

In Vitro Effects of Oil's Fatty Acids on T-Cell Function of Obese Men

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Abstract

Background: Dietary fatty acids have important homeostatic functions in regulating the immune response and may exert beneficial effects on immune alterations during obesity. **Objective:** To assess the *in vitro* effects of oil fatty acids, different oils (olive, linseed, Nigella, sunflower) were tested on T-lymphocyte proliferation, Th1- and Th2-type cytokine production, and intracellular oxidant/antioxidant status in obese patients. **Methods:** Peripheral blood lymphocytes were isolated using Histopaque and were *in vitro* cultured and stimulated by Con A in the presence or absence of the oils. Cell proliferation, interleukin-2, interleukin-4 and interferon- γ (INF γ) secretions and intracellular oxidative status (glutathione (GSH), malondialdehyde (MDA), carbonyl protein levels, catalase activity and micronuclei frequency) were investigated. **Results:** Abnormalities in lymphocyte function and intracellular oxidative stress were observed in obesity. Linseed oil induced a reduction in T-lymphocyte proliferation and IL-2 production while Nigella oil increased them in both obese and control groups. In addition, Nigella oil enhanced INF γ and IL-4 secretion. Olive and sunflower oils had no effect on lymphocyte proliferation and cytokine secretion in both groups. Linseed and Nigella oils induced an increase in T cell GSH concentrations and catalase activity with a concomitant decrease in MDA, carbonyl protein contents and micronuclei frequency especially in obese patients. **Conclusion:** Linseed and Nigella oils had beneficial effects on lymphocyte proliferation, cytokines secretions and redox status, while olive and sunflower oils had no effects on immune cell function in obesity.

Keywords

Obesity, Lymphocytes, Olive Oil, Linseed Oil, Nigella Oil, Sunflower Oil, Cytokines, Oxidant/Antioxidant Status

1. Introduction

Great progress has taken place in our knowledge of the physiologic and molecular mechanisms of fatty acids in health and disease. Fatty acids are important biological constituents with metabolic, structural and signaling roles and they can exert beneficial effects in various disorders. Fatty acids have been shown to modify biophysical parameters of cell membranes, including fluidity, permeability and domain formation, as well as cell division, signal transduction, membrane protein activities, and anti-proliferative control. Monounsaturated fatty acids (MUFAs) have favorable effects on the coagulation process, inflammation, and reduce fasting plasma glucose concentrations, and improve insulin sensitivity [1]. Oleic acid (C18:1 n-9), an important MUFA present in olive oil which is the main source of fat in the Mediterranean diet, may have healthy benefits on immune system and oxidative stress [1] [2] [3]. Oleic acid has been associated with a reduction in blood pressure and a lower incidence of hypertension [4]. Oleic acid reduces the incidence of cancer by inducing apoptosis in carcinoma cells, possibly related to an increase of free radical levels and caspase-3 activity [5].

Dietary n-3 polyunsaturated fatty acids (n-3 PUFAs) have been associated with the prevention of cardiovascular diseases and cancer [6] [7]. N-3 PUFAs are known as anti-obesity factors, with beneficial effects such as weight loss, lipid metabolism regulation by inhibiting lipogenesis, favoring lipolysis and fatty acid oxidation, reducing body fat deposition and suppressing preadipocyte differentiation [8] [9] [10]. They induce changes in membrane fatty acid composition, membrane fluidity, receptor distribution, prostaglandin biosynthesis which may influence T cell mediated immune responses [11] [12]. PUFAs have been shown to modulate lymphocyte proliferation, cytokine production, antigen presentation and natural killer cell activity [12] [13] [14] [15]. In fact, previous studies have demonstrated that n-3 PUFAs decrease T cell proliferation [14] [16], cytokine secretion [16], intracellular enzyme activity [17] and gene transcription [18]. Dietary n-3 PUFAs promote apoptosis in a T cell subset exhibiting a Th1 cytokine profile, affecting T cell proinflammatory function [19]. N-3 PUFA improved T cell intracellular oxidative status in diabetes and in gestational diabetes [20] [21]. α -linolenic acid (ALA, C18:3 n-3) is an important n-3 PUFA supplied by vegetable sources such as linseed oil. Linseed oil may play a beneficial role in the prevention and management of atherosclerosis, obesity, and the metabolic syndrome [22].

N-6 PUFAs regulate a wide variety of biological functions. Eicosanoids derived from n-6 PUFA are in general proinflammatory but they have important homeostatic functions in regulating both the promotion and resolution of inflammation in the immune response [23]. However, recent review has suggested that n-6 PUFAs have some anti-inflammatory actions such as those of the n-3 PUFA, with a reduction in serum CRP concentrations [24] [25] [26]. Higher plasma levels of n-6 PUFAs, mainly arachidonic acid, were associated with decreased plasma levels of serum proinflammatory markers, particularly interleu-

kin-6 and interleukin-1 receptor antagonist [27]. Favorable effects of n-6 PUFA on cholesterol levels, insulin sensitivity and the incidence of diabetes mellitus are well documented and would predict significant reductions in CHD risk [28]. A healthy diet may respect a balance between n-3 and n-6 fatty acids to equilibrate cell activity [29]. Nigella oil, rich in linoleic acid (LA, C18:2 n-6) and oleic acid, possesses anti-inflammatory, immune and anti-oxidant effects through enhancing the oxidant scavenger system [30] [31] [32]. Sunflower oil is also rich in linoleic and oleic acids. Some of the health benefits of sunflower oil include its ability to improve heart health, boost energy, strengthen the immune system, improve skin health, prevent cancer, lower cholesterol and reduce inflammation and oxidative stress [33] [34].

Obesity is associated with oxidative stress, as a result of increased formation of reactive oxidative substances and reduced antioxidant defense mechanisms [35]. It is also associated to an inflammatory state that may contribute to the development of lipid metabolism and tissue function alterations and to many obesity-related co-morbidities [36] [37] [38] [39] [40]. Immune dysfunction is often observed in obesity, with high rates of infections and low T- and B-cell proliferation [41]. We have previously observed altered *in vitro* lymphocyte proliferation and function in obese patients [42].

Linseed, olive, Nigella and sunflower oils are consumed in Mediterranean countries. To mimic human consumption, we have used these oils in spite of purified fatty acids and we have investigated their effects on immune function. We have previously shown that they have modulatory effects on *in vitro* lymphocyte function during gestational diabetes [21]. However, their effects on immune function during obesity remained to be discovered.

Therefore, in this study, we investigated the *in vitro* effects of oil fatty acids on lymphocyte proliferation, cytokine production and intracellular redox status in obese patients.

2. Material and Methods

2.1. Subjects

Between September 2013 and July 2014, 82 men who attended annual general health examinations were recruited from the department of endocrinology, University Hospital of Tlemcen (Algeria), with primary criteria including BMI 18 - 24 Kg/m² or ≥30 Kg/m², age range between 20 and 40 years, not taking any medication known to influence lipid metabolism and immune system and not having chronic diseases. Medical records were screened by specialist physicians. The study population selected consisted of 50 normal weight men (BMI 18 - 24 kg/m²) and 32 obese men (BMI ≥ 30 kg/m²). The participation to this study was voluntary and all subjects gave their written, informed consent. The study was approved by the Ethical Committee of the Tlemcen University Hospital and was performed according to the Declaration of Helsinki. The characteristics of the study population were well described previously [42] (Table 1).

2.2. Blood Samples

Fasting blood samples were collected from the arm veins in EDTA tubes for immediate lymphocyte isolation.

2.3. Isolation and *in vitro* Lymphocyte Proliferation Assay

Peripheral blood lymphocytes were obtained from venous blood using differential centrifugation by Histopaque 1077 according to the manufacturer's manual (Sigma-Aldrich, St. Louis, MO), as previously described [42]. Briefly, an aliquot of Histopaque 1077 medium is carefully layered with blood. The tube is then centrifuged ($400 \times g$) for 30 minutes at room temperature. The peripheral blood lymphocytes at the interface of plasma and Histopaque were collected and washed twice with RPMI 1640 culture medium (Gibco, USA). Cell viability was tested by Trypan blue exclusion method. Cells were cultured in triplicate in microtiter plates in complete RPMI 1640 with or without T-cell specific mitogen Concanavalin (Con A) (Sigma Aldrich) and maintained at 37°C in a 5% CO_2 humidified atmosphere for 48 h. For the purpose to determine the effects of oil fatty acids, lymphocytes were incubated with linseed oil, olive oil, Nigel oil and sunflower oil. The fatty acid composition and antioxidant contents of these oils are given in **Table 2**. Oil fatty acid composition was analyzed by gas liquid chromatography using a Becker gas chromatograph (Spiral-RD, Couternon, France) equipped with a 50-m capillary glass column packed with Carbowax 20 M (Becker Instruments, Downers Grove, IL). Tocopherol and carotenoid contents

Table 1. The study population characteristics.

	Control group (n = 50)	Obese group (n = 32)
Age (years)	31.62 ± 5.65	32.37 ± 4.82
BMI (kg/m^2)	23.00 ± 1.76	$33.84 \pm 2.00^{**}$
Glucose (mmol/L)	4.88 ± 0.22	$6.73 \pm 0.21^*$
Total cholesterol (mmol/L)	4.68 ± 0.35	$5.43 \pm 0.35^*$
Triglycerides (mmol/L)	1.22 ± 0.13	$1.82 \pm 0.17^*$

Values are means \pm SD. BMI: Body mass index ($\text{Weight}/\text{Height}^2$). Statistical comparison between obese and control groups was performed by Student t test. * $P < 0.01$, ** $P < 0.001$.

Table 2. Fatty acid composition and antioxidant contents of the oils used.

	Linseed oil	Olive oil	Nigel oil	Sunflower oil
SFA (%)	9	15	15	15
MUFA (%)	18	60	28	25
C18:2 n-6 (%)	14	22	55	54
C18:3 n-3 (%)	57	1	1	2
C20:4 n-6 (%)	2	2	1	4
Tocopherol (mg/kg)	570	288	361	80
Carotenoids (mg/Kg)	8	-	100	

Oil fatty acid composition was analyzed by gas liquid chromatography. Tocopherol and carotenoid contents were determined by reversed-phase HPLC.

were determined by reversed-phase HPLC (Hewlett Packard HP1100, Germany) and detected using an UV detector at 292 nm for tocopherol and 450 nm for carotenoids [43]. The oils were initially dissolved in ethanol. The stock solution of oil in ethanol was diluted in RPMI 1640 culture medium immediately before use (to minimize oxidation) and was added to the cultures at a final concentration of 30 μ M (final concentration of ethanol 0.1%). Control cells were mixed with equivalent amounts of ethanol to reach a final concentration of 0.1% ethanol. After incubation for 48 h and centrifugation at 1500 rpm for 10 minutes, the cells were removed and the supernatants were collected for cytokine analysis. The cells were counted and assessed for viability which was over 80% for all cultures in our experiments. Lymphocyte proliferation was evaluated by the widely method used, the MTT [3-(4,5-dimethyl thiazol-2-yl)-2,5-diphenyl tetrazolium bromide] (Sigma) assay as previously described [44]. The stimulation index (SI) was expressed as percentage of the control.

2.4. Interleukin-2, -4 and INF γ Assay

Interleukins (IL-2, IL-4) and interferon- γ (INF γ) concentrations in cell culture supernatants were measured by using commercially available ELISA kits (R & D System, Oxford, UK) as per instructions supplied with. Results are expressed as pg/mL. The Th1/Th2 ratio was evaluated as the INF γ /IL-4 ratio.

2.5. Intracellular Redox Markers

2.5.1. GSH Assay

After cell lysis, intracellular reduced glutathione (GSH) contents were measured by using a Bioxytech GSH-400 kit (OXIS International, Inc., Portland, OR, USA).

2.5.2. Catalase Activity Assay

Catalase activity was determined in lymphocytes using spectrophotometric analysis of the rate of hydrogen peroxide decomposition at 240 nm according to a previous method [45].

2.5.3. TBARS Assay

The widely marker used for lipid peroxidation, malondialdehyde (MDA) in cell cultures was measured spectrophotometrically using thiobarbituric acid according to a method described previously [46].

2.5.4. Protein Carbonyl Assay

Protein carbonyls were evaluated as biomarkers of protein oxidation by 2,4-dinitrophenylhydrazine reaction as previously reported [47].

2.6. Micronucleus Assay

To detect DNA damage, the micronucleus (MN) assay was used. The presence of MN was assayed after blocking cell cytokinesis by the actin inhibitor cytochalasin B (Sigma), following the method and the established criteria for MN evaluation as previously described [48] [49]. The procedure for MN count has been previously detailed [42].

2.7. Statistical Analysis

Results were expressed as mean \pm SD. A priori power analysis was performed to determine the sample size, using power and sample size calculator (Statistical solutions, Sigma). We calculated that a sample of 50 normal weight controls and 32 obese patients would give us a 90% power of detecting a 25% difference in measurements. The student's test was applied to observe the significant difference between obese and control subjects. The data were analyzed by one way ANOVA followed by LSD (least significant difference) test for assaying statistical differences between multiple incubations. The statistical analyses were done using STATISTICA software package (Statsoft, Paris, France). $P < 0.05$ was considered statistically significant.

3. Results

3.1. *In Vitro* Effects of Oils on Lymphocyte Proliferation

Con A mitogen-stimulated proliferative responses, expressed as stimulation indices, of lymphocytes incubated with oils are shown in **Table 3**. In general, obese patients showed a slight decrease in Con A-stimulated lymphocyte proliferation rate compared to the control value, but the difference was not statistically significant. In the presence of olive and sunflower oils, lymphocyte proliferation was not affected in both control and obese groups compared to non-supplemented Con A stimulated lymphocytes.

However, lymphocyte proliferation showed a decrease when treated with linseed oil ($P = 0.006$) and an increase ($P = 0.008$) when treated with Nigel oil for both obese and control groups.

3.2. *In Vitro* Interleukin-2, -4 and $INF\gamma$ Secretion

The balance between Th1 (IL-2 and $INF\gamma$) and Th2 (IL-4) phenotypes was evaluated by the secretion of cytokines IL-2, $INF\gamma$ and IL-4 (**Table 4**). No significant differences were found between IL-2 concentrations of Con A-stimulated lymphocytes from obese and control groups. Linseed oil induced a reduction in IL-2 secretion while Nigel oil enhanced it in lymphocytes from control and

Table 3. *In vitro* proliferative response (stimulation index) of Con A stimulated lymphocytes from obese and control subjects in the presence of oils.

	Control group	Obese group
Con A	212.33 \pm 18.25 ^b	205.28 \pm 15.92 ^b
Con A + Olive oil	222.30 \pm 11.30 ^b	198.97 \pm 15.16 ^b
Con A +Linseed oil	180.01 \pm 12.07 ^c	168.86 \pm 19.37 ^c
Con A + Nigel oil	260.23 \pm 11.18 ^a	254.08 \pm 11.39 ^a
Con A + sunflower oil	195.56 \pm 13.51 ^b	206.73 \pm 17.40 ^b
P (ANOVA)	0.030	0.005

The values are means \pm SD of triplicate assays from 32 obese patients and 50 healthy controls. Multiple comparisons were performed using ANOVA followed by the least significant difference (LSD) test. Letters a, b, c indicate significant differences between different incubations ($P < 0.05$).

Table 4. *In vitro* interleukin (IL-2, IL-4) and interferon gamma (INF- γ) production of Con A stimulated lymphocytes from obese and control subjects in the presence of oils.

	Control group	Obese group
IL-2 (pg/mL)		
Con A	2248.43 \pm 45 ^b	2223.39 \pm 84 ^b
Con A + Olive oil	2316.49 \pm 86 ^b	2246 \pm 67 ^b
Con A + Linseed oil	1606 \pm 56 ^c	1645 \pm 4.97 ^c
Con A + Nigel oil	2706.18 \pm 72 ^a	2704 \pm 78 ^a
Con A + sunflower oil	2182.11 \pm 94 ^b	2205 \pm 103 ^b
P (ANOVA)	0.020	0.004
IL-4 (pg/mL)		
Con A	58.29 \pm 4.59 ^a	31.02 \pm 2.66 ^{*b}
Con A + Olive oil	59.91 \pm 2.92 ^a	35.43 \pm 2.09 ^{*b}
Con A + Linseed oil	58.92 \pm 2.77 ^a	30.19 \pm 2.05 ^{**b}
Con A + Nigel oil	59.39 \pm 2.11 ^a	50.01 \pm 1.05 ^{*a}
Con A + sunflower oil	48.89 \pm 3.15 ^b	30.17 \pm 1.14 ^{**b}
P (ANOVA)	0.024	0.006
INF-γ (pg/mL)		
Con A	454 \pm 30.96 ^b	268.42 \pm 36 ^{**b}
Con A + Olive oil	455.61 \pm 48.31 ^b	270.48 \pm 27 ^{**b}
Con A + Linseed oil	318.47 \pm 32.19 ^c	276.31 \pm 38 ^b
Con A + Nigel oil	565.38 \pm 36.12 ^a	457.46 \pm 33 ^{*a}
Con A + sunflower oil	460.97 \pm 20.54 ^b	284.25 \pm 31 ^{*b}
P (ANOVA)	0.008	0.007

The values are means \pm SD of triplicate assays from 32 obese patients and 50 healthy controls. Multiple comparisons were performed using ANOVA followed by the least significant difference (LSD) test. Letters a, b, c indicate significant differences between different incubations ($P < 0.05$). Obese versus control within the same incubation by Student t test: * $P < 0.01$; ** $P < 0.001$.

obese subjects ($P = 0.020$). No effect of olive and sunflower oil supplementation was observed in either control or obese groups.

IL-4 concentrations secreted from Con A-stimulated lymphocytes were decreased in obese compared to control subjects ($P < 0.01$). In controls, supplementation with olive, linseed and Nigel oils had no effects on IL-4 secretion while sunflower decreased it ($P = 0.024$). In obese group, a significant rise in IL-4 amounts was observed in the presence of Nigel oil ($P = 0.006$) while the other oils had no effects (Table 4).

IFN γ concentrations were significantly lower in obese lymphocytes compared to controls (Table 4). IFN γ concentrations were unaffected by olive and sunflower oils, while increased in the presence of Nigel oil and decreased with linseed oil in the control group ($P = 0.008$). In the obese group, Nigel oil induced an increase in IFN γ secretion by lymphocytes ($P = 0.007$) while olive, linseed and sunflower oils had no effects.

In Con A-stimulated lymphocytes from obese patients, the Th1/Th2 ratio

measured as the ratio of $\text{INF}\gamma$ to IL-4 was similar to that found in controls (Figure 1). This ratio was unaffected by the addition of olive oil but was decreased by linseed oil and increased by Nigel and sunflower oil in the control group ($P = 0.004$). In the obese group, Th1/Th2 ratio was unaffected by olive, linseed, Nigel and sunflower oil treatments. Nevertheless, this ratio became higher in the presence of linseed oil in the obese group than in the control group ($P < 0.01$).

3.3. Lymphocyte GSH Content and Catalase Activity

There were no significant differences in GSH content of Con A-stimulated lymphocytes from the obese and control groups (Figure 2). Supplementation with olive and sunflower oils had no effect while linseed and Nigel oils induced a rise in lymphocyte GSH concentrations in both control and obese subjects ($P = 0.007$ and 0.005 , respectively). Indeed, in the presence of linseed and Nigel oils, GSH contents of obese lymphocytes became higher than that of controls ($P < 0.01$).

Lymphocyte catalase activity was reduced in obese subjects compared to the control group (Figure 2). Catalase activity was unaffected by addition of olive and sunflower oils and increased in the presence of linseed and Nigel oils in lymphocytes from control and obese patients ($P = 0.008$ and 0.004 , respectively). Supplementation with linseed and Nigel oils induced a normalization of enzyme activity in lymphocytes from obese patients.

3.4. Lymphocyte MDA and Protein Carbonyl Content

Lymphocyte MDA and carbonyl protein concentrations were increased in obese subjects compared to the control group (Figure 3).

These oxidative markers were unaffected by oils supplementation in control subjects.

However, in obese patients, the oils used induced a fall in lymphocyte MDA and protein carbonyl amounts ($P = 0.006$) except for the sunflower oil which had no effects.

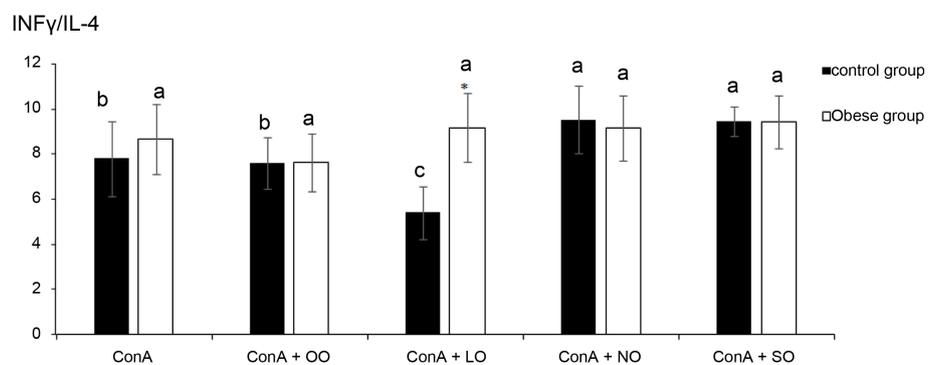


Figure 1. In vitro Th1/Th2 ($\text{INF}\gamma$ /IL-4) ratio of con A stimulated lymphocytes treated with oils from obese and control subjects. The values are means \pm SD of triplicate assays from 32 obese patients and 50 healthy controls. LO: linseed oil, NO: Nigel oil, OO: olive oil, SO: sunflower oil. Multiple comparisons were performed using ANOVA followed by the least significant difference (LSD) test. Letters a, b, c indicate significant differences between different incubations ($P < 0.05$). Obese versus control within the same incubation by Student t test: * $P < 0.01$.

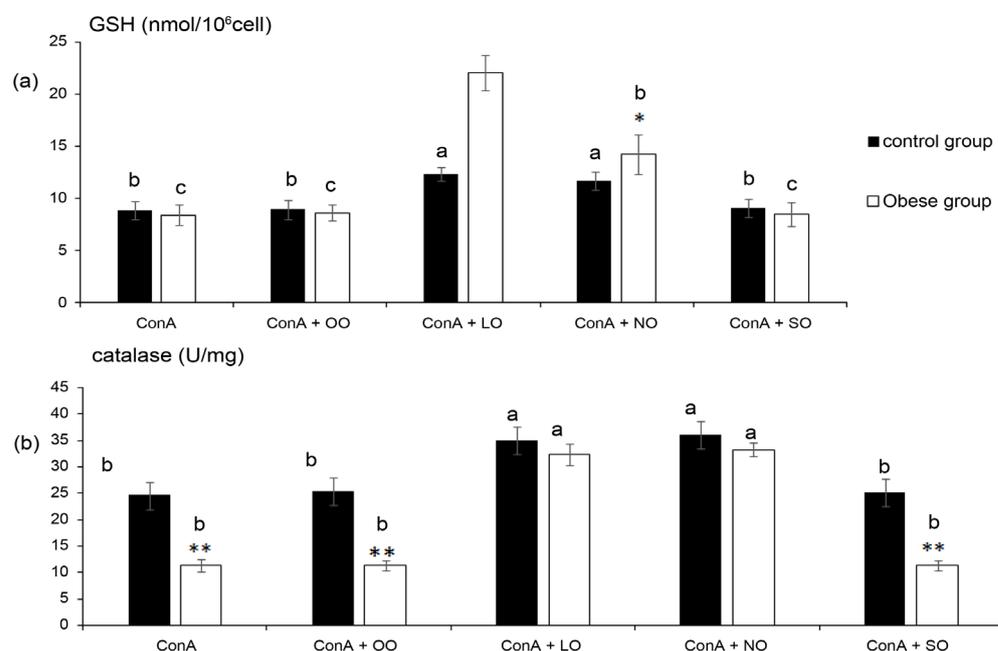


Figure 2. Intracellular antioxidant status of con A stimulated lymphocytes treated with oils from obese and control subjects. The values are means \pm SD of triplicate assays from 32 obese patients and 50 healthy controls. (a): GSH concentrations, (b): Catalase activity, LO: linseed oil, NO: Nigella oil, OO: olive oil, SO: sunflower oil. Multiple comparisons were performed using ANOVA followed by the least significant difference (LSD) test. Letters a, b, c indicate significant differences between different incubations ($P < 0.05$). Obese versus control within the same incubation by Student t test: * $P < 0.01$; ** $P < 0.001$.

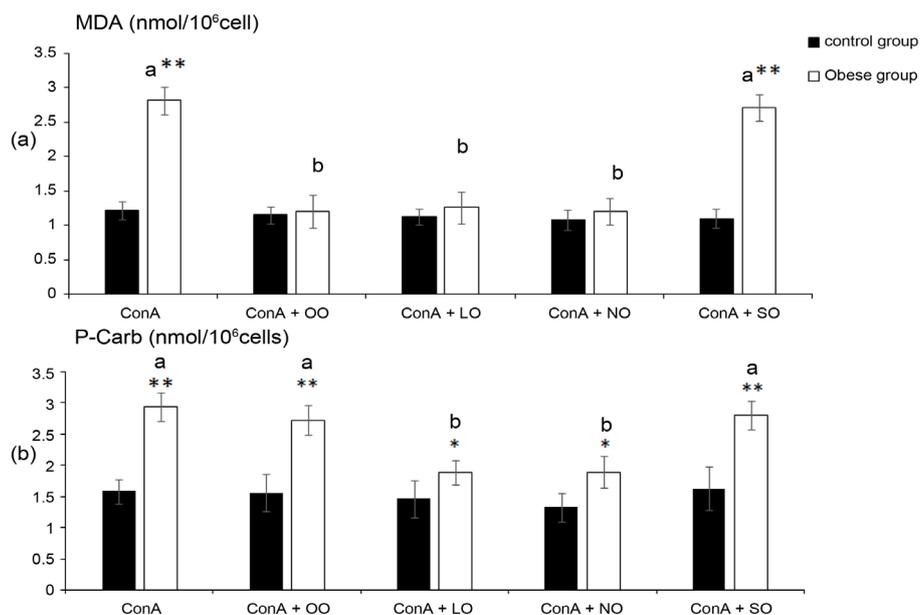


Figure 3. Intracellular oxidant status of con A stimulated lymphocytes treated with oils from obese and control subjects. The values are means \pm SD of triplicate assays from 32 obese patients and 50 healthy controls. (a): MDA concentrations, (b): Protein carbonyl concentrations, LO: linseed oil, NO: Nigella oil, OO: olive oil, SO: sunflower oil. Multiple comparisons were performed using ANOVA followed by the least significant difference (LSD) test. Letters a, b, c indicate significant differences between different incubations ($P < 0.05$). Obese versus control within the same incubation by Student t test: * $P < 0.01$; ** $P < 0.001$.

In the presence of olive, linseed and Nigel oils, MDA concentrations of obese lymphocytes became similar to control values except for the sunflower oil. However, protein carbonyl contents remained high in lymphocytes from obese patients treated with the different oils ($P = 0.002$).

3.5. Micronucleus Formation

Table 5 illustrates the induction of micronuclei (MN) by oils in stimulated T lymphocytes in obese subjects compared to the control group. The number of MN was increased in Con A-stimulated T lymphocytes in the obese group compared to the control group ($P < 0.01$). The addition of linseed and Nigel oil induced a decrease in MN number ($P = 0.006$) and no change with olive and sunflower oils in the lymphocytes of obese patients, but had no effect on MN in control subjects. In the presence of linseed and Nigel oils, MN number in the obese group was normalized to control values.

4. Discussion

In this study, we demonstrated that *in vitro* supplementation with olive, linseed, Nigel and sunflower oils modulated lymphocyte function after mitogen stimulation, cytokine secretion and intracellular oxidant/antioxidant status in obesity.

Our findings showed that Con A-stimulated lymphocyte proliferation in obese patients was similar to that found in control subjects, as previously reported [42]. This was concomitant with the normal IL-2 production by T cells after mitogen stimulation. However, IL-4 and IFN γ secretions by Con A-stimulated lymphocyte were reduced in obese patients compared with control subjects, reflecting an alteration in cytokine production during obesity. The INF γ /IL-4 ratio was unchanged in obese patients, reflecting no change in the profile of Th1/Th2 balance despite the defect in cytokine production.

In our study, lymphocytes of obese patients were exposed to an evident intracellular oxidative stress with a higher level of oxidant biomarkers, in agreement with the findings of previous studies [40] [50] [51] [52].

Table 5. Induction of micronuclei by oil fatty acids in Con A stimulated lymphocytes from obese and control subjects.

	Control group	Obese group
MN (%)		
Con A	4.38 \pm 0.45	7.56 \pm 0.55 ^{*a}
Con A + Olive oil	4.48 \pm 0.79	7.94 \pm 0.90 ^{*a}
Con A + Linseed oil	4.37 \pm 0.66	4.89 \pm 0.98 ^c
Con A + Nigel oil	4.58 \pm 0.63	4.62 \pm 0.36 ^c
Con A + sunflower oil	4.88 \pm 0.65	7.98 \pm 0.85 ^{*a}
P (ANOVA)	0.247	0.006

The values are means \pm SD of triplicate assays from 32 obese patients and 50 healthy controls. MN: micronuclei. Multiple comparisons were performed using ANOVA followed by the least significant difference (LSD) test. Letters a, b, c indicate significant differences between different incubations ($P < 0.05$). Obese versus control within the same incubation by Student t test: ^{*} $P < 0.01$.

In this study, our data have demonstrated that oil's fatty acids significantly affect the proliferation and cytokine secretion of T cells in both healthy and obese groups. Further, exposure to these nutrients resulted in a significant modulation of oxidative stress markers.

Our findings showed that fatty acids from linseed oil significantly reduced *in vitro* lymphocyte proliferation in both obese and control groups. However, fatty acids from olive and sunflower oils had no effects while Niger oil significantly enhanced T-cell proliferation in obese and control groups. The immunomodulatory effects of oils on *in vitro* IL-2 secretion supported those of T lymphocyte proliferation. The oil's fatty acid effects on IL-4 and INF- γ showed different kinetic profiles in lymphocytes from control and obese subjects.

The reduced lymphocyte proliferative response of linseed oil was in accordance with previous publications reporting that n-3 PUFA induced suppressive proliferative responses of lymphocytes [21] [53] [54]. After linseed oil addition, INF- γ /IL-4 ratio was not affected in lymphocytes from obese patients while it was reduced in control group. Linseed oil appeared to have generated a Th2-like phenotype, reflecting the anti-inflammatory effect of linseed oil as previously reported [20] [21] [55] [56], but only in controls.

Olive oil treatment had no effects on cytokine production in both control and obese groups, as previously reported [21]. Previous studies did not find significant differences in the proliferation of lymphocytes after olive oil supplementation [57] [58].

Our data have demonstrated that fatty acids from Niger oil induced an increase in lymphocyte proliferation and in IL-2 and INF γ secretion in both obese and control groups. Niger oil had no effect in IL-4 secretion in controls while it induced an increase in IL-4 production in obese patients. In addition, Niger oil induced a significant rise in Th1/Th2 ratio towards the Th1 type which reflects an inflammatory effect of this oil in control subjects. However, in obese patients, Niger oil had immunostimulatory effect on T Cells without affecting Th1/Th2 balance. Our results are in agreement with others studies [21] [59].

Our results showed that fatty acids from sunflower oil had no effects on lymphocyte proliferation and in IL-2 and INF γ secretion in both obese and control groups. Sunflower oil had no effect in IL-4 secretion in obese patients while it induced a decrease in IL-4 production in controls. This oil induced a significant rise in Th1/Th2 ratio generating a Th1 inflammatory phenotype in control subjects, without affecting Th1/Th2 balance in obese patients. Our results are in agreement with others studies [60].

In the current study, our findings showed that the presence of linseed and Niger oils induced an enhancement in GSH and Catalase activities in both obese and control groups, reflecting a favorable effects of these oils on intracellular antioxidant status. However, olive and sunflower oils had no effects on lymphocyte GSH contents in both groups. Olive and sunflower oils induced a significant reduction in lymphocyte catalase activity in obese patients, without affecting it in control group.

We observed that in the presence of linseed and Niger oils, the T cell oxidant markers, MDA and carbonyl proteins were reduced in obese patients, probably as a consequence of enhanced antioxidant defense. These results are in agreement with previous reports on antioxidant effects of linseed and Niger oils [21]. MDA and carbonyl protein contents were unaffected by these oils in controls, supporting a beneficial effect only in the presence of an evident oxidative stress such as shown in obesity.

Indeed, olive and sunflower oils had no effects on carbonyl protein levels while only olive oil reduced MDA levels in lymphocytes from obese patients. The beneficial effects of olive oil could be related to the high vitamin E content of this oil compared to that found in sunflower oil.

Previous reports suggested DNA damage in cells exposed to reactive oxygen species [61]. In our study, obesity was associated to a significant increase in the micronucleus (MN) frequency in lymphocytes, suggesting a cytotoxic effect of oxidative stress. The treatment with linseed and Niger oils induced a significant reduction in MN frequency in obese group. This favorable effect could be linked to the reduction in intracellular oxidative stress with linseed and Niger oils in obese patients. Indeed, linseed and Niger oils normalized MN frequency and may have then protective effect on cell damage during obesity. However, in the presence of olive and sunflower oils, MN frequency remained high in obese cells compared to controls, probably in relation with the persistent oxidative stress in these cells.

Some limitations of this study deserve to be mentioned. One limitation was the small sample size and obese characteristics. Obese patients were not separated according their BMI. Further studies are needed with large number size including obese patients with class 1, 2 and 3 obesity. The effects of oils on lymphocyte function were investigated *in vitro*. An *in vivo* study will be necessary to confirm our *in vitro* findings. The current study was conducted in male subjects and the findings should not be extrapolated to other groups such as women. It will be important to conduct similar dose a study in control and obese women.

5. Conclusions

Linseed, Niger, olive and sunflower oils displayed different *in vitro* immunomodulatory properties in control and obese subjects. Linseed oil possessed immunosuppressive properties while Niger oil had immunostimulatory effects on T cells with positive effects on oxidative stress in obesity, while olive and sunflower oils had weak effects.

The integration of linseed and Niger oils into a balanced diet could be a safe nutritional strategy that can attenuate lymphocyte alterations and oxidative stress associated with obesity.

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