

DIETARY FLAXSEED OIL SUPPLEMENTATION IMPROVES THE OXIDANT/ANTIOXIDANT STATUS IN OBESE AGED RATS

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ABSTRACT

The role of dietary flaxseed oil was investigated at 2,5% and 5% in the modulation of oxidant/ antioxidant status in obese aged rats. Aging male wistar rats randomly allocated into six groups of 10 rats each: a control diet, control flaxseed oil 2,5% diet, control flaxseed oil 5% diet, cafeteria diet, cafeteria flaxseed oil 2,5% oil diet, cafeteria flaxseed oil 5% diet.

Malondialdehyde and carbonyl proteins concentrations in plasma, erythrocytes, liver and adipose tissue were measured. Then, vitamin C level and antioxidant enzymes activities (catalase and glutathion peroxidase) were evaluated.

The results showed that malondialdehyde and carbonyl proteins levels were higher in cafeteria-diet group compared with controls, and lower in flaxseed oil diet groups compared to cafeteria-diet.

The decrease in vitamin C concentration and catalase and glutathion peroxidase activities were noted in aged obese rats, while these antioxidant parameters were improved in obese aged rats receiving flaxseed oil diet.

Aging and obesity were accompanied by increased in oxidative stress, which is characterized by reduction in the antioxidant enzymes activities and vitamin C level and increase in malondialdehyde and carbonyl proteins levels. Antioxidant status in aged obese rats was improved by flaxseed oil diet which might be helpful in preventing obesity complications in aging.

KEYWORDS: Aging, Cafeteria Diet, Flaxseed Oil, Oxidative Stress, Obesity

INTRODUCTION

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 (Haslam and James, 2005). Oxidative stress is highly correlated with a wide variety of inflammatory and metabolic disease states, including obesity (Sonta et al., 2004) and with cumulative damage in the body done by free radicals inadequately neutralized by antioxidants (Valdecantos et al., 2009). In fact, it has been shown that free radicals may adversely affect cell survival because of membrane damage through the oxidative damage of lipid, protein and irreversible DNA modification (Mishra, 2004). Aging is also associated with an increase in oxidative stress. Some investigators have expressed concern that elevations in lipid peroxidation with aging in humans are associated with increased levels of endogenous antioxidants (superoxide dismutase and catalase enzymes and vitamin E) (Kregel and Zhang, 2007). However, the rate of free radical formation has been shown to increase in older people and this may be at a rate that exceeds even the increased antioxidant capacity. Others researchers have found a decline in antioxidant status with aging (Marzani et al., 2004). In the other side,

epidemiological studies have shown association between the consumption of diets rich in fruits and vegetables and a lower risk of chronic diseases like cancer, heart disease (Steinmetz and Potter, 1993), obesity and diabetes (Gillman et al., 1995).

The beneficial effects of PUFAs on human health have been documented by numerous studies (Williams, 2000; Riediger et al., 2009). Therapeutic properties of plant extracts, used in traditional medicine, have been linked to their antioxidative activities. One promising antioxidant is Flaxseed (*Linum usitatissimum*) called linseed, which is a member of the Linaceae family. Flaxseed has stood out among the healthy foods because of its beneficial effects and the power to act on prevention of diseases (Fukumitsu et al., 2010). A number of investigations have demonstrated that diet supplemented with flaxseed oil has profound beneficial health effects in various pathologies (Carter, 1993). Some researchers consider flaxseed supplementation as a strategy to prevent cardiovascular disorders, based on its amount of fat ranging between 34 and 45.6%, of which omega-3 fatty acid represents 45–60% of the total fat content in this oil's seed (Bhathena et al., 2003). The main physiological benefits of flaxseed are attributed primarily to the high linoleic acid content which contributes to their antioxidant properties (Simopoulos, 1991).

Considering that older adults are already exposed to risk for lipid alterations and oxidant stress, the additional metabolic perturbations of obesity may aggravate the susceptibility to oxidant stress. To our knowledge, there are insufficient data regarding the relationship between obesity, aging, and oxidant/antioxidant status. The purpose of the present study is to evaluate the flaxseed oil effects on oxidative stress in obese rats during aging. Therefore, several markers of oxidative stress are assessed by measuring the concentrations of plasma vitamin C, malondialdehyde (MDA), carbonyl proteins, and the activities of catalase (CAT), and glutathione peroxidase (GPX) in aged obese rats.

MATERIALS AND METHODS

Animals and Experimental Protocol

Sixty aged male wistar rats 18 months old and weighing 320–350 g were used for this study. They were obtained from Pasteur institute (Algiers, Algeria). Rats were housed individually in wood-chip bedded plastic cages at constant temperature (25 C°) and maintained on a 12:12 h light/dark cycle. The rats had free access to water and were divided into 6 dietary groups of 10 each and fed diet *ad libitum* during 8 weeks of experimental period: Group 1, control (C) fed a control commercial diet produced by O. N.A.B (National Office of Food Cattle, Remchi, Tlemcen). Group 2, control flaxseed oil 2,5% (CL 2,5%) fed a control commercial diet enriched with flaxseed oil at 2,5%. Group 3, control flaxseed oil 5% (CL 5%) fed a control commercial diet enriched with flaxseed oil at 5%. Group 4, cafeteria group (CAF) fed a fat-rich hypercaloric diet. Group 5, cafeteria flaxseed oil 2,5% (CAF L2,5%) fed a fat-rich hypercaloric diet enriched with flaxseed oil at 2,5%. Group 6, cafeteria flaxseed oil 5% (CAF L 5%) fed a fat-rich hypercaloric diet enriched with flaxseed oil at 5%. The control diet (330 kJ/100 g) was composed of 25% of energy as protein, 65% of energy as carbohydrate, and 10% of energy as lipids (which contain 4% C14:0, 18% C16:0, 2% C16:1n-7, 5% C18:0, 22% C18:1n-9, 43% C18:2n-6, 5% C18:3n-3, and 1% C20:4n-6). 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 as published previously (Bouanane et al., 2009; Darimont et al 2004). The composition of the cafeteria diet (420 kJ/100 g) was 23% of energy as protein, 35% of energy as carbohydrates, and 42% of energy as lipids (which contain 7% C14:0, 25% C16:0, 1% C16:1n-7, 9% C18:0, 31% C18:1n-9, 24% C18:2n-6, 1% C18:3n-3, 1% C20:0, and 1% C22:0).

Pure flaxseed oil was 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, Liver and Adipose Tissue Samples

At the end of the experimental period (two months), after overnight fasting, in each diet group and at each experimental point, 10 rats from each group were anesthetized with pentobarbital (60 mg/kg of body weight). The blood was drawn from the abdominal aorta. Plasma was immediately used for 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 antioxidant enzyme activities.

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) and 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 redox markers determinations.

Determination of Tissue Oxidant / Antioxidant Status

The malondialdehyde levels, a marker of lipid peroxidation, was determined in plasma, erythrocytes and tissue supernatants by the procedure of Ohkawa et al. (1979) based on the reaction of MDA with thiobarbituric acid at 95°C. Carbonyl proteins (markers of protein oxidation) in Plasma, erythrocytes and tissues were assayed by the 2,4-dinitrophenyl hydrazine reaction (Levine et al.,1990) .

Catalase activity was measured on erythrocyte lysate by spectrophotometric analysis of the rate of hydrogen peroxide decomposition at 240 nm (Aebi 1974). Glutathione peroxidase activity was assessed by the method of Paglia and Valentine (1967), using cumene hydroperoxydes as substrate.

Determinations of Plasmatic Levels of Vitamin C

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

STATISTICAL ANALYSIS

Results are expressed as means \pm 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 Organs Weights and Food Intakes in Study Rats

The cafeteria diet was associated with increased body weight and weight gain compared to control diet, regardless of oil supplementation (Table 1). Supplementation with flaxseed oil at 2,5% and 5% induced a reduction in body weight and in weight gain in both control and obese aged rats. However, group fed on flaxseed oil at 5% diet showed the lowest body weight compared to those fed on the flaxseed oil at 2,5% diet. As expected, group fed on cafeteria diet had a higher

food intakes compared with control animals. Aged obese rats had a significantly higher adipose tissue and liver weight compared with the controls (Table1). Flaxseed oil supplementation reduced significantly adipose tissue and liver weights in cafeteria aged obese rats. However group fed on flaxseed oil at 5% diet showed the lowest adipose tissue weight compared to those fed on the flaxseed oil at 2,5% diet.

Plasma, Erythrocytes, Liver and Adipose Tissue Oxidant/Antioxidant Status

A significant increase in the MDA level (measure of lipid peroxidation) was found in the plasma, erythrocytes, liver and adipose tissue of aged obese rats compared to the control rats (table 2). The aged obese rats which were supplemented with flaxseed oil at 2,5% and 5%, showed significantly lower levels of MDA in plasma, liver and adipose tissue compared to the aged obese rats group. The Protein carbonyl levels (table 2) in plasma, erythrocytes, liver and adipose tissue were significantly increased in aged obese rats compared with controls. However, the groups which were supplemented with flaxseed oil at 2,5% and 5%, showed significantly lower levels of PCAR in plasma, liver and adipose tissue compared to the aged obese group.

In the other hand, antioxidant enzymes activities (catalase and glutathione peroxidase) showed a decrease in obese aged rats when compared to the control aged rats. Then, administration of flaxseed oil at 2,5% and 5% to obese aged rats lead to enhance in catalase and glutathione peroxidase activities (figure 1).The plasma vitamin C levels (figure 2) were significantly influenced by the diet. The obese groups showed decrease levels of vitamin C. However, the groups which were supplemented with flaxseed oil, showed significantly higher levels of vitamin C compared to the obese aged rats and group fed on flaxseed oil at 5% diet showed higher levels of vitamin C compared to those fed on the flaxseed oil 2,5% diet.

Table 1: Characteristics of the Study Rats

	Control Aged Rats			Obese Aged Rats			P (Anova)
	C	CL2,5	CL5%	CAF	CAFL2,5%	CAFL5%	
Body weight (g)	521.66±5.13 ^c	491.43±4.36 ^d	443.33±4.27 ^e	600.25±5.23 ^a	552.71±7.31	515.30±6.22 ^c	0.0001
Weight gain (g)	154±7.57 ^c	111±4.21 ^c	78.12±5.62 ^d	200.95±8.51 ^a	175±9.32 ^b	164.36±8.63 ^b	0.001
Food intake (g/day/rat)	39.08±1.42 ^b	22.53±1.30 ^c	21.88±2.54 ^c	45.18±2.37 ^a	21.48±2.32 ^c	21.95±1.77 ^c	0.001
Liver weight (g)	21.19±1.43 ^c	20.13±1.23 ^c	20.52±1.78 ^c	24.31±1.27 ^a	21.21±1.09 ^b	22.67±1.12 ^b	0.001
Adipose tissue weight (g)	7.63±0.57 ^d	7.25±0.11 ^d	5.39±0.88 ^e	19.08±1.90 ^a	14.42±1.39 ^b	10.26±1.6 ^c	0.0001

Values are presented as means ± standard deviations (SD). C: aged rats fed control diet. CL2,5%: aged rats fed control diet enriched with flaxseed oil at 2,5% .CL5% : aged rats fed control diet enriched with flaxseed diet at 5% . CAF: aged obese rats fed cafeteria diet. CAFL2,5%: aged obese rats fed cafeteria diet enriched with flaxseed oil at 2,5%. CAFL5%: aged obese rats fed cafeteria diet enriched with flaxseed oil at 5%.Values with different superscript letters (a, b, c, d, e) are significantly different (P < 0.05).

Table 2: MDA and Protein Carbonyl Levels in Plasma, Erythrocytes, Liver and Adipose Tissue in the Study Rats

	Control Aged Rats			Obese Aged Rats			P (Anova)
	C	CL2,5	CL5%	CAF	CAFL2,5%	CAFL5%	
MDA Plasma (µmol/l)	2,86±0,19 ^d	2,59±0,1 ^e	2,02±0,1 ^f	5,44 ±0,38 ^a	3,74±0,36 ^b	3,07±0,1 ^c	0.0001
MDA Erythrocyte (µmol/l)	3,01 ±0,29 ^d	2,44±0,6 ^e	2,10±0,8 ^e	4,95 ±0,56 ^a	3,91±0,05 ^b	3,53 ±0,11 ^c	0.0001
MDA Liver (nmol/g wet tissue)	4,37± 0,51 ^b	1,66±0,3 ^d	1,42±0,3 ^d	5,90± 0,81 ^a	2,63± 0,4 ^c	2,28±0,21 ^d	0.001
MDA Adipose tissue (nmol/g wet tissue)	3,04± 0,24 ^c	2,53±0,6 ^d	2,33±0,5 ^d	4,67± 0,26 ^a	3,88±0,5 ^b	3,38±0,08 ^c	0.001
PCAR Plasma (µmol/l)	3,24± 0,06 ^b	2,4± 0,08 ^c	2,97±0,12 ^b	5,36± 0,24 ^a	3,42±0,06 ^b	3,17±0,09 ^b	0.0001
PCAR erythrocyte (µmol/l)	1,45 ±0,19 ^b	1,31±0,04 ^b	1,11±0,04 ^b	2,75 ±0,29 ^a	1,82±0,06 ^b	1,71± 0,10 ^b	0.001
PCAR Liver (n mol /g wet tissue)	4,02± 0,09 ^b	3,10±0,19 ^d	1,47 ±0,13 ^f	5,26 ±0,34 ^a	3,4± 0,08 ^c	2,87± 0,04 ^c	0.001
PCAR Adipose tissue (nmol/g wet tissue)	2,82 ±0,08 ^b	1,94±0,10 ^d	1,65±0,13 ^e	3,59± 0,05 ^a	2,22±0,11 ^c	2,11± 0,09 ^c	0.001

Values are presented as means ± standard deviations (SD). C: aged rats fed control diet. CL2,5%: aged rats fed control diet enriched with flaxseed oil at 2,5% .CL5% : aged rats fed control diet enriched with flaxseed diet at 5% . CAF: aged obese rats fed cafeteria diet. CAFL2,5%: aged obese rats fed cafeteria diet enriched with flaxseed oil at 2,5%. CAFL5%: aged obese rats fed cafeteria diet enriched with flaxseed oil at 5%.Values with different superscript letters (a, b, c, d, e) are significantly different (P < 0.05).

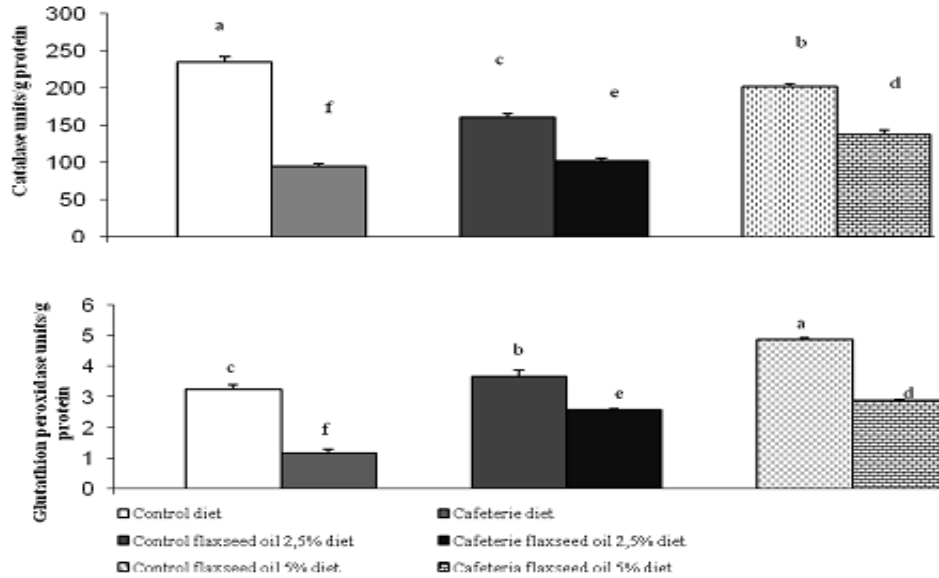


Figure 1: Catalase and Glutathion Peroxidase Activities in the Study Rats

Values are presented as means ± standard deviations (SD). C: aged rats fed control diet. CL2,5%: aged rats fed control diet enriched with flaxseed oil at 2,5% .CL5% : aged rats fed control diet enriched with flaxseed diet at 5% . CAF: aged obese rats fed cafeteria diet. CAFL2,5%: aged obese rats fed cafeteria diet enriched with flaxseed oil at 2,5%. CAFL5%: aged obese rats fed cafeteria diet enriched with flaxseed oil at 5%.Values with different superscript letters (a, b, c, d, e) are significantly different (P < 0.05).

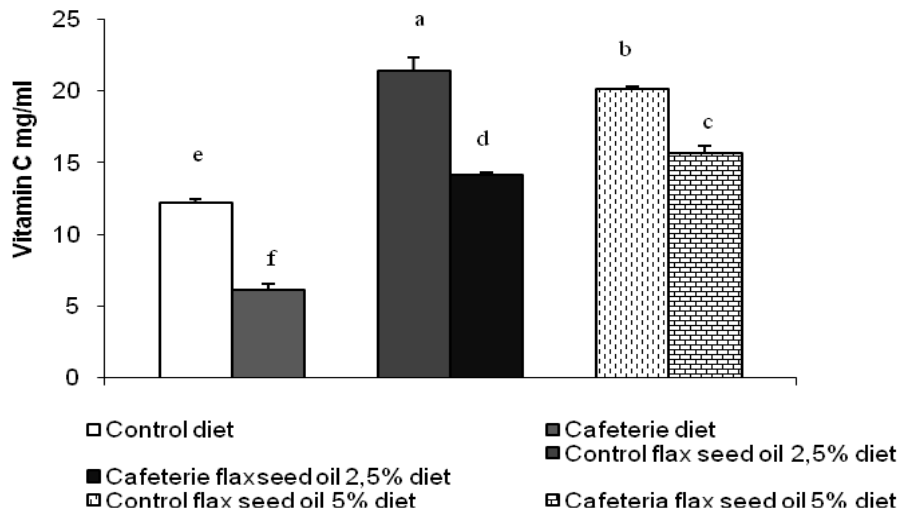


Figure 2: Plasma Vitamin C in the Study Rats

Values are presented as means ± standard deviations (SD). C: aged rats fed control diet. CL2,5%: aged rats fed control diet enriched with flaxseed oil at 2,5% .CL5% : aged rats fed control diet enriched with flaxseed diet at 5% . CAF: aged obese rats fed cafeteria diet. CAFL2,5%: aged obese rats fed cafeteria diet enriched with flaxseed oil at 2,5%. CAFL5%: aged obese rats fed cafeteria diet enriched with flaxseed oil at 5%.Values with different superscript letters (a, b, c, d, e) are significantly different (P < 0.05).

DISCUSSIONS

The present study was undertaken to ascertain the beneficial effects of flaxseed oil, in obese rats during aging on oxidant /antioxidant status. It is well known that oxidative stress is elevated during aging, these alterations were observed

in obesity and they would be worsened in the aging obesity-association. Aging and obesity are usually associated with increasing level of oxidation. An imbalance between the formation and removal of reactive oxygen species (ROS) and the development of oxidative stress plays an important role in aging and age-associated diseases (Keaney, 2003). ROS inflicts the macromolecules like proteins, lipids, carbohydrates and nucleic acids, thereby inactivates enzymes transporters, damages DNA and the transcriptional machinery. Additionally it initiates the chain reactions that peroxidize polyunsaturated fatty acids in membrane phospholipids (Friedman, 2000). Considerable experimental evidence supports the idea that ROS plays a key role in the pathophysiological process of major organs and tissue damage in aged rats (Kumaran et al., 2009). The damage to the organs by ROS is evidenced by the elevation of biomarkers in serum of aged rats (Hashimoto et al., 2009).

The body has an effective defense mechanism to prevent and neutralize the free radical-induced damage. This is proficient by a set of endogenous antioxidant enzymes such as GPX and CAT. These enzymes constitute a mutually supportive team of defense against ROS (Amresh et al., 2009). In the present study, cafeteria diet was given to aged rats for 8 weeks to induce dietary obesity; cafeteria diet feeding induced an increase in total food that they may explain the higher body and adipose depot weight in agreement of previous study (Bouanane et al., 2009; Bouanane et al., 2010).

Flaxseed oil supplementation modulated several liver and adipose parameters in both control and obese old rats, with beneficial effects including lower body weight, lower adipose fat deposition, reduced oxidative stress. Flaxseed oil appeared to be more effective in metabolic improvements especially in obese old rats. Flaxseed oil enriched diets resulted in a reduction in food intakes with a concomitant decrease in body weight in both control and obese groups. This effect is more pronounced with flaxseed oil supplementation at 5%. The decrease in body weight was accompanied by a reduction in adipose tissue weight in obese old rats (Keaney et al., 2003). Also, the obese aged rats presented an intracellular oxidative stress. In fact, the elevated levels of plasmatic erythrocyte hepatic, adipose tissue MDA and of protein carbonyls suggested an increased lipid peroxidation and protein oxidation in the aged obese rats, in agreement with previous studies (Vincent and Taylor, 2006; Bouanane et al., 2009). In the present study, such destruction of membrane lipids possibly accounted for the observed increase in MDA levels in liver and adipose tissue besides, inadequate levels of antioxidants to scavenge peroxy radicals during aging (Aydin et al., 2010) could also have added to the increased level of MDA in the aged rats. These data from the present study agree with the findings of earlier investigations (Jayakumar and Thomas, 2007). The observed diminution in the MDA and PC levels in aged obese rats following administration of the flaxseed oil is one indicator of the antioxidant activity of this oil due to their high content of n-3 PUFAs (Simopoulos, 1991).

The amounts of the small molecular-weight antioxidants and the activities of the free radical scavengers are altered and inadequate in the aging condition (Aydin et al., 2010). In the present investigation, a significantly lower of vitamin C and activity of the enzymes catalase and glutathione peroxidase were observed in aged obese rats and the diminution in the activities of these enzymes has been well documented (Kumaran et al., 2009; Aydin et al., 2010). Administration of flaxseed oil in obese aged rats elevated the levels of vitamin C and the antioxidant enzymes, indicating the antioxidant potential of the flaxseed oil (Makni et al., 2008).

CONCLUSIONS

Our study found that flaxseed oil could attenuate lipid and protein oxidation and preserve anti-oxidation capacity. Therefore, dietary interventions such as flaxseed oil could present an opportunity for developing new strategies to treat obesity during aging.

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