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Fatty acids modulate in vitro T cell function in obese children

Radjaa Kaouthar Meziane[1], Hafida Merzouk[1], Meriem Saker[1], Samira Baba Ahmed[2] and Michel Narce[3]

[1]Departement De Biologie, Faculte Des Sciences, Universite De Tlemcen, Tlemcen, Algeria
[2]Service De Pediatrie, Centre Hospitalo-Universitaire De Tlemcen, Tlemcen, Algeria
[3]Umr 866 « Lipides, Nutrition, Cancer », Faculte Des Scienses De La Vie, 6 Boulevard Gabriel, Dijon, France

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ABSTRACT

In this work, we determine the in vitro effects of different fatty acids (MUFA, n-3 PUFA and n-6 PUFA) on T lymphocyte proliferation, on membrane fatty acid composition, and the secretion of interleukins 2. The T lymphocytes were isolated from children's blood, control and obese living in Tlemcen area. These cells were incubated in presence of oils (fish oil, olive oil, and nigel oil), and then stimulated by a "mitogen" agent (Concanavaline A during 48 hours). At the end of the treatment, the cells were counted and the surnageant was used for interleukin 2 assay. The cells were used for membrane fatty acid composition. A reduction of cell proliferation, basal or stimulated by mitogen agent (Con A) was observed in obese children compared to controls. The presence of oils reduced the lymphoproliferation in obese children as well as in controls. Indeed, these oils, especially fish oil, decreased interleukin 2. The fatty acid analysis by chromatography showed that the presence of oils leads to change in the phospholipid fatty acid composition in obese children and controls. All these results indicated that fatty acids (MUFA, n-3 PUFA and n-6 PUFA) can modulate lymphocyte T activity and the secretion of cytokines in obese children.

Keywords: infantile obesity, polyunsatured fatty acids, interleukins 2, lymphocytes T.

INTRODUCTION

The problem of childhood obesity has grown considerably in recent years [1]. The causes of obesity are complex and include genetic, biological, behavioral and cultural factors [2]. Childhood obesity can lead to life-threatening conditions including diabetes, high blood pressure, heart disease, sleep problems, cancer, and other disorders. Some disorders would include liver disease, early puberty or menarche, eating disorders such as anorexia and bulimia, skin infections, and asthma and other respiratory problems [3]. Studies have shown that overweight children are more likely to grow up to be overweight adults [4].

Many studies have also shown that obesity is associated with a chronic inflammatory condition characterized by increased circulating levels of several inflammatory proteins (CRP, fibrinogen, etc..) and numerous cytokines inflammatory (TNF, IL6 and leptin). The biological functions of these adipokines and the consequences of the increase in their circulating concentrations suggest that they may play a role in the establishment and development of many complications of obesity [5].

Obesity is also accompanied by signs of immune deficiency characterized by atrophy of lymphoid organs, a decrease of bactericidal activity of phagocytes, impaired delayed hypersensitivity reaction and increased susceptibility infections [6].

The influence of fatty acids from dietary oils on inflammatory and immune response has been the subject of numerous studies. Polyunsaturated fatty acids omega-3 (PUFA n-3) and omega-6 (PUFA n-6) have been particularly studied.

The addition of unsaturated fatty acids (AGI) in culture medium of immune cells or dietary supplementation with polyunsaturated fatty acids (PUFAs) affect various parameters of the immune response [7], including lymphocyte proliferative response to mitogens [8], cytokine production, NK activity and expression of surface molecules on T cells [9]. Dietary n-3 PUFA induce changes in membrane fatty acid composition specially alterations in lipid raft composition, membrane fluidity, receptor distribution,and the production of eicosanoids [10]. Thus, the immunomodulatory effects of polyunsaturated fatty acids (APGI) can be used in the prevention and treatment of complications associated with obesity.

Therefore, the present study was undertaken to assess in vitro effects of three oils on the proliferation of T lymphocytes, the fatty acid composition of their membranes, and the secretion of interleukins 2.

The oils tested are fish oil, olive oil, and nigel oil.

MATERIALS AND METHODS

Subjects

A total of 26 control children and 20 obese children were recruited from schools elementary and middle of Tlemcen (Algeria). The study population was composed of children (aged 6–14 years). Height and weight were measured according to international standards. Obesity is defined by calculating the body mass index (BMI weight/height2, kg/m2) from the BMI curves that are in the health records. Children whose BMI is above the 97th percentile of BMI curves were considered obese. Children's parents completed a self-administered questionnaire that provided information on general family background characteristics and children's physical activity. Children's dietary intake was measured using a 24-h dietary recall. The characteristics of study population are given in table 1.

Laboratory Methods

Lymphocyte Proliferation Assay

Peripheral blood lymphocytes were isolated from heparinized venous blood using differential centrifugation (400g for 40 min) on a density gradient of Ficoll-Paque (pharmacia biotech, UK). The peripheral blood lymphocytes (PBL) at the interface of plasma and Ficoll-Paque were collected and washed twice with RPMI 1640 culture medium (Gibco, USA). After washing and counting, the cells were resuspended in a tissue culture medium at $4x10^6$ cells/ml. For

proliferation assay, $4x10^5$ cells were cultured in triplicate in 200 µl of medium RPMI 1640 containing 25 mM HEPES buffer supplemented with 10% heat-inactivated fetal calf serum, L - Glutamine (2mM), penicillin (100 UI/ml) and streptomycin (100 µg/ml) with or without concanavalin A (Sigma, St Louis, MO) at a final concentration of 5 µg/ml. This concentration of Con A was found optimal to activate T cells (result not show). Cultures were performed in 96-well flat-bottomed microtiter plates (Nunc, Paris, France) and maintained at 37C° in humidified 5% CO_2 atmosphere for 48 h. This culture time was optimal for cytokine secretion. The cells were incubated in the presence of three oils in order to evaluate their effects on lymphocyte proliferation. The oils tested are fish oil, olive oil, and nigel oil.

Table 1 Characteristics of the study groups

	Controls	Obese Children
Number	26	20
Sex (male / female)	14/12	12/8
Age (years)	9.85 ±0.63	10.30 ±0.27
Height (m)	1.42 ±0.02	1.40 ±0.02
Weight (kg)	34.21 ±1.73	53.30 ±1.88 ***
BMI (kg/m2)	17.03 ±0.50	26.07 ±0.53 ***

*Values are means± SD. The significance of differences between two groups was determined by Student's t test. *P< 0.05; *** P <0.001, obese subjects versus controls BMI body mass index*

The stock solution of each oil at 10 mM TG was previously prepared in absolute ethanol and is kept at -20 C ° until use. From each stock solution, a solution of 300 µM TG is used for the various incubations. After incubation, cells were harvested by washing with RPMI 1640 medium. Cell viability was monitored by direct cell counts, and confirmed by [3-(4,5-Dimethyl thiazole-2-yl)-2,5-diphenyl tétrazolium bromide] (MTT, Sigma) assay as descripted by Mosmann [11].

Interleukin-2

Aliquots of culture supernatants were used to quantitate IL-2 by using commercially available ELISA kits (Genzyme, Cambridge, MA, USA), as per instructions furnished by the manufacturer. The results are expressed as pg/ml.

Analysis of Lymphocyte Phospholipid Fatty Acids

Lipids from T lymphocytes extrated according to the method of Bligh and Dyer [12]. Phospholipids were separated on silica gel by thin layer chromatography. Fatty acid composition was analysed by gas-liquid chromatography as previously reported [13].

Statistical Analysis

Data are expressed as mean ± SEM. Statistical analysis were carried out using STATISTICA (version 4.1, Statsoft, Paris, France). The significance of the differences between two groups was determined by Student's t test. Multiple comparisons were performed using ANOVA followed by the least significant difference (LSD) test. P< 0.05 was considered to represent statistical significant differences.

RESULTS AND DISCUSSION

To gain further insight into the immunomodulatory effects of fatty acids, the present study was conduced to examine in vitro proliferation of T lymphocytes from obese children and from healthy subjects, cultured with fish oil, olive oil, and nigel oil.

Our study was performed on lymphocytes of obese children and controls cultured in vitro in the presence or absence of various edible oils to determine abnormalities of lymphocyte function in obesity and effects of polyunsaturated fatty acids (PUFA) on the proliferation of T lymphocytes, the fatty acid composition of their membranes, and the secretion of interleukins 2.

The oils tested are: fish oil rich in polyunsaturated fatty acids (PUFA), the most important are eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), olive oil rich in oleic acid , and nigel oil rich in linoleic and oleic acid.

Effects of fatty acids on T cell Blastinogenesis

Figure 1 shows that mitogen induced cell proliferation, as expressed by cell number, was significantly diminished in obese children, as compared to controls. Con A, a T cell-specific mitogen, significantly stimulated lymphocyte proliferation in both obese and control subjects. Addition of insuline to culture medium potentiated Con A-stimulated T cell proliferation in all groups.

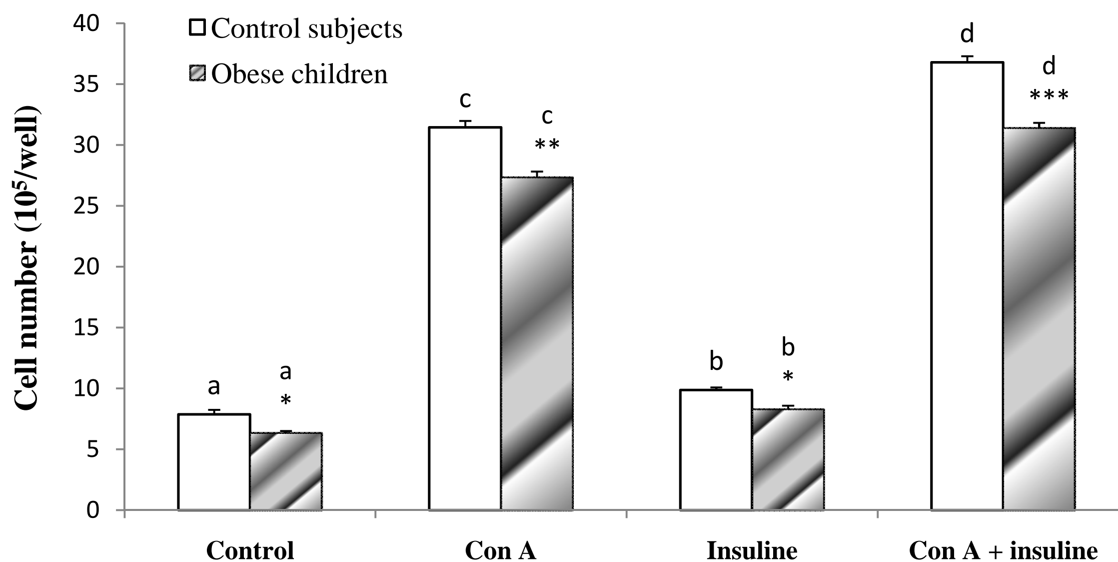

Fig. 1 Resting and mitogen-stimulated T cell proliferation in obese and control subjects. 4x10^{5} cells per well were incubated in 96 well microplates in the presence of concanavalin A (Con A, 5µg/ml) an insulin (Ins, 5µg/ml). Cultures were performed in triplicate for 48h. Proliferation was monitored by direct cell counts, and confirmed by MTT method. The values are mean ± SE of triplicate assays of subjects composed of following numbers: controls, 26; obese children, 20. The significance of the differences between two groups was determined by Student's *t* test. Multiple comparaisons were performed using ANOVA followed by the least significantly difference (LDS) test. The values in obese children are significantly different (*P< 0.05; **P< 0.01; *** P <0.001) as compared to respective control subjects in each incubation. *a, b, c, d* denote significant differences between different incubations within a group (P< 0.05).

Several studies have shown that obese people had low proliferative response through the mitogen [14-16].

The reduction in cell proliferation observed in obese children may be partly related to a state of insulin resistance. In fact, insulin modulates cell differentiation and lymphocyte proliferation [17]. Moreover, our results show that insulin, added to the incubation medium, potentiates the effect of mitogen and proliferation of T and B lymphocytes.

Some studies have shown that DHA, EPA, oleic acid, linoleic acid and inhibit in vitro proliferation of lymphocytes stimulated by mitogens. This inhibition depends on the

concentration and degree of unsaturation of the fatty acid used. Most fatty acids are inhibitors of the n-3 [18].

This is in the same direction as our results, since the three oils used (fish oil, cumin oil, olive oil), fish oil is more immunosuppressive in obese children and witnesses in the presence of mitogen (Fig.2). Several studies have shown that EPA and DHA inhibit lymphocyte proliferation by inhibiting the activity of MAP kinase [19].

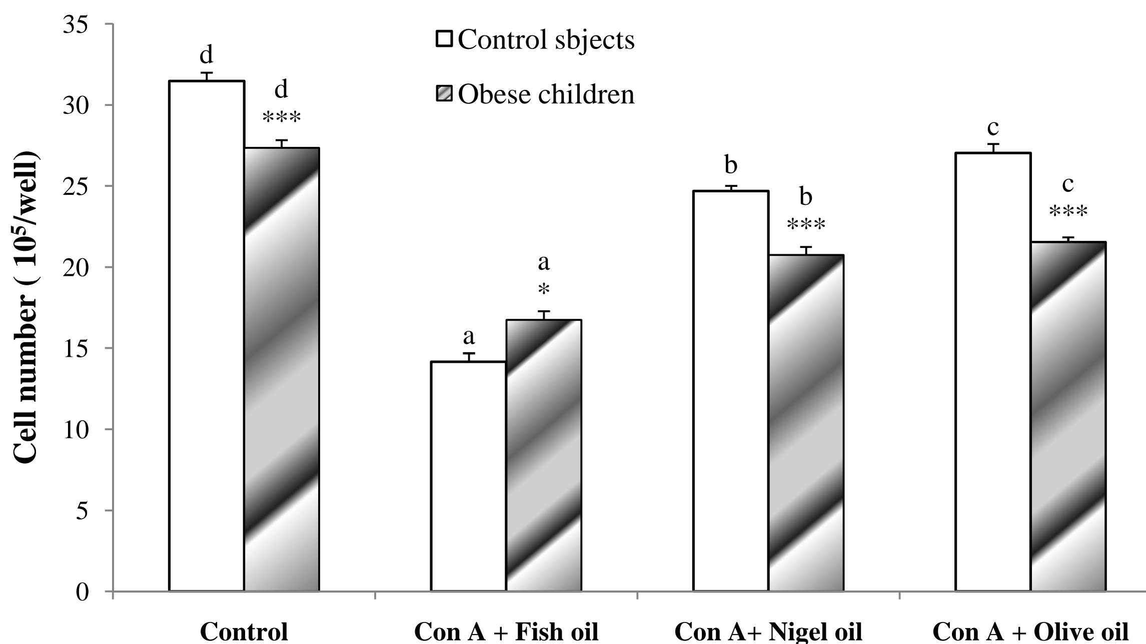

Fig. 2 Effects of fish oil, olive oil, and nigel oil on mitogen-stimulated T cell proliferation in obese and control subjects. 4x10^5 cells per well were incubated in 96 well microplates in the presence of the following agents: concanavalin A (Con A, 5µg/ml), fish oil (300 µM TG), olive oil (300 µM TG), nigel oil (300 µM TG), insulin (Ins, 5µg/ml). The values are mean ± SE of triplicate assays. The values in obese children are significantly different (*P< 0.05; **P< 0.01; *** P <0.001) as compared to respective control subjects in each incubation. *a, b, c, d* denote significant differences between different incubations within a group (P< 0.05)

Tableau 2: Production of interleukin 2 (IL-2) by stimulated lymphocytes from control children and obese children

	Control children (Pg / ml)	Obese children (Pg / ml)
Basal proliferation	558.34 ± 66 [a]	498.83 ± 59.01 [a]
Con A	4235.89 ± 255 [d]	2034.55 ± 111 [c] *
Con A + Insulin	6752.33 ± 377.22 [e]	3771.54 ± 349.14 [d] *
Con A + Fish oil	975.38 ± 155.32 [b]	1502.17 ± 165.14 [b] *
Con A+ Nigel oil	2679.56 ± 345 [c]	1893.44 ± 246 [c] *
Con A + Olive oil	3250.94 ± 337.04 [c]	2404.39 ± 301.95 [c] *

Values are means ± SE. Total of 4x10^5 cells per well were incubated in 96 well microplates in the presence of the following agents: concanavalin A (Con A, 5µg/ml), fish oil (300 µM TG), olive oil (300 µM TG), nigel oil (300 µM TG), insulin (Ins, 5µg/ml) in triplate assays. Different letters (a, b, c, d) denote significant differences between different incubations within a group (P< 0.05).
** Comparison between obese and control groups *P< 0.05*

Effects of fatty acids on Interleukin- 2 (IL-2) Production

Obesity not only affects the functionality of immune cells but also their pattern of cytokine secretion (20). Indeed, our results show that the production of IL-2 by lymphocytes stimulated by mitogens is low in obese children compared to control children (Table 2). A deficit in the

production of IL-2 or the expression of their membrane receptors may lead to the low proliferative response observed in obese children.

Our results also show a reduced production of IL-2 by lymphocytes stimulated in the presence of oils mainly fish oil in children and obese controls. EPA and DHA inhibit the production of proinflammatory cytokines: IL-6, IL-1 and TNF-α, however, this effect is not observed with polyunsaturated fatty acids (PUFAs) of n-6 series. This inhibition is likely due to incorporation into the membranes of mononuclear cells [21].

Fatty Acid Composition of Lymphocyte Phospholipids

The basal fatty acid composition of phospholipid of lymphocytes from obese childrens was significantly different from that of controls (Table 3). In fact, obese childrens showed a significant increase in the proportion of C16 : 0 and a significant decrease in the proportion of C20 : 4n -6 in their lymphocyte phospholipids as compared to controls.

The reduction of C20: 4n -6 obese children may be related to a state of insulin resistance, since insulin stimulates the activity of desaturases. On the other hand, the reduction of C20: 4n -6 may be due to increased use of this fatty acid by preadipocytes during their differentiation into adipocytes [22].

Lymphocytes stimulated with Con A showed a significant increase in the proportion of C18 : 1 and a significant decrease in the proportion of C16 : 0 and C18 : 2n-6 compared to basal fatty acid composition, in both obese and control groups.

Changing the membrane composition is one mechanism by which PUFA may modulate the inflammatory response [23]. Thus, incubation in the presence of nigel oil rich in linoleic acid (n-6) increased arachidonic acid content of mononuclear cells. In contrast, incubation of cells in the presence of fish oil (n-3) decreases the production of metabolites of arachidonic acid and promotes the production of metabolites of n-3.

Table 3 Fatty acid composition of lymphocyte phospholipids in obese children and control subjects

	Controls	Obese
16 : 0		
Basal	34.41 ± 1.60^{c}	37.74 ± 1.05 $*,^{d}$
Con A	27.78 ± 1.19^{b}	30.95 ± 1.24 $*,^{c}$
Fish oil	28.82 ± 1.23^{b}	30.99 ± 1.50 $*,^{c}$
Nigel oil	23.96 ± 1.12^{a}	25.52 ± 1.10 $*,^{a}$
Olive oil	24.26 ± 1.14^{a}	27.93 ± 1.16 $*,^{b}$
16 : 1		
Basal	03.07 ± 0.45^{b}	02.06 ± 0.30^{a}
Con A	05.12 ± 0.54^{c}	04.60 ± 0.35^{c}
Fish oil	05.32 ± 0.44^{c}	03.61 ± 0.32^{b}
Nigel oil	02.36 ± 0.28^{a}	02.10 ± 0.25^{a}
Olive oil	02.35 ± 0.21^{a}	02.21 ± 0.25^{a}
18 : 0		
Basal	19.60 ± 1.10^{c}	18.77 ± 1.02^{c}
Con A	23.66 ± 1.16^{d}	24.03 ± 1.20^{d}
Fish oil	16.12 ± 0.32^{a}	16.08 ± 0.36^{a}
Nigel oil	17.48 ± 0.35^{b}	18.77 ± 0.34^{c}
Olive oil	18.55 ± 0.52^{c}	17.42 ± 0.32^{b}
18 : 1		
Basal	14.74 ± 1.12^{a}	15.24 ± 1.19^{a}
Con A	17.56 ± 1.22^{b}	17.37 ± 1.25^{a}

Fish oil	16.75 ± 1.25 [b]	16.32 ± 1.15 [a]
Nigel oil	17.82 ± 1.14 [b]	17.63 ± 1.15 [a]
Olive oil	32.54 ± 1.25 [c]	31.84 ± 1.29 [b]
18 : 2n-6		
Basal	17.14 ± 1.22 [a]	17.36 ± 1.18 [b]
Con A	14.71 ± 1.12 [a]	14.42 ± 1.08 [a]
Fish oil	16.11 ± 1.23 [a]	18.51 ± 1.21 [b]
Nigel oil	31.86 ± 1.25 [b]	30.26 ± 1.29 [c]
Olive oil	12.78 ± 1.24 [a]	13.65 ± 1.21 [a]
20 : 5n-3		
Basal	01.54 ± 0.46 [a]	01.32 ± 0.35 [a]
Con A	01.73 ± 0.52 [a]	01.54 ± 0.45 [a]
Fish oil	08.20 ± 0.52 [b]	07.62 ± 0.41 [b]
Nigel oil	01.33 ± 0.22 [a]	01.32 ± 0.25 [a]
Olive oil	01.41 ± 0.25 [a]	01.35 ± 0.25 [a]
22 : 6n-3		
Basal	01.21 ± 0.38 [a]	01.24 ± 0.39 [a]
Con A	01.62 ± 0.46 [a]	01.46 ± 0.44 [a]
Fish oil	03.56 ± 0.21 [b]	03.42 ± 0.24 [b]
Nigel oil	01.09 ± 0.25 [a]	01.24 ± 0.36 [a]
Olive oil	01.19 ± 0.45 [a]	01.21 ± 0.22 [a]

Values are means ± SE. values are expressed as a percentage of total fatty acids. Total of $4x10^5$ cells per well were incubated in 96 well microplates in the presence of the following agents: concanavalin A (Con A, 5µg/ml), fish oil (300 µM TG), olive oil (300 µM TG), nigel oil (300 µM TG), insulin (Ins, 5µg/ml) in triplate assays. Fatty acid composition was determined in resting lymphocytes before any addition (basal), after mitogen addition (Con A), and after oil addition (fish oil, olive oil, and nigel oil). Values with the same letter are not significantly different.
*Comparison between obese and control groups *P< 0.05*
Different letters (a, b, c, d) denote significant differences between different incubations within a group (P< 0.05)

Arachidonic acid is the most represented in the membranes. Thus, it is the main substrate for the synthesis of eicosanoids: leukotrienes of series 4 and 5-HETE, prostaglandins and thromboxanes of series 2. Eicosanoids are involved in modulating the intensity and duration of inflammatory responses. Arachidonic acid content of inflammatory cells is strongly correlated with their ability to produce eicosanoids such as prostaglandin E2 [24].

In conclusion, the present study demonstrates that childhood obesity is associated to in vitro reduced T lymphocyte proliferation, IL-2 secretion and altered fatty acid composition of T lymphocyte membrane phospholipids. The lymphocytes of obese children respond to the effects of fatty acids in the same way that cells of controls, showing the immunomodulatory role of these PUFAs. However, cells of the obese are more susceptible to the effects of PUFAs, especially n-3 family, which tend to enhance lymphocyte proliferation. The beneficial effects of n-3 PUFA on T cell functions in Childhood Obesity could be attributed to their suppressive action and modulation of cytokine secretion.

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