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Fatty acids composition of plasma phospholipids and triglycerides in children with cystic fibrosis. The effect of dietary supplementation with an olive and soybean oils mixture

Composizione in acidi grassi dei fosfolipidi plasmatici e dei trigliceridi in bambini con fibrosi cistica. Effetto di un'integrazione alimentare con una miscela di olio di oliva e soia

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Riassunto

La maggior parte dei pazienti affetti da fibrosi cistica presenta una carenza di acidi grassi essenziali (EFA) particolarmente evidente nei fosfolipidi plasmatici. È noto da tempo che la ridotta disponibilità di EFA modifica profondamente la distribuzione degli acidi grassi nelle diverse classi lipidiche plasmatiche e nei tessuti e può determinare profondi cambiamenti nella fluidità di membrana e nei meccanismi di comunicazione cellulare. Nel presente studio sono presentati i risultati di una nuova strategia mirata alla realizzazione di un integratore di acidi grassi facilmente reperibile e a basso costo, che possa essere impiegato quotidianamente dai pazienti affetti da CF con carenze di EFA. A tal fine, è stata studiata la composizione in acidi grassi dei fosfolipidi e dei trigliceridi plasmatici, in pazienti che facevano uso di un supplemento dietetico costituito da una miscela di olio di soja (50%) e di olio extravergine di oliva (50%), e ne sono stati valutati gli aspetti clinici. Lo studio comprendeva 14 soggetti, affetti da CF, di età compresa tra i 6 e i 15 anni con insufficienza pancreatica e portatori di uno o due alleli per la mutazione ΔF508. I soggetti sono stati accoppiati in base all'età e assegnati casualmente ad un gruppo supplementato con la miscela d'olio (n = 7) o a un gruppo di controllo (n = 7). A differenza del gruppo di controllo i pazienti che facevano uso della miscela d'olio hanno mostrato un aumento significativo della percentuale relativa di C 18:1 nei trigliceridi e una diminuzione significativa degli acidi grassi saturi (C 16:0, C 17:0, C 18:0, C 22:0). Inoltre, il rapporto tra LA e AA è aumentato significativamente nel gruppo che faceva uso del supplemento dietetico. Anche nei fosfolipidi del gruppo supplementato con la miscela d'olio la percentuale relativa di C 18:1 è aumentata significativamente, così come quella di acido palmitico, mentre sono diminuite le percentuali relative dei più importati acidi grassi polinsaturi (PUFA). Questi risultati evidenziano come l'acido oleico possa essere facilmente assorbito e incorporato nei fosfolipidi plasmatici dei pazienti con CF, con insufficienza pancreatica ma che fanno uso di enzimi pancreatici, mentre l'incorporazione di LA risulta meno evidente. A livello clinico è stato notato che, nonostante sia evidente una riduzione di PUFA nei fosfolipidi plasmatici, i soggetti sottoposti alla dieta sperimentale non hanno riportato modificazioni significative nel quadro patologico complessivo. Non sono state evidenziate differenze tra i due gruppi in nessuno degli indici clinici monitorati (altezza, peso, BMI, test di Schwachman-Kulczycki e FEV 1s).

Abstract

Cystic fibrosis (CF) is characterized by abnormal levels of essential fatty acids (EFA) in plasma phospholipids. The reduced availability of EFA has been reported to alter patterns of circulating and tissue esterified acids and may determine profound changes in membrane fluidity and cell signaling mechanisms. In the current study, the results of a new strategy aimed at the realization of a practical, low cost integrator, for daily use in the dietary management of FC subjects, are reported. We investigated the plasma phospholipids and triglycerides fatty acids composition of CF patients subjected to a dietary supplement constituted of a mixture of 50% extra virgin olive oil and 50% soybean oil and studied the clinical effects of this supplementa-

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tion. The study included fourteen young subjects, aged between 6 and 15 years, affected by cystic fibrosis, with pancreatic insufficiency and heterozygotes or homozygotes for the ΔF508 mutation. The subjects were matched by age and randomly assigned to either an oil mixture supplemented (OM) group (n = 7), or to a control (C) group (n = 7). In contrast to the control group, the patients with supplemented diet achieved significant increases of the relative amount of C18:1 in the triglycerides as well as a significant decrease in saturated fatty acids (C 16:0, C 17:0, C 18:0, C 22:0). Moreover, the ratio between LA acid and AA significantly increased in the triglycerides of the OM group. In the phospholipids of the OM group, the relative amount of C 18:1 and of palmitic acid increased significantly whereas the relative amount of the most important polyunsaturated fatty acids (PUFA) decreased. These results show that oleic acid can be absorbed and incorporated into the plasma triglycerides of CF patients receiving pancreatic enzymes, whereas poor incorporation of LA occurs. Despite the reduction in the relative amounts of phospholipid PUFA, the supplemented subjects did not reported adverse effects There were no significant differences between groups in the clinical indexes recorded (height, weight, BMI, Schwachman-Kulczycki score and FEV 1s). The results of this study showed that the supplementation with a mixture of extravirgin olive and soybean oil was safe in seven CF patients treated during a 2-months period and no negative clinical effects were evident. However, further clinical trials will be necessary in order to better evaluate the consequence of the observed changes in plasma fatty acids composition in a longer testing period.

Introduction

Cystic fibrosis (CF) is characterized by abnormal levels of essential fatty acids (EFA) in plasma phospholipids. Linoleic acid (LA) and docosahexaenoic acid (DHA) have been reported to be lower and palmitoleic acid (POA) higher in CF patients than healthy subjects[1-7]. Moreover, recent studies found that membrane-bound arachidonic acid (AA) levels increased, whereas docosahexaenoic acid levels decreased in the pancreas, lungs, and ileum of CF knock-out mice[8-9]. These defects have been attributed to low fat diet[10,11], fat malabsorption[12-14], abnormal lipid turnover in cell membranes[15], altered desaturase activity[14,16,17], increased oxidation of fatty acids[18], increased production of eicosanoids[19,20], abnormality in Ca^{++} induced AA release[21], defective control of EFA utilization[22] by cystic fibrosis transmembrane regulation protein (CFTR), and unpaired Cl^- conductance[23].

The reduced availability of EFA has been reported to alter patterns of circulating and tissue esterified acids and may determine profound changes in membrane fluidity[24,25] and cell signaling mechanisms[9,26].

Alterations in these processes may be particularly important for cells responding to chronic lung infections and for pancreatic insufficiency that are frequently found in CF patients. A few years ago, when the clinical definition of CF was first introduced, average survival did not exceed the pediatric age. Nowadays, many CF patients survive until an adult age due to ever advancing dietary management and therapeutical techniques[27].

Relatively recent improvements in the dietary management of children affected by CF include the introduction of both enteric-coated microsphere pancreatic enzyme preparations, and a diet with about 40% of energy from lipids.

The effects of administration of lipids with different fatty acid composition on EFA availability and on the production of prostaglandins 2 (PG_2) and of leukotrienes B4 (LTB_4) in CF patients[28,29] have been investigated, as well. Different lipid composition of dietary supplements have been investigated, but two kinds of strategies are often suggested by literature. One tries to correct poor EFA pattern with supplements rich in LA[30]. Some authors reported that patients with this kind of supplemented diets achieved significant increases of energy intake, weight for height, body fat, as well as LA in plasma phospholipids[31,32]. Other authors reported that poor EFA pattern was not corrected or was difficult to correct because of malabsorption or other metabolic deficiencies[33,34].

The second dietary strategy aims at the suppression of proinflammatory eicosanoids. This is generally achieved by providing a supplement of long chain polyunsaturated ω-3 fatty acids with fish oil[35-39]. The dietary management with fish oil resulted in increased eicosapentaenoic acid (EPA) and DHA in plasma and tissues and reduced plasma LTB_4, and may provide some benefits with relatively few adverse effects, but there is still insufficient evidence to recommend routine use of supplements of omega-3 fatty acids in people with cystic fibrosis[40].

A different approach in the dietary treatment of CF could involves the use of extra virgin olive oil. The processing technology used for extra virgin olive oil is a mechanical cold crushing without any refining or organic solvent extraction step and preserves its peculiar content of natural phenolic compounds. Recent literature data showed that the daily use of extra virgin olive oil has irrefutably beneficial effects in healthy subjects. Its peculiar polyphenols showed protective effects in different animal models of inflammation[41] and possess an array of potentially beneficial lipoxygenase-inhibitory, prostaglandin-sparing, and antioxidant properties[42,43]. Moreover, recent report suggest that enrichment of LDL with olive oil monounsaturated fatty acids reduces the lipoprotein susceptibility to oxidation[44].

Tabella 1
SUBJECTS CHARACTERISTICS

	Olive and Soybean oil mixture	Control
n	7	7
Age (years)	10±3.8	11±3.9
Male:female	4:3	4:3
Genotype		
ΔF508 homozygotes (subjects number)	4	3
ΔF508 heterozygotes (subjects number)	3[a]	4[b]

a: the other alleles carried were N1303 (1 subjects), G85E (1 subject), G542X (1 subject); b: the other alleles carried were N1303 (2 subjects), G85E (1 subject)

Tabella 2
FATTY ACIDS, TOTAL POLYPHENOLIC COMPOUNDS AND VITAMIN E CONTENT OF THE OIL MIXTURE

Compounds	Content
C 16:0 (g/100 g)	10.6
C 16:1 (ω-7) (g/100 g)	0.5
C 18:0 (g/100 g)	3.2
C 18:1 (g/100 g)	49.6
C 18:2 (ω-6) (g/100 g)	26.2
C 18:3 (ω-3) (g/100 g)	3.7
C 20:0 (g/100 g)	0.3
C 20:1 (ω-9) (g/100 g)	0.6
Vit. E (mg/100 g)	46
Polyphenols (mg/100 g)	25.5

In the current study, the results of a new strategy aimed at the realization of a practical, low cost integrator, for daily use in the dietary management of FC subjects, are reported. We investigated the plasma phospholipids and triglycerides fatty acids composition of CF patients subjected to a dietary supplement constituted of a mixture of 50% extra virgin olive oil and 50% soybean oil and studied the clinical effects of this supplementation.

Subjects

The study included fourteen young subjects, aged between 6 and 15 years, affected by cystic fibrosis, with pancreatic insufficiency and heterozygotes or homozygotes for the ΔF508 mutation. The patients were recruited from the Pediatric Division of "G. Salesi" Hospital (Ancona, Italy), matched by age and randomly assigned to either an oil mixture supplemented (OM) group (n = 7), or to a control (C) group (n = 7). The main subjects characteristics are presented in Table 1. Therapies prescribed to patients before the experimental period were not suspended. All the subjects required regular pancreatic enzyme supplementation and received antibiotics therapy when exacerbation of their pulmonary disease occurred. Four subjects of the OM and three of the C groups received recombinant human deoxyribonuclease I (Pulmozyme). Two subjects of the OM and three of the C groups were taking antileukotrienes (Montelukast). Subjects receiving insulin, corticosteroids and other drugs affecting fat mass were excluded, as well as patients without ΔF508 allele or with chronic intestinal pseudobstruction, renal insufficiency, abnormal liver function or any metabolic disorder.

Methods

Dietary intake

Dietary intake was assessed by using a 7-d food (household measures) diary. Subjects were given instructions and formatted recording sheets to document the timing, the quantity and the types of food consumed. The general approach to the nutritional management of CF patients was a high-fat, high-energy diet designed to achieve 120-150% of the recommended daily allowance (RDA). Lipid intake represented 30-40% of total energy intake. The visible portion of dietary lipids of OM was substituted by dietary supplement, constituted of a mixture of 50% extravirgin olive oil and 50% soybean oil, in reason to achieve 8-10% of total energy intake. The mixture was fed to OM for 2 months. The fatty acids, total polyphenolic compounds and vitamin E content of the oil mixture is reported in Table 2. Body weight, height and the forced expiratory volume in 1 s (FEV_1) of the subjects were recorded, using standard techniques at the beginning and at the end of the dietary supplemented period.

Materials

L-α-phosphatidylcholine dipalmitoyl (PC), L-α-phosphatidylethanolamine dipalmitoyl (PE), L-α-phosphatidylinositol ammonium salt from bovine liver (PI), N-palmitoyl-D-sphingomyelin (Sph) and L-α-lysophosphatidylcholine from egg yolk (L-PC) were purchased from Sigma Chemicals Co. (St. Louis, MO).
Pre-coated silica gel plates were purchased from Merck (Darmstadt, Germany). HPLC grade solvents were purchased from BDH Ltd (Poole, UK). All other chemicals, with noted exceptions, were obtained from Sigma Chemicals Co. (St. Louis, MO).

Plasma collection and lipid extraction

Blood samples were obtained by venopuncture. Clotting was prevented with EDTA and the plasma fraction collected by centrifugation at 1500 g for 20 min at 4°C. Plasma samples were stored at -20°C for lipid analysis. Total lipids were extracted from plasma according to the method of Folch[45].

Two drops of 1:1 (v/v) aqueous hydrochloric acid were added to 500 mg of serum. The acidified serum was vigorously mixed with 10 ml of chloroform/methanol (2:1, v/v); the solution was filtered under vacuum and 2 ml of 0.88% KCl were added. The chloroform layer was recovered and the solvent was evaporated under nitrogen. The lipid extract was redissolved in 500 ml of chloroform/methanol/water (5:5:1, v/v/v) for further analysis.

Phospholipid analysis

Phospholipid classes were resolved by high-performance liquid chromatography (HPLC) on a Hypersil Si (150 mm x 4.6 mm i.d., 3 μm particle size) column (Phenomenex, Torrance, USA). The separation was obtained with a gradient elution starting at 100% A, decreasing to 0% A in 10 min, held at 0% A for 15 min and then back to 100% A in 5 min. The mobile phase A was $CHCl_3$/MeOH/NH_3 (80:19.5:0.5, v/v), and the mobile phase B was $CHCl_3$/MeOH/H_2O/NH_3 (60:34:5.5:0.5, v/v). The flow rate was 0.8 ml/min. The HPLC system consisted of a degassing unit (Gastorr GT-103), a ternary gradient module (Jasco LG-980-02), a pump module (Jasco PU-980), and a light scattering detector (Polymer Labs PL-EMD 960). Peak identification was carried out by comparing retention times with those of pure standards provided by Sigma Chemical Co.
Calibration curves for quantitative HPLC analyses were run with the commercial standards of PC, L-PC, PI, PE, and Sph. Standard solution contained 0.2-1 mg $*ml^{-1}$ for Sph, 0.06-0.28 mg $*ml^{-1}$ for PE, 0.1-0.4 mg $*ml^{-1}$ for PI, 0.02-0.1 mg $*ml^{-1}$ for L-PC, and 0.4-1 mg $*ml^{-1}$ for PC, and were injected with an increasing concentration order in each run. Three replicates were run for each concentration. Regression analyses were done with a linear function; r^2 varied between 0.988 for PC to 0.997 for Sph and L-PC.

Fatty acid analysis

Phospholipids and triglycerides were separated by thin layer chromatography (TLC) using pre-coated silica gel G plates (20x20x0.25 cm) and *n*-hexane/diethylether (60:40, v/v) as developing solvent.
The separated lipid classes were detected under UV light after nebulization with 1% solution of 2',7'-dichlorofluorescin in ethanol. The plates were scraped off and lipids were extracted from silica: the triglyceride band was recovered with diethylether and phospholipids were recovered with chloroform/methanol/water (5:5:1, v/v/v).
Fatty acid methyl esters were prepared from phospholipids and triglycerides by acid-catalyzed transmethylation according to Christie[46].
After extraction with *n*-hexane, the methyl esters were concentrated under nitrogen. The analysis was performed with a Chrompack (Middelburg, The Netherlands) CP-9003 gas chromatograph, on a SP 2340 (30 m x 0.32 mm i.d., 0,20 μm film thickness) column (Supelco, Bellefonte, CA), equipped with an on-column temperature programmed injection system (TP-OCI) and flame-ionization detector (FID). The carrier gas was helium and the oven temperature was maintained at 60°C for 3 min, then raised to 220°C in 55 min. The TP-OCI temperature was maintained at 60°C for 6 min, then raised to 220°C in 8 min.
Peak identification was carried out by comparing retention times (RTs) with those of pure standards provided by Sigma Chemicals Co. (St. Louis, MO) and Supelchem Inc. (Bellefonte, PA), and by comparison with the results published in literature[47,48].

Oil mixture analysis

The crude oil was saponified according to the procedures detailed in "Norme Grassi e Derivati" (Method NGD C 12-1976)[49]. The vitamin E content was detected in the unsaponifiable fraction by gas chromatography using a Carlo Erba (Milano, Italy) HRGC 5160 Mega instrument in the conditions reported by Frega et al.[50]. The fatty acid composition was determined in the saponifiable matter, in the condition reported above, following the treatment with diazomethane (CH_2N_2)[51] to convert free fatty acids into their methyl ester. Total polyphenolic compounds were detected via the spectrofotometric procedure reported by Montedoro et al.[52].

Statistical analysis

Results are expressed as mean ± standard deviation (SD). All variables were recorded at inclusion (day 0) and after two months (day 60). Data were compared with respect to time (day 60 versus day 0) and between groups (OM versus control group) by using the Student's t test.

Results

Data on height, weight, puberty status, clinical status and lung function were recorded. BMI distribution of subjects participating in the present study were categorized in the ideal range for age. Schwachman-Kulczycki score and FEV 1s suggested that the CF subjects were not too severely affected. Mean BMI and FEV 1s of OM group and C group recorded at the beginning and at the end of the experimental period are reported in Table 3. There were no significant differences between groups in the clinical indexes recorded. There were no significant differences on plasma phospholipids composi-

Tabella 3

CLINICAL INDEXES OF THE PATIENTS SUPPLEMENTED WITH OIL MIXTURE (OM) AND OF THE CONTROL GROUP (C)*

	OM (n = 7) Mean ± SD		C (n = 7) Mean ± SD	
	Day 0	Day 60	Day 0	Day 60
Weight (kg)	36.1±15.6	37.3±15.5	37.7±15.2	38.1±15.6
Height (cm)	137.5±25.6	139.9±24.8	138.6±26.7	139.7±27.7
BMI (kg • m^{-2})	18.2±2.4	18.1±2.4	18.8±2.1	18.7±2.2
FEV 1s (%)	95.8±14.5	90.7±15.2	91.2±88.5	88.5±27.2
Shwachman-Kulczycki score[a]	87.1±7.0	88.6±6.3	82.1±10.7	82.9±12.5
Antibiotic therapy (days)[b]	–	13.4±6.8	–	21.7±11.5

OM: oil mixture group; C: control group; BMI: Body Mass Index; FEV 1s: forced expiratory volume in 1s; a: possible score range from 0 to 100, the lowest being the worst; b: days of antibiotic therapy prescribed to patients from the beginning to the end of the experimental period

Tabella 4

EFFECT OF DIETARY SUPPLEMENT ON PLASMA PHOSPHOLIPIS COMPOSITION

Phospholipids (μg/g plasma)*	OM (n = 7)		C (n = 7)	
	Day 0	Day 60	Day 0	Day 60
Phosphatidylethanolamine	27.6±6.1	36.8±9.6	27.4±6.2	29.5±9.8
Phosphatidylinositol	72.7±4.2	75.6±5.6	73.0±3.8	71.6±6.4
Phosphatidylcholine	741.4±76.3	685.2±60.3	715.9±105.4	650.5±155.4
Sphingomyelin	299.6±52.3	317.1±29.4	301.2±42.6	292.8±47.7
Lysophosphatidylcholine	89.4±24.2	132.6±76.7	77.8±18.1	74.0±18.3
Total Phospholipids	1231.8±128.3	1293.9±148.4	1195.4±160.3	1118.3± 207.7

OM: oil mixture group; C: control group; *: mean ± SD

tion as reported in Table 4. There were significant differences between groups in the fatty acid composition of plasma triglycerides (Table 5) and phospholipids (Table 6). In contrast to the control group, the patients with supplemented diet achieved significant increases of the relative amount of C18:1 in the triglycerides (respectively from 40.3 ± 5.5 to 51.7 ± 9.7 in the OM group, and from 43.9 ± 4.9 to 41.6 ± 5.1 in the C group), as well as a significant decrease in saturated fatty acids (C 16:0, C 17:0, C 18:0, C 22:0). Moreover, the ratio between LA acid and AA significantly increased in the triglycerides of the OM group.

In the phospholipids of the OM group, the relative amount of C 18:1 increased significantly from 13.4 ± 1.5 to16.4 ± 2.3. The relative amount of palmitic acid also increased significantly from 28.5 ± 2.2 to 31.0 ± 1.9 and the relative amount of the most important PUFA decreased significantly: AA and dihomo-γ-linolenic acid (C 20:3 ω6) decreased respectively from 3. 5 ± 0.7 to 2.8 ± 0.6 and from 8.6 ± 1.1 to 6.5 ± 1.2; DHA decreased from 2.2 ± 0.7 to 1.6 ± 0.4.

Discussion

The objective of this 2-months study was to evaluate, in children affected by CF, aged between 6 and 15 years, the clinical effects and the impact on the plasma fatty acid composition of a dietary supplement consisting of a mixture of 50% extravirgin olive oil and 50% soybean oil. The mixture was high in monounsaturated fatty acids, especially in oleic acid (OA) and LA.

Tabella 5

EFFECT OF DIETARY SUPPLEMENT ON FATTY ACID COMPOSITION OF PLASMA TRIGLYCERIDES

Fatty acids (%)[a]	OM (n = 7)		C (n = 7)		P	
	Day 0	Day 60	Day 0	Day 60	Groups[b]	Time[c]
C 14:0	2.7±1.0	1.5±0.8	1.9±0.9	2.5±0.8	0.01	NS
C 16:0	31.1±6.0	26.6±5.4	26.7±4.6	28.4±4.1	0.007	0.02
C 16:1	3.5±1.3	2.5±1.0	3.2±1.3	3.9±1.5	0.05	NS
C 17:0	0.9±0.2	0.6±0.2	0.8±0.2	0.8±0.1	0.02	0.007
C 18:0	6.1±0.8	4.6±1.0	5.9±1.3	5.9±0.7	0.03	0.006
C 18:1	40.3±5.5	51.7±9.7	43.9±4.9	41.6±5.1	0.007	0.03
C 18:2 ω6	12.5±5.5	10.6±3.4	14.4±5.5	13.9±6.5	NS	NS
C 20:0	0.3±0.1	0.3±0.1	0.2±0.1	0.2±0.1	NS	NS
C 20:3 ω6	0.2±0.1	0.1±0.1	0.2±0.1	0.2±0.1	NS	NS
C 20:4 ω6	0.7±0.2	0.5±0.2	0.7±0.2	0.7±0.2	NS	0.05
C 22:0	0.7±0.2	0.4±0.2	0.7±0.1	0.7±0.1	0.01	0.02
C 22:6 ω3	0.4±0.2	0.3±0.2	0.5±0.2	0.5±0.1	NS	NS
C 20:4/C 20:3	4.1±1.5	4.2±1.0	4.3±1.9	3.6±1.6	NS	NS
C 18:1/C 18:2	3.7±1.5	5.9±2.7	3.6±1.6	4.1±1.7	NS	NS
C 18:2/C 20:4	18.0±6.2	23.6±3.6	19.5±6.0	19.3±7.6	0.005	0.01
C 20:4/C 22:6	2.0±1.1	2.0±0.8	1.5±0.7	1.5±0.5	NS	NS
Tot. ω6/tot. ω3	38.5±19.5	51.0±23.2	32.9±21.3	31.2±14.5	NS	NS
Tot. saturated	41.8±6.9	32.6±6.4	37.5±5.8	38.8±5.4	0.001	0.008
Tot. polyunsaturated	13.8±5.7	11.2±4.1	15.6±6.2	13.9±6.1	NS	NS

OM: oil mixture group; C: control group; P: Student's test value (NS, P > 0,05); a: mean ± SD of the relative amount; b: OM versus C groups; c: OM at day 60 versus OM at day

Tabella 6

EFFECT OF DIETARY SUPPLEMENT ON FATTY ACIDS COMPOSITION OF PLASMA PHOSPHOLIPIDS

Fatty acids (%)a	OM (n = 7)		C (n = 7)		P	
	Day 0	Day 60	Day 0	Day 60	Groups[b]	Time[c]
C 14:0	0.6±0.3	1.0±0.7	0.5±0.1	0.6±0.2	NS	NS
C 16:0	28.5±2.2	31.0±1.9	29.6±2.7	29.8±2.4	0.004	0.002
C 16:1	1.0±0.6	1.2±0.3	1.3±0.5	1.2±0.6	NS	NS
C 17:0	0.5±0.1	0.6±0.1	0.5±0.1	0.5±0.1	NS	NS
C 18:0	15.0±2.1	15.1±1.3	15.1±1.7	15.0±1.6	NS	NS
C 18:1	13.4±1.5	16.4±2.3	13.8±1.7	13.9±1.8	0.03	0.009
C 18:2 ω6	18.9±2.2	17.4±2.1	17.7±2.0	17.9±2.3	NS	NS
C 18:3 ω3	0.6±0.2	0.5±0.2	0.6±0.1	0.6±0.1	NS	NS
C 20:3 ω6	3.5±0.7	2.8±0.6	3.3±0.7	3.2±0.8	0.04	0.02
C 20:4 ω6	8.6±1.1	6.5±1.2	8.6±1.1	8.1±1.5	0.03	0.007
C 22:0	1.4±0.3	1.1±0.3	1.3±0.2	1.3±0.2	NS	0.03
C 20:5 ω3	0.4±0.1	0.3±0.1	0.4±0.1	0.4±0.1	NS	NS
C 24:0	1.3±0.4	1.2±0.5	1.2±0.3	1.3±0.2	NS	NS
C 24:1	3.5±0.6	2.8±0.8	3.6±0.4	3.6±0.8	NS	NS
C 22:5 ω3	0.6±0.1	0.4±0.1	0.5±0.1	0.5±0.2	NS	NS
C 22:6 ω3	2.2±0.7	1.6±0.4	2.0±0.7	2.0±0.7	0.02	0.02
C 20:4/C 20:3	2.6±0.6	2.3±0.4	2.7±0.7	2.6±0.8	NS	NS
C 18:2/C 20:4	2.2±0.5	2.7±0.5	2.1±0.3	2.2±0.3	NS	0.04
C 18:3/C 22:6	0.3±0.1	0.4±0.1	0.3±0.1	0.3±0.1	NS	NS
C 18:3/C 20:5	1.3±0.4	1.7±1.0	1.6±0.4	1.8±0.5	NS	NS
C 18:2/C 18:3	36.3±11.5	34.7±10.7	30.4±9.0	31.2±8.9	NS	NS
C 20:4/C 22:6	4.1±1.1	4.3±1.0	4.7±1.1	4.3±0.8	NS	NS
C 20:4/C 20:5	20.6±4.4	19.0±2.5	22.3±5.6	23.6±5.4	NS	NS
Tot. ω6/tot. ω3	8.5±1.7	9.7±2.1	8.9±1.7	8.8±1.7	NS	NS
Tot. saturated	47.4±1.6	50.1±1.7	48.3±3.2	48.5±2.7	0.04	0.01
Tot. polyunsaturated	34.7±2.3	29.5±3.0	33.0±3.4	32.8±4.4	0.05	0.02

OM: oil mixture group; C: control group; P: Student's test value (NS, P > 0,05); a: mean ± SD of the relative amount; b: OM versus C groups; c: OM at day 60 versus OM at day 0

After two months of dietary treatment, the relative amount of all C 18:1 isomers increased in the triglycerides of the supplemented subjects, particularly at the expanse of saturated fatty acids. However the relative amount of LA remained unchanged despite the high LA content of the dietary supplementation (Table 2). These results showed that oleic acid can be easily absorbed and incorporated into the plasma triglycerides of CF patients receiving pancreatic enzymes, whereas the intestinal uptake of LA could be impaired. In fact, the ratio between C 18:1 and LA in plasma triglycerides of the OM group was 3.7 ± 1,5, at the inclusion, and 5.9 ± 1,5, at the end of the experimental period, whereas it was only 1.9 in the mixture. This could be attributed to impaired intestinal absorption or to abnormal metabolism of LA[33,34]. Increased levels of C 18:1 were found also in plasma phospholipid fatty acids, particularly at the expanse of PUFA. Because the essential fatty acids status in the plasma of CF patients is often compromised by deficiencies in PUFA, a worsening of the disease could have been expected from the reduction in the relative amounts of phospholipid PUFA recorded in this study. However, the supplemented subjects did not reported adverse effects despite this reduction in the relative amounts of PUFA in the phospholipids. Notably, the ratio between ω6 and ω3 fatty acids remained unchanged. No complications resulted from administration of the oil mixture to these CF patients. In addition, the antibiotic therapy in occurrence of respiratory exacerbation episodes was shorter in the OM group with respect to the control group, in most cases.

The results of this study showed that the supplementation with a mixture of extravirgin olive and soybean oil was safe in seven CF patients treated during a 2-months period and no negative clinical effects were evident. However, further clinical trials will be necessary in order to better evaluate the consequence of the observed changes in plasma fatty acids composition in a longer testing period. It will be a major challenge for the future to modify the oil mixture fatty acids composition in order to achieve a better profile in the plasma fatty acids of CF patients and to investigate changes in the fatty acids composition of CFTR regulated tissues.

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