Available online at www.scholarsresearchlibrary.com

Scholars Research Library

***J. Nat. Prod. Plant Resour.*, 2013, 3 (2):61-71**
(http://scholarsresearchlibrary.com/archive.html)

ISSN : 2231 – 3184
CODEN (USA): JNPPB7

Beneficial effects of dietary olive and linseed oils on serum and tissue lipids and redox status in the aging obese rat

Amina Ayad[1], Hafida Merzouk[1]*, Yamina Baba Hamed[2], Sid Ahmed Merzouk[3], Joseph Gresti[4] and Michel Narce[4]

[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 Chemistry, Faculty of Sciences, University Abou-Bekr Belkaïd, Tlemcen 13000, Algeria
[3]Department of technical sciences, Faculty of Engineering, University Abou-Bekr Belkaïd, Tlemcen 13000, Algeria
[4]INSERM UMR 866, "Lipids Nutrition Cancer", University of Burgundy, Faculty of Sciences, 6 Boulevard Gabriel, Dijon 21000, France

---

ABSTRACT

The aim of the present work was to study how dietary olive and linseed oils modulate the rat metabolic responses to cafeteria diet during aging. Male older rats (ten months aged) weighing 350 to 400g were fed a standard chow or a cafeteria diet containing either olive oil (5%) or olive-linseed oils (2.5% olive, 2.5% linseed) for two months. Changes in serum glucose, cholesterol and triglyceride levels, liver and adipose tissue lipids and fatty acid composition, hepatic triglyceride lipase (HTGL), adipose tissue lipoprotein lipase (LPL) and hormone-sensitive lipase (HSL) activities and intracellular redox status (glutathione, malondialdehyde and carbonyl proteins) were determined at the end of the experiment. The cafeteria diet intake led to higher energy intake with higher body weight, hyperglycemia, hyperinsulinemia, hyperleptinemia and hyperlipidemia, liver and adipose lipid accumulation, alterations in lipolytic enzyme activities (high HSL and low HTGL activities) and intracellular oxidative stress (high malondialdehyde and carbonyl protein levels) in older rats. Olive oil and olive-linseed oils supplementation modulated liver and adipose tissue protein, cholesterol and triglyceride contents in both control and obese older rats, with beneficial effects resulting in lower energy intake and lower body weight, lower adipose fat deposition, decreased lipids, upregulated lipolytic enzyme activities and reduced intracellular oxidative stress. The combination olive-linseed oils appeared to be more effective in metabolic improvements especially in obese older rats. In conclusion, olive-linseed oils supplementation induced lower energy intake associated to an improvement of metabolic alterations observed in obesity during aging in rats.

Key words: aging, cafeteria diet, linseed oil, metabolism, obesity, olive oil.

Abbreviations
ALA: α-linolenic acid
C: control rats fed standard chow
CO: control rats fed the olive supplemented control diet
COL: control rats fed the olive-linseed supplemented control diet
DTNB: 5,5-dithiobis-2-nitrobenzoic acid
GSH: reduced glutathione
HSL: hormone sensitive lipase
HTGL: hepatic triglyceride lipase
LPL: lipoprotein lipase
MDA: malondialdehyde
MUFA: monounsaturated fatty acid
O: obese rats fed cafeteria diet
OL: obese rats fed the olive supplemented cafeteria diet
OOL: obese rats fed the olive-linseed supplemented cafeteria diet
PNPB: *p*-nitrophenylbutyrate

PUFA: polyunsaturated fatty acid
SFA: saturated fatty acid
TBA: thiobarbituric acid
VLDL: very low density lipoprotein

INTRODUCTION

Obesity is an important metabolic disorder characterized by reduced insulin sensitivity and lipid metabolism abnormalities, in both animal models and humans [1]. Several lipid abnormalities have been observed including plasma and tissue cholesterol and triglyceride accumulation with altered fatty acid metabolism. Additionally, lipid alterations have been considered as a contributory factor to oxidative stress in obesity [2]. In fact, obesity is associated with increased production of reactive oxygen species as well as reduced antioxidant defense mechanisms [3]. In obese patients, the increase in oxidative damage may be a consequence of hyperglycemia, hyperlipidemia, increased tissue lipid levels, inadequate antioxidant defenses, increased rates of free radical formation and chronic inflammation [3]. Aging is also associated with abnormal lipid metabolism and increased systemic oxidative stress [4]. Elevations in oxidative products with aging are normally associated with increased levels of endogenous antioxidants [5]. However, the free radical formation has been shown to increase within aging skeletal and myocardial muscle and liver at a rate that exceeds the increased antioxidant capacity of the tissue [5,6]. Oxidative stress is associated with increased susceptibility to the onset of age-related diseases [5]. Since older adults are at risk for lipid alterations and oxidant stress, the additional presence of obesity could enhance these metabolic abnormalities. We have previously shown that obese rats had increased oxidative stress which was more pronounced with advancing age [7].

On the other hand, modification of dietary fat composition may influence metabolic disorders associated with obesity [8]. Saturated fat- rich diets resulted in a reduction in insulin sensitivity and an increase in serum cholesterol and triglyceride levels and in body weight [9]. However, diets containing unsaturated fatty acids reduce the risk of developing metabolic diseases [10]. Olive oil, the main source of fat in the Mediterranean diet, is rich in oleic acid, a monounsaturated fatty acid (MUFA), and may have health benefits [11]. Recent studies have shown that diets rich in MUFAs have favorable effects on the coagulation process, inflammation, reduce fasting plasma glucose concentrations and improve insulin sensitivity [11].

In the same way, considerable interest has been generated over the last decade on the potential role of *n*-3 polyunsaturated fatty acids (PUFAs) in the prevention of metabolic diseases. The modulation of these fatty acids in the diets plays an important role in the prevention and treatment of coronary heart diseases, hypertension, auto-immune disorders and cancer [12,13].

An important source of dietary n-3 PUFA is α-linolenic acid (ALA, 18:3 n-3), supplied by vegetable sources such as linseed oil. Evidence has shown that *n*-3 fatty acids lower both plasma cholesterol and triglycerides and are useful in treating dyslipidemia in diabetes [13,14]. The effect of PUFAs on insulin sensitivity is well known [15]. There is some experimental evidence that *n*-3 PUFA-enriched diets lead to changes in energy balance and in body weight, with beneficial effects on insulin resistance [15,16]. The administration of *n*-3 PUFAs -rich diets increased lipid peroxidation [17] while the olive-rich diet resulted in less oxidative stress in rat tissues compared with a diet based on sunflower oil [18].

The liver and adipose tissue play an important role in fatty acid metabolism. Most of oleic acid and ALA are stored in adipose tissue [19]. These fatty acids are also used in the carbon recycling pathway for the *de novo* lipid synthesis or in beta-oxidation or converted to long-chain PUFAs in numerous mammalian tissues, including liver [20]. The impact of dietary fatty acid composition on liver and adipose tissue lipid metabolism has been examined by some authors [20,21,22]. However, although it is well documented that the consumption of diets high in MUFA or in n-3 PUFA can improve metabolic alterations, their beneficial effects on obesity during aging have not been elucidated. Indeed, most studies have employed only one high-fat formula in contrast with standard chow and did not analyze the influence of the combination of different fatty acids in the model.

This paper is the first attempt to understand how the combination of dietary n-3 PUFA and MUFA affects the metabolic responses of aged rat to cafeteria diet, with special attention to liver and adipose tissue lipid profiles and oxidant / antioxidant status. To mimic human diets, we used the combination of two oils, olive and linseed oils. The cafeteria feeding is believed to be a reliable model of dietary obesity in humans and we have previously shown that offering rats a variety of snack-type foods induced obesity, oxidative stress and several liver and adipose tissue metabolic alterations [23,24]. Our hypothesis is that the type of dietary fat is an important factor modulating obesity-related changes in aging.

MATERIALS AND METHODS

Animals and experimental protocol

Older male wistar rats (aged 10 months, n = 48), weighing 350 to 400g, were obtained from Animal Resource Centre (Algeria). Animals were housed at 20 ± 2°C with 2 - 3 in each cage, and maintained on a 12:12 h light/dark cycle. Rats were randomly assigned to one of 6 experimental diets. The control group (control, C, n=8) was fed standard laboratory chow (ONAB, Algeria). In group 2 (control olive, CO, n=8), rats were on standard chow supplemented with olive oil (5%). In group 3 (control olive linseed, COL, n=8), rats were on standard chow supplemented with the combination of olive-linseed oils (2.5% olive oil and 2.5% linseed oil). The cafeteria group 4 (diet induced obese, DIO, n=8) was fed a palatable rich-fat diet. In group 5 (diet induced obese olive, DIOO, n=8), rats were on cafeteria diet supplemented with olive oil (5%), and in group 6 (diet induced obese olive linseed, DIOOL, n=8), rats were on cafeteria diet supplemented with the combination of olive-linseed oils (2.5% olive oil and 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 grinded 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). 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 fatty acid composition of the six diets is listed in Table 1. Pure linseed and olive oils were obtained from INRA (INRA, Algeria). Fresh food was given daily and food intake and body weights were recorded. Rats were killed after 8 weeks of feeding.

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.

Blood and tissue samples

At the end of the experimental period (two months), the animals were kept for overnight fasting. They were anaesthetized with intraperitoneal injection of sodium pentobarbital (60 mg/kg of body weight). The blood was drawn from the abdominal aorta, and serum was used for glucose and lipid determinations. The liver and abdominal (perirenal and epididymal) white adipose tissue were removed, washed with ice-cold saline, quickly blotted and weighed. An aliquot of each tissue was homogenized in an Ultraturrax homogenizer (Bioblock Scientific, Illkirch, France) for lipid extraction. A second aliquot of tissues was homogenized in 10 volumes of ice-cold 10 mmol/l phosphate-buffered saline (pH 7.4) containing 1.15% KCl. The homogenate was subjected to a 6000 g centrifugation at 4°C for 15 min. The supernatant fractions were collected and used for biochemical and redox markers determinations. For lipoprotein lipase (LPL) activity, tissue homogenates in 0.9% (w/v) NaCl containing heparin (Sigma, St. Louis, MO, U.S.A.) were prepared as described by Mathe et al. [25]. Another aliquot of adipose tissue portion was homogenized in ice cold buffer containing 0.25M sucrose, 1 mM dithiothreitol and 1 mM EDTA, pH 7.4, supplemented with 20 mg/ml leupeptin, 2 mg/ml antipain and 1 mg/ml pepstatin, and was used for the adipose hormone-sensitive lipase (HSL) assay as described by Kabbaj et al. [26].

Chemical analysis

Serum glucose was measured using the Trinder glucose kit (Sigma). Serum and tissue triglyceride and cholesterol were measured using colorimetric enzymatic kits (Roche Diagnostics). For these enzymatic methods, the interassay CV (coefficient of variance) was in the range of 1.7 to 3%. Serum HDL- and LDL/VLDL-Cholesterol concentrations were determined by an enzymatic calorimetric test provided by Bioassay Systems Kits, with an interassay CV of 2 to 3.5%. Plasma creatinine, urea and uric acid were measured using enzymatic colorimetric methods (Kits from BioAssay Systems, CA), with an interassay CV of 1.3 to 2.5%. Serum insulin, adiponectin and leptin concentrations were analysed using RIA kits with antibodies to authentic rat insulin, adiponectin and leptin respectively (Linco Research), with an interassay CV of 3 to 5%. Total lipids of liver and adipose tissue were extracted by chloroform/methanol (2:1, v/v). Lipids were transmethylated and fatty acids were analyzed by gas–liquid chromatography as previously described [23]. Protein contents of tissue homogenates were determined by the method of Lowry et al. [27], with BSA as the standard.

Determination of lipolytic activities

To estimate adipose hormone sensitive lipase (HSL; EC 3.1.1.3) activity, a spectrophotometric esterase assay based on the hydrolysis of PNPB (*p*-nitrophenylbutyrate) was used as described by Kabbaj et al. [26], with an interassay CV of 4.5%. Hepatic triglyceride lipase (HTGL) and adipose tissue lipoprotein lipase (LPL) activities were assayed in the supernatants containing heparin-releasable lipases described by Nilsson-Ehle and Ekman [28], with an interassay CV of 4%. We have previously reported details on these enzymatic methods [24,29].

Determination of tissue oxidant / antioxidant status

The tissue malondialdehyde (MDA) levels, a marker of lipid peroxidation, were determined in tissue supernatants by the method of Draper and Hadley [30] based on the reaction of MDA with thiobarbituric acid (TBA) at 95 ^{o}C. Carbonyl proteins (markers of protein oxidation) were assayed by the 2,4-dinitrophenyl hydrazine reaction as described previously [31]. Tissue reduced glutathione (GSH) levels were measured using a Bioxytech GSH-400 kit (OXIS International, Inc., Portland, OR, USA).

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 individual effects of the diets and the oil supplementations were distinguished by two-way ANOVA. The significance level was set at $P < 0.05$. These calculations were performed using STATISTICA version 4.1 (STATSOFT, Tulsa, OK).

RESULTS

Body and organ weights, food and energy intakes in study rats

The cafeteria diet was associated with increased body weight and weight gain compared to standard chow, regardless of oil supplementation (Table 2). Supplementation with olive oil or with the combination of olive–linseed oils induced a reduction in body weight and in weight gain in both control and obese aged rats. Both diet induced obese (DIOO) and control (CO) rats from groups fed on olive diets showed the lowest body weight compared to those fed on the standard basal diets (C, DIO) or the olive-linseed supplemented diets (COL, DIOOL) (P=0.0001). As expected, diet induced obese rats in the cafeteria fed groups had a higher food and energy intakes compared with control animals. Food and energy intakes in the control and diet induced obese rats fed with the olive oil (CO, DIOO) or the olive–linseed oil (COL, DIOOL) supplemented diets were significantly lower than those in the rats fed on the standard diets (C, DIO) (P=0.001).

Compared with the controls, aged diet induced obese rats had a significantly higher adipose tissue and liver weight (Table 2). Oil supplementation significantly reduced adipose and liver weights in diet induced obese rats ($P \leq 0.001$). Diet induced obese rats fed on the olive -linseed diet (DIOOL) had significantly decreased adipose tissue than those fed on the olive diet (DIOO). Liver weight did not differ between control rats in the three diets C, CO and COL. However olive-linseed diets exerted an adipose tissue weight-decreasing action in control and diet induced obese groups. Two-way ANOVA indicated significant effects of diet and oil supplementation for all these parameters (P< 0.01).

Serum glucose, lipid and hormone levels in study rats

Serum glucose levels were significantly higher in aged diet induced obese rats fed on basal cafeteria diet (DIO) than control groups (C, CO, COL) (P=0.001). Oil supplementation induced a significant reduction in glycemia in both control and obese rats, with a more distinct fall on the olive oil supplemented diets compared to the olive-linseed oil diets especially in the diet induced obese rats (Table 2). Two-way ANOVA revealed an effect for both the diet (P=0.002) and the oil supplementation (P=0.006) on serum glucose concentrations.

Serum cholesterol, triglyceride and LDL/VLDL-C levels were increased in diet induced obese rats compared to controls (Table 2). These values were significantly decreased with oil supplementation of the basal diets in both control and diet induced obese rats (P<0.001). A comparable reduction was obtained with olive oil and olive-linseed oil combination with respect to serum cholesterol and LDL/VLDL-C. However, the fall in serum triglyceride was most pronounced on the olive-linseed oil diet compared to olive oil diet. There was no difference in serum triglyceride levels among control and diet induced obese groups fed on olive-linseed supplemented diets. There were significant effects (two-way ANOVA) of the diet and oil supplementation on cholesterol (P= 0.006 and P= 0.007 respectively), triglyceride (P= 0.004 and P= 0.002 respectively) and VLDL/LDL-C ((P= 0.007 and P= 0.008 respectively) concentrations. Neither the diet nor the oil supplementation affected HDL-C levels in the rats, as revealed by two-way ANOVA.

Serum insulin, adiponectin and leptin levels were increased in diet induced obese rats compared to controls (P=0.001) (Table 2). These values were significantly decreased with oil supplementation in diet induced obese rats but not in controls. Hormone variations were more apparent in the olive-linseed oil diet than in olive oil diet. Two-way ANOVA revealed that the oil supplementation did not influence insulin, leptin and adiponectin concentrations

(P=0.320); however, these hormone concentrations were affected by the diet (P<0.005) and by the interaction between the diet and oil supplementation (P<0.002).

Table 1. Fatty acid composition of experimental diets

	Control diet (C)	Control olive diet (CO)	Control olive linseed diet (COL)	Cafeteria diet (DIO)	Cafeteria olive diet (DIOO)	Cafeteria olive linseed diet (DIOOL)
(% fatty acids)						
SFA	29	22	20	44	39.50	34
C18:1 n-9	20	30	28	28	33	31
C18:2 n-6	46	44	36	27	26.50	23
C18:3 n-3	3	3	15	1	1	12
C20:4 n-6	2	1	1	0	0	0
n-9 / n-3	7	7	1.87	28	33	2.58

SFA: saturated fatty acids. Fatty acid composition was analyzed by gas liquid chromatography as mentioned in material and method section.

Table 2. Characteristics of the study rats

	Control rats			Diet induced obese rats			*P*
	C	**CO**	**COL**	**DIO**	**DIOO**	**DIOOL**	***(ANOVA)***
Body weight (g)	541.66±6.73^{c}	463.33±5.47^{e}	501.43±6.76^{d}	616.67±8.13^{a}	530.00±7.31^{c}	565.71±6.1^{b}	0.0001
Weight gain (g)	166±9.77^{b}	97.42±7.92^{d}	131±6.31^{c}	250.25±9.11^{a}	170.36±8.03^{b}	185±14.22^{b}	0.001
Food intake (g/day/rat)	44.38±1.67^{b}	28.13±1.38^{d}	31.88±1.04^{c}	48.88±2.07^{a}	31.88 ±2.12^{c}	33.75±1.77^{c}	0.001
Energy Intake (Kcal/day/rat)	171.66±8.89^{b}	108.58±7.18^{d}	123.05±6.01^{c}	277.51±13.08^{a}	180.73±9.3^{b}	191.52±8.9^{b}	0.001
Liver weight (g)	20.90±1.43^{c}	19.63±1.23^{c}	20.61±1.31^{c}	26.01±1.20^{a}	23.21±1.09^{b}	23.30±1.13^{b}	0.001
Adipose tissue weight (g)	8.57±0.67^{c}	7.45±0.31^{d}	6.39±0.58^{e}	18.78±1.20^{a}	13.22±1.09^{b}	9.06±0.64^{c}	0.0001
Serum characteristics							
Glucose (g/L)	1.02±0.08^{b}	0.79±0.07^{c}	0.81±0.06^{c}	1.45±0.04^{a}	0.90 ±0.05^{c}	1.14±0.06^{b}	0.001
Cholesterol (g/L)	1.29±0.03^{b}	0.81±0.02^{c}	0.80±0.03^{c}	1.86±0.03^{a}	1.23±0.02^{b}	1.22±0.04^{b}	0.001
Triglycerides (g/L)	1.14±0.03^{b}	0.73±0.01^{c}	0.57±0.01^{d}	1.97±0.03^{a}	1.15±0.03^{b}	0.57±0.01^{d}	0.0001
HDL-C (g/L)	0.63±0.08	0.51±0.06	0.50±0.07	0.54±0.06	0.60±0.05	0.63±0.06	0.124
LDL/VLDL-C (g/L)	0.64±0.05^{b}	0.30±0.04^{c}	0.31±0.05^{c}	1.30±0.06^{a}	0.62±0.04^{b}	0.57±0.05^{b}	0.001
Insulin (ng/mL)	1.36±0.11^{d}	1.34±0.06^{d}	1.32±0.09^{d}	2.33±0.16^{a}	1.86±0.14^{b}	1.52±0.15^{c}	0.001
Adiponectin(µg/mL)	3.68±0.32^{d}	3.12±0.43^{d}	3.10±0.37^{d}	6.56±0.24^{a}	5.37±0.26^{b}	4.79±0.21^{c}	0.001
Leptin (ng/mL)	4.67±0.36^{d}	4.22±0.41^{d}	4.38±0.31^{d}	8.45±0.45^{a}	6.63±0.33^{b}	5.22±0.28^{c}	0.001

Values are presented as means ± standard deviations (SD). C: aged rats fed control diet. CO: aged rats fed control olive diet. COL: aged rats fed control olive-linseed diet. DIO: aged diet induced obese rats fed cafeteria diet. DIOO: aged diet induced obese rats fed cafeteria olive diet. DIOOL: aged diet induced obese rats fed cafeteria olive-linseed diet. Values with different superscript letters (a, b, c, d, e) are significantly different (P < 0.05).

Table 3. Liver and adipose tissue lipid and protein contents of the study rats

	Control rats			Diet induced obese rats			
	C	**CO**	**COL**	**DIO**	**DIOO**	**DIOOL**	***P* (ANOVA)**
Liver							
Lipids (mg/g)	101.50±13.80^{c}	50.72±6.57^{d}	100.62±15.38^{c}	188.39±14.33^{a}	142.19±15.43^{b}	132.23±18.72^{b}	0.001
Proteins (mg/g)	149.41±12.60^{c}	39.00±6.46^{e}	57.36±6.57^{d}	152.12±11.86^{a}	121.29±11.17^{b}	88.14±12.15^{c}	0.0001
Cholesterol (mg/g)	21.40±2.22^{b}	10.53±1.66^{d}	9.24±1.15^{d}	36.93±2.86^{a}	13.40±1.48^{c}	14.94±1.62^{c}	0.0001
Triglycerides (mg/g)	39.60±2.63^{b}	20.60±1.84^{d}	19.24±1.36^{d}	59.00±1.60^{a}	28.20±2.23^{c}	20.03±1.05^{d}	0.001
Adipose tissue							
Lipids (mg/g)	225.00±16.85^{f}	123.61±17.88^{d}	143.38±13.01^{d}	345.05±14.24^{a}	226.95±12.36^{b}	178.14±15.43^{e}	0.001
Proteins (mg/g)	54.25±3.85^{b}	34.75±1.29^{d}	35.0±1.05^{d}	61.53± 3.59^{a}	56.71±3.58^{b}	50.86±4.73^{c}	0.01
Cholesterol (mg/g)	6.70 ±0.88^{b}	4.78±0.46^{c}	2.23±0.27^{e}	9.87±0.82^{a}	6.36±0.45^{b}	3.08±0.70^{d}	0.0001
Triglycerides (mg/g)	83.50±5.16^{c}	51.59±3.25^{d}	49.2±5.15^{d}	167.60±7.07^{a}	95.10±4.05^{b}	76.36±3.63^{c}	0.001

Values are presented as means ± standard deviations (SD). C: aged rats fed control diet. CO: aged rats fed control olive diet. COL: aged rats fed control olive-linseed diet. DIO: aged diet induced obese rats fed cafeteria diet. DIOO: aged diet induced obese rats fed cafeteria olive diet. DIOOL: aged diet induced obese rats fed cafeteria olive-linseed diet. Values with different superscript letters (a, b, c, d, e) are significantly different (P < 0.05).

Liver and adipose tissue lipid and protein contents and fatty acid composition

Older diet induced obese rats (DIO) had significantly higher liver and adipose tissue lipid contents than control rats (C) (P=0.001) (Table 3). The olive oil supplemented diet significantly decrease liver lipid levels while the olive-linseed oil diet did not affect these levels in control rats. Adipose tissue lipid contents were decreased by oil supplementation with a more distinct fall on the olive oil supplemented diet in control rats (CO).

Liver lipid contents were decreased in diet induced obese rats by oil supplementation. Adipose tissue lipid contents were also reduced by oil supplementation with a more distinct fall on the olive-linseed oil diet in diet induced obese rats (DIOOL). Two-way ANOVA showed significant effects of the diet and the oil supplementation on liver and adipose tissue lipid contents (P<0.006).

Table 4. Fatty acid composition of liver and adipose tissue of the study rats

	Control rats			Diet induced bese rats			
	C	**CO**	**COL**	**DIO**	**DIOO**	**DIOOL**	***P* (ANOVA)**
Liver							
SFA	42.23±1.34^{b}	29.67±1.41^{e}	25.94±1.33^{f}	47.97±1.48^{a}	39.67±1.22^{c}	31.06±1.58^{d}	0.0001
C18:1 n-9	17.68±1.01^{d}	28.23±1.05^{a}	25.26±1.12^{b}	18.02±1.02^{d}	22.02±1.48^{c}	20.51±1.66^{c}	0.0001
C18:2 n-6	19.22±1.21^{b}	19.92±1.20^{b}	17.96±1.04^{c}	22.39±1.11^{a}	20.67±1.35^{b}	19.12±1.08^{b}	0.001
C18:3 n-3	0.50±0.06^{b}	0.44±0.05^{b}	7.88±1.01^{a}	0.46±0.07^{b}	0.67±0.14^{b}	7.73±0.93^{a}	0.001
C20:4 n-6	17.31±1.23^{a}	18.51±2.55^{a}	16.92±1.33^{a}	9.15 ±1.04^{c}	14.26±1.11^{b}	17.02±1.25^{a}	0.001
C20:5 n-3	3.46 ±0.54^{b}	2.98 ±0.58^{b}	5.85 ±0.56^{a}	1.63 ±0.39^{c}	1.54 ±0.42^{c}	4.53 ±0.77^{a}	0.001
Adipose tissue							
SFA	31.74±1.22^{c}	22.54±1.15^{e}	23.17±1.23^{e}	36.62±1.11^{a}	34.13±1.04^{b}	28.70±1.25^{d}	0.0001
C18:1 n-9	28.73±1.06^{d}	40.19±1.44^{a}	35.66±1.67^{b}	33.84±1.24^{c}	37.87±1.57^{b}	36.14±1.63^{b}	0.0001
C18:2 n-6	26.99±1.41^{b}	26.30±1.67^{b}	25.08±1.84^{b}	29.82±1.35^{a}	18.22±1.22^{d}	22.07±1.21^{c}	0.001
C18:3 n-3	0.85±0.10^{c}	0.64±0.06^{c}	5.48±0.88^{a}	0.58±0.07^{c}	0.52±0.08^{c}	3.09±0.54^{b}	0.001
C20:4 n-6	10.74±1.72^{a}	9.33±1.31^{a}	8.22±1.83^{a}	3.14±0.55^{b}	8.38±1.77^{a}	8.23±1.62^{a}	0.01
C20:5 n-3	0.60±0.05^{b}	0.50±0.07^{b}	1.88±0.26^{a}	0.53±0.04^{b}	0.50±0.06^{b}	1.60±0.41^{a}	0.01

Values are presented as means ± standard deviations (SD). C: aged rats fed control diet; CO: aged rats fed control olive diet; COL: aged rats fed control olive-linseed diet; DIO: aged diet induced obese rats fed cafeteria diet; DIOO: aged diet induced obese rats fed cafeteria olive diet; DIOOL: aged diet induced obese rats fed cafeteria olive-linseed diet; SFA: saturated fatty acids. Values with different superscript letters (a, b, c, d, e, f) are significantly different (P < 0.05).

Liver protein contents in diet induced obese rats were similar to those in controls (Table 3). However, adipose tissue protein contents were high in diet induced obese rats (P=0.01). Oil supplementation significantly decreased liver and adipose protein levels in aged control rats (CO and COL versus C), and in aged diet induced obese rats (DIOO and DIOOL versus DIO), with a pronounced effect of olive-linseed combination in obese group. Although two-way ANOVA showed significant diet effects on adipose tissue protein contents (P=0.02) and no effect on liver protein contents, oil supplementation and the interaction effect between diet and oil were significant on tissue protein contents (P<0.005).

The obese rats had a higher accumulation of cholesterol and triglycerides in both liver and adipose tissue than controls whatever the diet fed (P≤0.001) (Table 3). The olive and olive-linseed diets induced a similar reduction of the concentrations of liver cholesterol and triglycerides, and adipose triglycerides in control rats, and liver cholesterol in obese rats. However, adipose cholesterol contents in control rats, liver triglycerides and adipose cholesterol and triglycerides in diet induced obese rats were more decreased with the olive-linseed diet than the olive diet. Two-way ANOVA indicated significant effects of diet and oil supplementation for liver and adipose cholesterol and triglyceride contents (P<0.01).

As expected, liver and adipose tissue fatty acid concentrations reflected dietary fatty acid composition (Table 4). The proportions of hepatic and adipose saturated fatty acids (SFA) and monounsaturated fatty acids (MUFA) were significantly increased, whereas polyunsaturated fatty acids (PUFA) were decreased in diet induced obese aged rats (DIO) compared with controls (C), except for high C18:2 n-6 proportions in diet induced obese rats (P≤0.001). The olive oil supplementation induced a significant decrease in SFA and an increase in MUFA in liver and adipose tissue lipids of both aged control and diet induced obese rats (CO versus C and DIOO versus DIO). The olive-linseed oils supplementation exerted a significant decrease in SFA and an increase in MUFA, C18:3n-3 and C20:5n-3 proportions in control and obese liver and adipose lipids (COL versus C and DIOOL versus DIO). In addition to these modifications, both DIOO and DIOOL rats showed also significant lower C18:2n-6 proportion and higher

C20:4n-6 proportion than in DIO rats. Two-way ANOVA indicated significant effects of diet and oil supplementation for fatty acid composition (P<0.005).

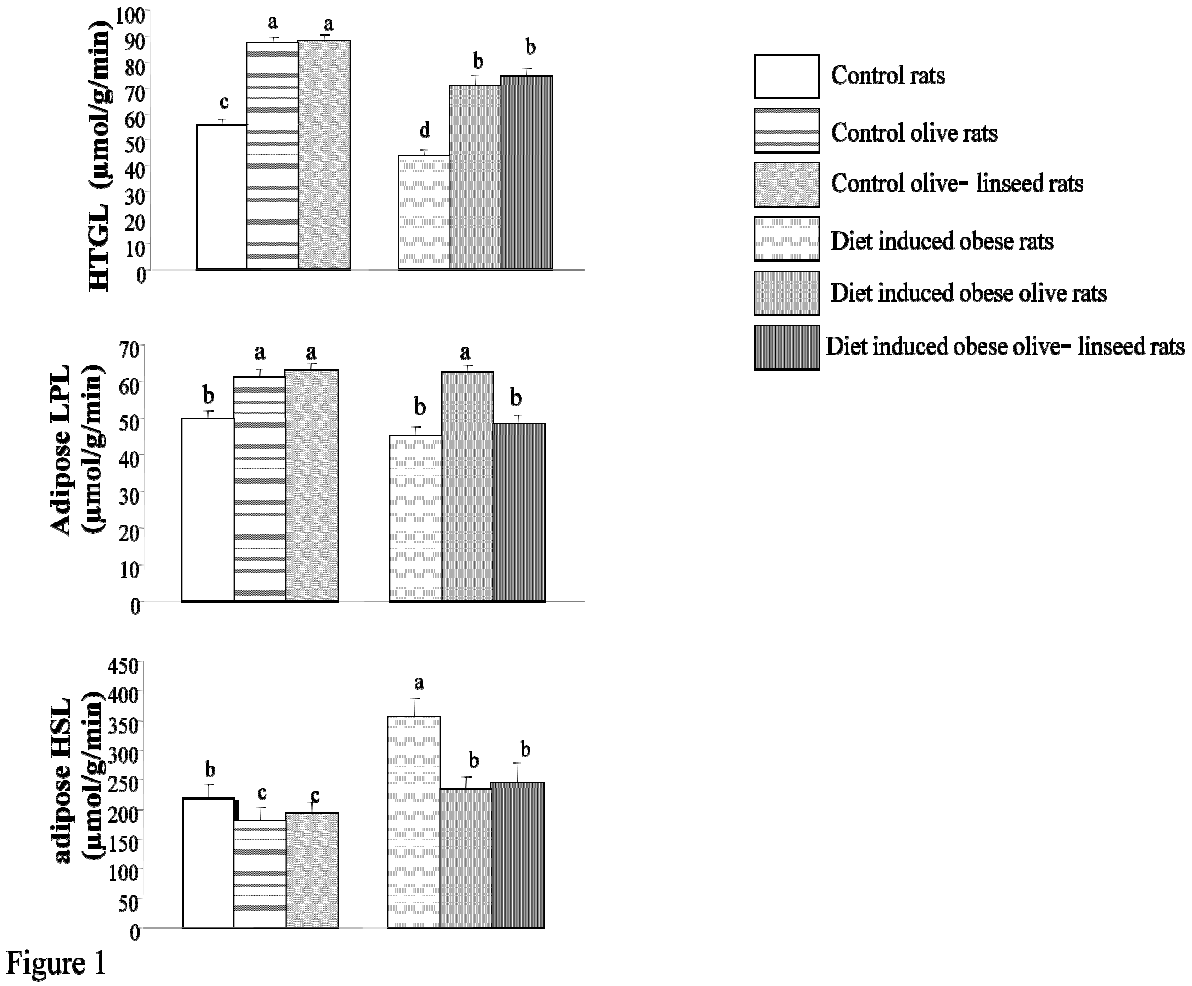

Figure 1. Hepatic triglyceride lipase 'HTGL), adipose lipoprotein lipase (LPL) and hormone sensitive lipase (HSL) activities in the study rats.

Values are presented as means ± standard deviations (SD). Values with different superscript letters (a, b, c) are significantly different (P < 0.05).

Liver and adipose tissue lipolytic activities

The aged DIO rats had low HTGL activity and high adipose tissue HSL activity compared to control values whatever the diet fed (P=0.001) (Figure 1). Olive oil and olive-linseed oils supplementation of the diets induced a significant enhancement of liver lipase activity and a significant fall of adipose HSL activity in both control and diet induced obese rats. The values obtained after olive oil addition were comparable to those with olive-linseed oils supplementation in both control and obese rats.

Adipose tissue LPL activity did not vary significantly between DIO and C rats (Figure 1). Olive oil diets enhanced adipose LPL activity in both aged obese (DIOO versus DIO) and control (CO versus C) rats. Olive-linseed diets induced an increase in adipose LPL in control rats (COL versus C) but not in obese rats (DIOOL versus DIO). Two-way ANOVA showed significant effects of diet and oil supplementation for lipolytic activities (P<0.003).

Liver and adipose tissue oxidant/antioxidant status

In liver, carbonyl protein and MDA levels were increased in diet induced obese compared to control rats, whatever the diet fed (P<0.004) (Figure 2). Olive oil as well as olive-linseed oils induced a similar fall in hepatic carbonyl protein and MDA levels in obese and control rats. The variations in hepatic glutathione amounts didn't reach statistical significance between the different groups. The effects of diet and oil supplementation and the interaction effect between diet and oil supplementation were significant on hepatic carbonyl protein and MDA levels (Two-way ANOVA, P≤0.001).

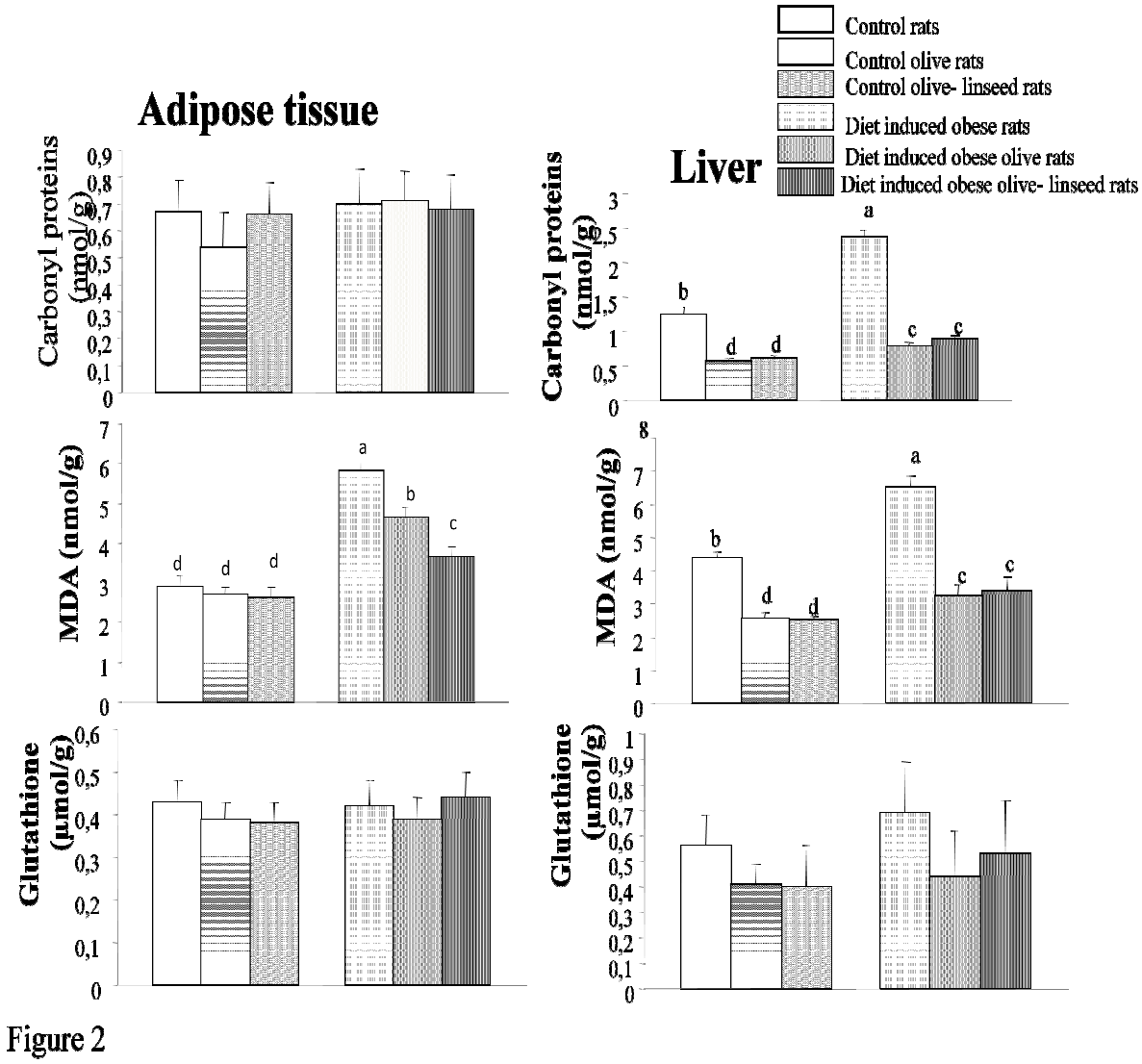

Figure 2. Liver and adipose tissue oxidant/antioxidant status in the study rats.
Values are presented as means ± standard deviations (SD). MDA: Malondialdehyde. Values with different superscript letters (a, b, c) are significantly different (P < 0.05).

In adipose tissue, protein carbonyl and glutathione levels were unaffected by obesity or by oil supplementation (Figure 2). However, adipose MDA concentrations were increased in diet induced obese rats versus controls regardless of the diet fed (P=0.001). Although oil supplementation had no significant effect on adipose MDA levels in controls, it reduced these levels in diet induced obese rats with a most pronounced effect on olive-linseed oils supplementation (P=0.001). . The interaction effect between diet and oil supplementation were significant on adipose MDA levels (Two-way ANOVA, P=0.001).

DISCUSSION

In this study, we aimed to verify whether diet supplementation in olive oil or in olive–linseed oils combination have the potential to counteract the effects of a cafeteria diet on liver and adipose tissue lipids, lipolytic activities and redox status during aging. We also aimed to prove that olive–linseed oils combination has a more beneficial effect than olive oil supplementation. It is well known that during aging, metabolic dysfunctions take place, leading to altered lipid and glucose metabolism, insulin resistance and organ damage. All these alterations are also observed in obesity, and they would be worsened in the aging – obesity association.

In the present study, palatable cafeteria diet was given to older rats for 8 weeks to induce dietary obesity. Cafeteria diet feeding induced an increase in total food and energy intakes that may explain the higher body and adipose depot weights, an increase in serum glucose, insulin, leptin, adiponectin and lipid concentrations, hepatic cholesterol and triglyceride accumulation, in agreement with previous studies [7,23,24,32]. The old obese rats presented low hepatic lipase activity compared to controls. Knowing the role of HTGL in hydrolyzing lipoprotein lipids and in facilitating

the uptake of lipoprotein lipids by cell surface receptors [33], reduced HTGL activity could explain high cholesterol and triglyceride concentrations observed in obese rats. Low HTGL activity may limit hepatic lipid uptake. However, despite low HTGL activity, hepatic cholesterol and triglyceride levels were increased in these obese rats. Indeed, in our present study, diet induced obese older rats presented significant increases in adipose tissue cholesterol and triglyceride with alterations in adipose lipolytic enzyme activities such as an increase in HSL activity, as previously shown in younger rats [24]. Normal adipose LPL activity was seen in our obese older rats. It can be suggested that high adipose tissue HSL activity and lipolysis with normal LPL activity may be a biological adaptation to a metabolic alteration, i.e. saturation of the capacity for triglyceride storage. In our study, pronounced changes in the fatty acid composition of liver and adipose lipids were also observed in older obese rats. These old diet induced obese rats presented a significant decrease in liver and adipose lipid PUFA contents, balanced by increases in SFA and MUFA. These observations are in agreement with our previous data in younger obese rats [23,24]. It is interesting to note that aged rats responded to the cafeteria diet in a manner similar to that with young rats.

These obese older rats presented also an intracellular oxidative stress. In fact, the elevated levels of hepatic and adipose MDA and of hepatic protein carbonyls suggested an increased lipid peroxidation and protein oxidation in these aged obese rats, in agreement with previous studies [2,3,7]. Oxidative stress in cafeteria fed rats may be generated by exacerbated nutrient oxidation, as previously reported [34]. However, levels of tissue glutathione (GSH), one of the most important cellular antioxidant defense mechanisms, remained unchanged between control and obese rats. A fall in GSH content of aging tissues and also in obesity has been previously established [35]. In our study, control rats as well as obese rats were old and might have low hepatic and adipose GSH contents compared to their respective younger rats. It is interesting to note that obesity in the aged rats did not induce a further fall in tissue GSH levels.

The present study showed that olive oil and olive-linseed oils supplementation modulated several liver and adipose parameters in both control and obese old rats, with beneficial effects including lower energy intake, lower body weight, lower adipose fat deposition, decreased lipids and reduced oxidative stress. The combination olive-linseed oils appeared to be more effective in metabolic improvements especially in obese old rats. Olive or olive-linseed oils enriched diets resulted in a reduction in food and energy intakes with a concomitant decrease in body weight in both control and obese groups. This effect was more pronounced with olive oil supplementation. A satiating effect of olive oil intake might explain this weight loss [36]. The decrease in body weight was accompanied by a reduction in adipose tissue weight due to a depletion in triglycerides and cholesterol that was more apparent with olive-linseed combination especially in obese old rats. Oil supplementation also induced a fall in adipose HSL activity and an enhancement in adipose LPL activity that could not explain the lipid depletion in adipose tissue. Olive oil and olive-linseed oils supplementation reduce adiposity in old animals despite stimulating fatty uptake and reducing lipolysis. Reduced fat mass could be explained mechanistically by increased β-oxidation and reduced de novo lipogenesis in white adipose tissue as previously reported [21]. Some researchers on young rats showed that a diet rich in oleic acid caused either an increase [37] or a decrease in adipose LPL activity [38] while PUFAs caused either a decrease [38] or an increase in this LPL activity concomitantly with an increase in lipolysis via HSL activity [39]. Our results are novel in obese old rats and showed that the combination olive-linseed oils had no effect on adipose LPL activity but reduce lipolysis. These alterations in adipose lipolytic activities seemed to be related to improvement in insulin sensitivity in obese older rats, and this observation corroborated previous clinical studies [11-13,15]. Reduction of fatty acid efflux by adipose tissue reduces the availability of fatty acids as a substrate for triglyceride synthesis and VLDL production in hepatocytes. In fact, oil supplementation induced a concomitant improvement in hepatic parameters, in particular reduce liver lipids suggesting improving hepatic steatosis in obese older rats, especially with olive-linseed combination. These results agree with previous findings [22]. Oil supplementation also caused an increase in HTGL activity in both control and obese old rats. However, despite high HTGL activity, oil supplementation reduced hepatic lipid accumulation both by stimulating β-oxidation, by suppressing fatty acid synthesis and by enhancing cholesterol secretion into bile, as previously reported [40].

Moreover, olive oil and olive-linseed oils supplementation were also associated to a reduction in serum glucose, cholesterol, triglyceride and VLDL/LDL-C levels, especially in cafeteria fed obese older rats, in agreement with previous reports [8,10,12-14]. Indeed, oil supplementation induced a decrease in serum insulin, leptin and adiponectin concentrations in cafeteria diet fed rats, especially with olive-linseed combination. Liver and adipose tissue fatty acid composition was modified by the oils added to the diets. The increase in MUFA and the reduction of SFA proportions in olive oil group, as well as the high MUFA, C18:3n-3 and its elongated product, C20:5n-3 with low SFA proportions in olive-linseed oils group reflected the difference in fatty acid composition of the diets, as previously reported [17,19,22]. In addition, in obese older rats, oil supplementation induced a reduction in tissue C18:2n-6 proportion and a concomitant increase in C20:4n-6 proportion, reflecting an amelioration of desaturase activities. These fatty acid modifications were accompanied by a fall in intracellular oxidative stress in both control and obese rats. In fact, oil supplementation caused a reduction in hepatic protein carbonyl and MDA levels, and in

adipose MDA contents, especially in obese old rats. It has been demonstrated that olive oil-rich or ALA-rich diets decrease the rate of peroxidation and production of free radicals with a concomitant increase in antioxidant enzyme activities [14,27,18,38,40]. It is known that the type of dietary fat is an important factor modulating oxidative stress in aging [18]. We showed for the first time a positive effect of olive-linseed oils combination on liver and adipose tissue redox status in the aging obesity.

It is important to note that lower food intake in control and obese rats fed on olive or olive-linseed oils supplemented diets implicated lower fatty acid amounts ingested by these rats than those fed on non supplemented diets. Food restriction could have beneficial effects on metabolic parameters [41,42]. However, animals were allowed free access to all diets. Indeed, the animals were fed on isocaloric (386 Kcal/100 g diet for control and 523 Kcal/100 g for cafeteria) diet containing identical amounts of all dietary constituents, except the quality of dietary fats (different oil). Reduced food intake in oil supplemented fed rats was then a consequence of oil supplementation in control and cafeteria diet groups. On the other hand, similar food intake was observed in obese rats fed on olive and olive-linseed oil diets, reflecting similar quantity of fatty acids. Our results demonstrated then the importance of the MUFA-*n-3* PUFA combination in obtaining more favorable beneficial effects in aging obesity rather than simply reducing the levels of fatty acids ingested.

In conclusion, our results provided evidence that olive-linseed oils supplementation induced lower energy intake associated to an improvement of metabolic alterations observed in obesity during aging in rats. Therefore, dietary interventions such as olive-linseed oils combination could present an opportunity for developing new strategies to treat obesity during aging.

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

The authors declare that they have no conflicts of interest.

REFERENCES

[1] K Marinou; D Tousoulis; AS Antonopoulos; E Stefanadi; C Stefanadis, *International Journal of Cardiology*, **2010**, 138, 3-8.
[2] JF Keaney; MG Larson; RS Vasan; PWF Wilson; I Lipinsk; D Corey, *Arterioscler Thromb Vasc Biol*, **2003**, 23, 434-439.
[3] HK Vincent; AG Taylor, *Int J Obes*, **2006**, 30, 400–418.
[4] GA Kelley; KS Kelley; ZV Tran, *Prev Cardiol*, **2005**, 8, 206-214.
[5] BP Yu; HY Chung, *Mech Ageing Dev*, **2006**, 127, 436-443.
[6] B Marzani; O Pansarasa; F Marzatico, *Basic Appl Myol*, **2004**,14, 37-44.
[7] S Bouanane; NB Benkalfat; FZ Baba Ahmed; H Merzouk; N Soulimane Mokhtari; S Merzouk; J Gresti; C Tessier; M Narce, *Clin Sci*, **2009**, 116, 669–680.
[8] KC Hayes, *Br J Nutr*, **2000**, 84:397-399.
[9] MG Volk, *Alternative Medicine Review*, **2007,** 12, 228-445.
[10] JJ Moreno; MT Mitjavilab, *J Nutr Biochem*, **2003**, 14, 182-195.
[11] MI Covas ; V Ruiz-Gutiérrez ; R De la Torre ; A Kafatos ; RM Lamuela-Raventos ; J Osada ; RW Owen ; F Visioli, *Nutr Rev*, **2006**, 64, 20-30.
[12] MA Alsaif, *Pak J Nutr*, **2004**, 3, 56-63.
[13] J Breslow, *Am J Clin Nutr*, **2006**, 83, 1477-1482.
[14] K Prasad, *Drug News and Perspectives*, **2000**, 13, 99-104.
[15] LH Storlien; LA Baur; AD Kriketos; DA Pan, GJ Cooney; AB Jenkins, *Diabetologia*, **1996**, 39, 621-631.
[16] SC Cunnane; KR McAdoo, DF Horrobin, *Br J Nutr*, **1986**, 56, 87-95.
[17] S Miret; MP Saiz; MT Mitjavila, *Br J Nutr*, **2003**, 89, 11-18.
[18] JJ Ochoa ; JL Quiles ; S Ibanez ; E Martınez ; M Lopez-Frıas ; JR Huertas, *J Bioenerg Biomembr*, **2003**, 35, 267–275.
[19] LKM Summers; SC Barnes; BA Fielding; C Beysen; V Ilic; SM Humphreys SM; KN Frayn, *Am J Clin Nutr*, **2000**, 71, 1470–1447.
[20] SC Cunnane; MA Ryan; CR Nadeau; RP Bazinet; K Musa-Veloso; U McCloy, *Lipids*, **2003**, 38, 477-484.
[21] MHG Gaiva; RC Couto; LM Oyama; GEC Couto; VLF Silveira; EB Riberio; CMO Nascimento, *Br J Nutr*, **2001**, 86, 371-377.
[22] O Hussein; M Grosovski; E Lasri; S Svalb; U Ravid; N Assy, *World J Gastroenterol*, **2007**, 13, 361-368.

[23] S Bouanane; H Merzouk; NC Benkalfat ; N Soulimane ; SA Merzouk; J Gresti; CH Tessier; M Narce, *Metab Clin Exp*, **2010**, 59, 1701-1709.
[24] NC Benkalfat; H Merzouk; S Bouanane; SA Merzouk; J Bellenger; J Gresti; CH Tessier; M Narce, *Clin Sci*, **2011**, 121, 19-28.
[25] D Mathe; C Serougne; J Ferezou; B Lecuyer, *Ann Nutr Metab*, **1991**, 35, 165-173.
[26] O Kabbaj; SR Yoon; C Holm; J Rose; ML Vitale; MR Pelletier, *Biol Reprod*, **2003**, 68, 722–734.
[27] OH Lowry; NJ Rosebrough; AL Farr; RJ Randall, *J Biol Chem*, **1951**, 193, 265–275.
[28] P Nilsson-Ehle; R Ekman, *Artery*, **1977**, 3, 194–209.
[29] H Merzouk; S Madani; D Chabane sari; J Prost; M Bouchenak; J Belleville, *Clin Sci*, **2000**, 98, 21-30.
[30] H Draper; M Hadley, *Methods Enzymol*, **1990**, 186, 421-431.
[31] RL Levine; D Garland; CN Oliver; I Climent; AG Lenz; BW Ahn; Shaltiel S; Stadtman ER, *Methods Enzymol*, **1990**, 186, 464–478.
[32] HA MacQueen; DA Sadler; SA Moore ; S Daya; JY Brown; DEG Shuker; M Seaman; WS Wassif , *Nutr Res*, **2007**, 27, 38-47.
[33] S Santamarina-Fojo; H Gonzalez-Navarro; L Freeman; E Wagner; Z Nong, *Arterioscler Thromb Vasc Biol*, **2004**, 24, 1750-1754.
[34] FI Milagro; J Campion; JA Martinez, *Obesity*, **2006**, 14, 1118–1123.
[35] R Christon; R BelcadiHaloå; G Durand, *J Nutr*, **1995**, 125, 3062-3070.
[36] GJ Schwartz; J Fu; G Astarita; X Li; S Gaetani; P Campolongo; V Cuomo, *Cell Metab*, **2008**, 8, 281-288.
[37] JS Perona; V Ruiz-Gutierrez, *J Agric Food Chem*, **2004**, 52, 4227–4233.
[38] N Hwalla Baba; Z Ghossoub; Z Habbal, *Nutr Res*, **2000**, 20, 1113-1123.
[39] CC Tai; ST Ding, *J Nutr Biochem*, **2010**, 21, 357–363.
[40] GE Rasmy, *JGEB*, **2007**, 5:9-17.
[41] T Armeni; G Principato; JL Quiles; C Pieri; S Bompadre; M Battino, *J Bioenerg Biomembr*, **2003**, 35:181–191.
[42] TA Bowman; SK Ramakrishnan; M Kaw ; SJ Lee; PR Patel; VK Golla, *Endocrinology*, **2010**, 151, 5157–5164.