0.79±0.3 ^{c}	1.063±0.15 ^{b}
Carbonyl proteins plasma (µmol/L)	0.6±0.09 ^{b}	1.32±0.1 ^{a}	0.62±0.09 ^{b}	0.57±0.07 ^{b}
Carbonyl proteins Erythrocyte (µmol/L)	1.56±0.11 ^{b}	3.73±0.43 ^{a}	1.59±0.13 ^{b}	1.57±0.18 ^{b}

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

3.3. Effect of linseed oil on blood antioxidative capacity

3.3.1. Mothers

As shown in Figure 1, plasma vitamin C levels, erythrocyte GSH and CAT activity were lower in cafeteria-fed mothers at day 21 and 51 compared to control mothers. At day 21, administration of linseed oil significantly augmented the vitamin C levels in plasma of CAFL and CL groups when compared to their respective controls. In contrast, linseed oil did not affect maternal plasma vitamin C levels at day 51. Then, linseed oil supplementation to cafeteria –diet-fed rats enhanced GSH levels and catalase activity in mothers at day 21 and 51.

3.3.2. Offspring

Pups from cafeteria fed mothers had significantly lower plasma vitamin C levels at day 30 and day 90 compared with control offspring (Figure 1). However, the group (CL and CAFL) which were supplemented with linseed oil showed significantly higher levels of vitamin C compared to their respective controls at day 30 and day 90. No significant changes in levels of vitamin were noted between CAFL and CL pups at day 30. Erythrocyte catalase was deceased in obese pups throughout adulthood. At weaning, CAT activity was enhanced in CAFL group when compared with cafeteria-fed pups. On the contrary, any changes were noted between CAFL and CL and between CL and control groups.

At adulthood, linseed oil supplementation increased CAT activity in both control and cafeteria-fed rats compared to their controls (Figure 1).

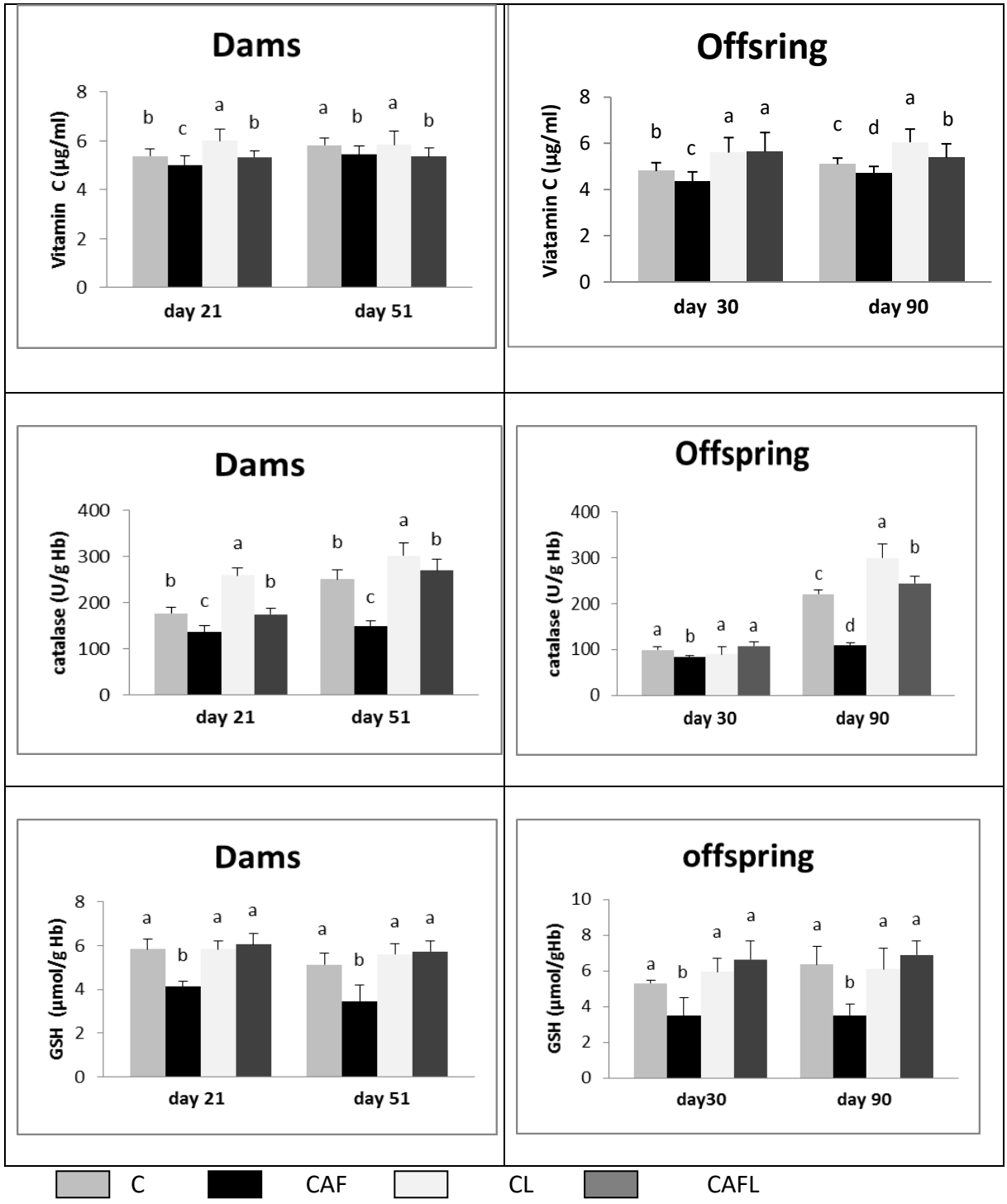


Figure 1. Effects of linseed oil on the levels of vitamin C, GSH and activity of CAT enzyme activity in obese and control mothers and their offspring.

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

4. Discussion

The present study examined the impact of maternal dietary supplementation with linseed oil on blood oxidative stress in rats during gestation and lactation and in their offspring. This study showed that the increased weight gain of adult offspring of cafeteria-diet-fed dams, both males and females, was associated with the development of obesity, hyperleptinaemia and hyperlipidaemia. These offspring also had an increase in oxidative stress with alterations in oxidant/antioxidant status similar to that found in their cafeteria-diet-fed mothers. This observation provides a support for the hypothesis that *in utero* events related to maternal overnutrition may adversely affect the oxidant/ antioxidant balance and may predispose the offspring to oxidative stress *in utero* and in later life (Bouanane et al., 2009; Moll et al., 2017).

In this study, experimental obesity was produced by cafeteria diet in pregnant and lactating rats. Rats fed this cafeteria diet (CAF) had an increase in food and energy intakes and were heavier than rats fed control standard diet (C), in agreement with previous studies on younger rats (Benkalfat et al., 2011). CAF induced hyperglycemia and hyperlipidemia in dams and their pups. Also, oxidative stress was associated with increased food, energy intakes and fat accumulation.

Our results showed that cafeteria–fed dams and their offspring had an imbalanced oxidant/antioxidant system characterized by alteration in antioxidant defense mechanisms and by an increase in oxidative stress markers, such as protein carbonyls, MDA in plasma and erythrocyte and plasma diene conjugates (DC) suggested an increase in lipid peroxidation and protein oxidation in these obese rats and their offspring in agreement with previous studies (Bouanane et al., 2009). Juvenile overweight and obesity have been linked to high levels of oxidative stress (Tran et al., 2012); obese subjects show higher oxLDL and TBARs levels than control subjects (D'Archivio et al., 2012).

Protein carbonyl contents reflect the amount of oxidative stress that the animal has been exposed to during a long time period. In offspring of cafeteria-diet-fed dams, increased plasma and erythrocyte protein carbonyl levels indicated that free-radical-mediated oxidative damage occurred at an early stage of development. A short-term high fat (HF) diet (30 days, 90 days) provokes the development of the oxidative stress, accompanied by the activation of lipid peroxidation, reducing the GSH buffering capacity of antioxidant protection (Denisenko and Novgorodtseva, 2013). GSH is one of the most important cellular antioxidant defense mechanisms (Yang et al., 2012).

As erythrocytes contain more than 95% of the blood GSH (Fang et al., 2002), GSH plays a critical role for protecting erythrocytes against oxidative stress (Yang et al., 2012; Adeoye et al., 2016). In fact, levels of erythrocyte GSH were reduced in obese mother rats and their pups compared to controls.

A fall in GSH content in obesity has been previously established. Hyperphagia-induced obesity by cafeteria diet also promotes a decrease in GSH levels (Goutzourelas et al., 2018).

The levels of plasma vitamin C were lower in cafeteria-fed mothers and their offspring than controls. In our study, low levels of vitamin C could reflect their high utilization rate, suggesting that this vitamin may be used to reduce oxidative stress in obese rats in agreement with previous studies on aging obese rats (Laissouf et al., 2013).

Accordingly, in 72 healthy obese subjects, we found that plasma total antioxidant capacity and vitamin C values were lower than those measured in lean subjects, these individuals also showed higher oxidized/reduced glutathione (GSSG/GSH) ratio. Obese adults with a history of weight fluctuations, show the most serious vitamin C deficiency (Savini et al., 2013).

Pregnant, lactating obese rats and their pups showed also lower catalase activity than controls.

Antioxidant enzymes may be consumed or inactivated in high oxidative conditions; however, ROS (reactive oxygen species) are sometimes produced in higher amounts than the antioxidant systems can scavenge (Bouanane et al., 2009).

Our results suggest that oxidative stress occurred during intra-uterine life, persisted through adulthood in offspring of cafeteria-diet-fed rats, and might be related to maternal oxidative stress, overfeeding, hyperglycaemia and hyperlipidaemia.

On the other hand, many studies have found a positive effect of specific food consumption on redox balance and/or inflammatory biomarkers in obese children and adolescents (Bradlee et al., 2010; Tang et al., 2021). Evidence for a protective role of ω-3 PUFA on cardio-vascular diseases arises both from *in vitro* and *in vivo* studies (Savini et al., 2013).

Linseed oil is 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 EPA and docosahexaenoic DHA) (Xu et al., 2012; Tang et al., 2021).

Linseed oil supplementation had beneficial effects including lower body weight, decreased plasma glucose and lipids and reduced oxidative stress. These observations corroborated with previous reports (Rasmy, 2007; Xu et al., 2012; Ayad et al., 2013).

The positive effects of n-3 PUFA-based diets on less weight gain, lipid and glucose metabolism, such as lower plasma total cholesterol, LDL-C, triglyceride and glucose levels have been described extensively before, and were explained by reduced resorption of dietary calories, enhanced insulin secretion from pancreatic beta-cells, enhanced thermogenesis and increased fatty acid oxidation (Rasmy, 2007).

It has been demonstrated that ALA rich diet decreased the rate of peroxidation and production of free radicals and dietary linseed or linseed oil reduce inflammation, oxidative lung damages, lipid peroxidation by decreasing reactive oxygen species (Rezaei and Heidarian, 2013).

In this study, CAFL-diet-fed mothers and their offspring showed a reduction in plasma DC, plasma and erythrocyte MDA and PC than controls. This reduction after linseed oil supplementation could be due to an increase in the body’s antioxidant capacity leading to reduce lipid and protein oxidation. MDA is an oxidative modification of cellular macromolecules, which can induce cell apoptosis, cell necrosis, as well as tissue damage (Ross et al., 2007).

In the present study, linseed oil reduced MDA levels in erythrocyte of obese pregnant and lactating dams and their offspring compared to their controls. This phenomenon indicated that linseed oil contained a high content of n-3 PUFA and many double bonds in n-3 PUFA avoided damage on cell and membrane form oxidative stress (Ross et al., 2007). The n-3 PUFAs acquired by the fetus are derived from maternal dietary intake and metabolism (Haggarty, 2002), but the precise molecular mechanisms which regulate fatty acid delivery from maternal to fetal circulations remain poorly understood (Duttaroy, 2009).

Then, Yang et al. (2012) suggested that linseed oil reduced MDA levels in human erythrocytes at high glucose level. In addition, erythrocyte GSH levels were increased by linseed oil supplementation in obese mothers and their pups compared to their controls suggesting that linseed oil may enhance antioxidant capacity in erythrocyte. Similar findings were reported in previous studies in erythrocytes of obese aging rats (Ayad et al., 2013; Laissouf et al., 2013). Other study observed that linseed oil could transfer phenolic hydrogen to a peroxyl free radical of a peroxidized PUFA. This mechanism can inhibit the radical chain reaction for preventing the peroxidation of PUFA in cellular or subcellular membrane phospholipids (Fang et al., 2002). Vitamin C was high in CAFL-diet-fed pregnant dams and their offspring at day 30 and day 90, suggesting that this vitamin was less used related to low oxidative stress after oil supplementation.

Administration of linseed oil in obese pregnant and lactating mothers and their offspring elevated catalase activity, indicating the antioxidant potential of linseed oil. Similar results were found by Laissouf et al. (2013) in aging rats. Also, another study suggest that administration of linseed oil and Lipoic acid combination significantly elevated the activities of antioxidant enzymes (CAT and glutathione peroxidase) as well as level of GSH which resulted in pronounced enhancement of plasma total antioxidant capacity (Xu et al., 2012).

5. Conclusion

In conclusion, the result in the present study strongly suggested that administration of linseed oil during pregnancy and lactation could attenuate lipid peroxidation and protein oxidation and preserve antioxidant capacity in dams and could have beneficial effect on the progeny. Therefore, dietary interventions such as linseed oil could present an opportunity for developing new strategies to treat obesity during pregnancy.

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Author Contribution Statement

Abdelkader Abid Djamila: Carried out the experiment, analysed the data and wrote the manuscript; Mokhtari-Soulimane Nassima: Designed the study, planned the experiment protocol, supervised the findings of this work and corrected the manuscript; Merzouk Hafida: Contributed to supervision of the findings.
All authors discussed the results and contributed to the final manuscript

Conflict of interest

All authors claim that there was no conflict of interest policy.

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