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Original Article

Fatty Acid Supplementation Improves Respiratory, Inflammatory and Nutritional Parameters in Adults with Cystic Fibrosis

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ABSTRACT

Introduction and aims: Chronic inflammation plays a major role in lung deterioration in cystic fibrosis (CF) patients and anti-inflammatory strategies have beneficial effects. To study the changes seen after a one-year course of low-dose dietary supplements with a mixture of fatty acids in adult patients with CF in chronic inflammation, pulmonary status (lung function, respiratory exacerbations and antibiotic consumption), quality of life and anthropometric parameters.
Patients and method: Seventeen adult subjects with CF received 324mg of eicosapentaenoic, 216mg of docosahexaenoic, 480mg of linoleic and 258mg of gammalinolenic acid daily. We assessed inflammation markers, spirometry parameters, number and severity of respiratory exacerbations, antibiotic consumption, quality of life (St George's QoL), anthropometric parameters and serum phospholipid fatty acid composition.
Results: At the end of the treatment period TNF alpha levels fell significantly and its soluble receptors (60 and 80) rose significantly. Levels of IgG and IgM anti-oxidised LDL antibodies fell significantly. Spirometry improved significantly. Annual respiratory exacerbations and days of antibiotic treatment fell significantly. The improvement in QoL was not significant. Serum levels of docosahexaenoic, total omega-3 and linoleic acid rose significantly and more favourable profiles were seen in monoenoic acids, arachidonic acid and the arachidonic/docosahexaenoic ratio. The fat-free mass and hand grip dynamometry improved significantly.
Conclusions: Low-dose supplements of n-3 and gammalinolenic fatty acids over a long period (one year) appears to improve pulmonary status (lung function, respiratory exacerbations and antibiotic consumption), inflammatory and anthropometric parameters in adults with CF.

La suplementación con ácidos grasos mejora parámetros respiratorios, inflamatorios y nutricionales en adultos con fibrosis quística

Palabras clave:
Fibrosis quística
Inflamación
Tratamiento antiinflamatorio
Función pulmonar
Reagudizaciones respiratorias
Ácidos grasos
Omega-3
Suplementación
Factor de necrosis tumoral

RESUMEN

Introducción: La afectación pulmonar es la causa más importante de morbimortalidad en la fibrosis quística (FQ) y la inflamación-infección bronquial crónica condiciona el deterioro progresivo de la función pulmonar. Los tratamientos destinados a reducir la respuesta inflamatoria podrían ser beneficiosos.
Objetivos: Valorar el efecto de la suplementación oral durante un año en pacientes adultos con FQ, de una combinación de ácidos grasos sobre parámetros respiratorios, antropométricos, inflamatorios, de calidad de vida y sobre el perfil de AG de los fosfolípidos séricos (AGFS).

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Pacientes y método: Diecisiete pacientes recibieron diariamente durante un año: 324 mg de ácido eicosapentaenoico, 216 mg de ácido docosahexaenoico, 480 mg de ácido linoleico y 258 mg de ácido gammalinolénico. Se valoraron marcadores inflamatorios, parámetros espirométricos, reagudizaciones respiratorias, consumo de antibióticos, calidad de vida (St. George), antropometría y los AGFS.
Resultados: Al final del tratamiento se observó, de forma significativa, una disminución de reagudizaciones y del consumo de antibióticos con mejoría de los parámetros espirométricos, de la masa magra y la dinamometría. Concomitantemente, se observó una reducción significativa de los anticuerpos anti-LDL oxidada (inmunoglobulina [Ig] G e IgM) y de los niveles séricos del factor de necrosis tumoral α, así como un incremento de sus receptores solubles. Los niveles de AGFS mejoraron con un aumento significativo de DHA, AG-omega-3 y ácido linoleico, y un descenso de monoinsaturados y del cociente araquidónico/DHA.
Conclusiones: La suplementación con una mezcla definida de AG durante un año parece mejorar los parámetros respiratorios (espirométricos, reagudizaciones y consumo de antibióticos), inflamatorios y antropométricos en pacientes adultos con FQ.

Introduction

Most morbidity and mortality associated with cystic fibrosis (CF) are related to pulmonary involvement, characterised by repeated bronchial infections and an exaggerated inflammatory response. People with CF suffer a significant increase of inflammation of the airways, with elevation of activated neutrophils and proinflammatory cytokines and a decrease of anti-inflammatory cytokines. Treatment of airway obstruction and respiratory infections, control of inflammation and appropriate nutritional support are key factors to delay lung damage.

Chronic inflammation plays an essential role in the pulmonary deterioration of patients with CF and, in this sense, previous studies have shown that these patients frequently present abnormal levels of essential fatty acids (EFA) in serum, plasma and blood cell membranes, as also in histological samples, and the normalisation or modification of this fatty acid (FA) pattern could reduce chronic inflammation.[1-6] The most significant characteristic of subjects with CF, in comparison with the general population, is the finding of low levels of linoleic acid (LA) and docosahexaenoic acid (DHA) with a relative increase of the percentage of monounsaturated FAs and eicosatrienoic acids.[2-6] Our study group has observed these same alterations in the serum of a group of adults with CF in comparison with control subjects of similar age, sex and body composition, in spite of good nutritional status, absence of vitamin E deficiency and adequate food intake.[3] These findings support the hypothesis that this anomalous profile could be secondary to mutation of the cystic fibrosis transmembrane conductance regulator (CFTR) gene and that a Mediterranoean type diet, although a good starting point for these patients, is not capable of modifying their plasma FA profile.[3]

The decrease seen in bioavailability of these EFAs could induce significant changes in the composition of cell membrane FAs with the corresponding functional deterioration. These alterations could favour, in part, the deterioration of organs specifically affected in CF (such as the lung).[3,7] Although these alterations have been known to exist for more than 40 years, the subject once more attracted interest when Freedman et al described a restoration of pancreatic morphology after supplementation with high doses of DHA in mouse models with CF.[5]

During recent years, other attempts have been made to normalise or modify the FA pattern in patients with CF by means of diet supplementation with different FAs, with the aim of reducing chronic inflammation. Polyunsaturated FAs of the omega-6 family have been used (such as Gamma-linolenic acid [GLA])[8] and others of the omega-3 family (n-3) (fish oil, eicosapentaenoic acid [EPA] and DHA).[2,4,9-19] These studies have shown that it is possible to modify the FA profile of plasma and cell membranes with oral supplementation.[2,4,8-10,12,16,19] Some studies have also shown improvement in some nutritional, clinical, spirometric[2,8,10,13] and inflammatory[10,13,14,18] parameters, at least in the short and medium term. However, long term effects on the evolution of the disease or quality of life are not known. Furthermore, the most appropriate FA content (type and optimum dosage) of the supplementation is currently unknown, as is also the appropriate duration of supplementation; further research on the subject is necessary.

Some authors have proposed using, instead of supplementation with high doses of a single n-3-FA, combinations of n-3 and Gamma-linolenic acid (GLA) that could act synergically[1,20] at different levels of the proinflammatory eicosanoid production cascade. Our work hypothesis is that intake of a combination of EFAs could modulate inflammation, reduce the number and severity of exacerbations, and slow down the deterioration of lung function. For this reason, the objective of this study was to assess the long term effect (one year) of oral supplementation with a combination of several FAs in low doses in adult patients with CF on: the number and severity of exacerbations of respiratory infections and spirometric parameters; b) quality of life; c) nutritional parameters; d) FA profile of serum phospholipids; and e) different oxidation and inflammation markers.

Patients and Methods

Patients

The study included patients that presented CF diagnostic criteria and that came to regular follow-up consultations (every 2 to 3 months) at the CF Adult Unit of the Carlos Haya University Hospital Complex (Málaga) according to the directives of the European Consensus on CF.[21] Patients were screened (sequentially and prospectively), for inclusion during a 7 month period, from amongst those that attended for annual assessment (respiratory and nutritional) for CF. The Ethics and Clinical Research Committee of the Carlos Haya Hospital approved the study and all the participants gave their informed consent in writing.

Inclusion Criteria

Subjects of 16 years of age or over, who had completed their pubertal development (Tanner V), with CF, with 2 or more years of follow-up at the unit and who had come in for consultation at least 4 times during the year prior to beginning supplementation. Patients were excluded if they had received prior oral supplementation with n-3-FAs, if they suffered from renal failure, if they were taking oral contraceptives, oral glucocorticoids or non steroid anti-inflammatory drugs (NSAIDs), if they had a prothrombin time < 90%, if their platelet count was < 50,000/mm^3, if they were suffering from severe liver failure (determined by ultrasound: severe cirrhosis/fibrosis or portal hypertension or persistent elevation of serum aminotransferase

levels) or had suffered an episode of massive or life-threatening haemoptisis, if they had a forced expiratory volume in one second expressed in percentage with respect to expected theoretical value that was less than 20%, or if they had received organ transplants or were on the waiting list for a transplant (lung, liver, heart or combined).

Study Design: A one year observation period was established, during which the same variables were prospectively collected that were subsequently analysed during the year of supplementation. A prospective intervention study was performed giving 6 daily capsules of Synerbiol (Nutergia S. A.), 2 with each main meal, for 12 months. Synerbiol has the following composition per pearl-like capsule: EPA (54mg), DHA (36mg), LIN (80mg), GLA (43mg) and vitamin E (10mg). The follow-up period was 12 months. Compliance and possible adverse effects (diarrhoea, steatorrhoea, abdominal pain, nausea, vomiting, gastroesophageal reflux, taste of fish or haemorrhage) were assessed every 3 months with monthly visits programmed by telephone; the containers of the study drug were checked and possible side effects registered. During the 12 month period with diet supplementation no patient began treatment with macrolides, although 6 of them received treatment with azithromycin before the beginning of the observation period. To compare the concentration of FA of serum phospholipids and inflammation and oxidation markers (see below), a *control group* was selected with the same number of people - healthy and of similar characteristics as to age, sex and nutritional status- from the Pizarra nutritional study in the province of Málaga.[22]

Pulmonary Variables

A simple basal spirometry was carried out at 6 months from the beginning of supplementation and after one year of supplementation. Spirometry readings were also collected from the clinical histories of the annual follow-ups performed 12 and 24 months before the beginning of supplementation (in a context of clinical stability). Forced vital capacity and FEV_1 were measured in ml and as a percentage of the expected theoretical value for persons of a reference population of the same sex, age, weight and height.[23] Severity of disease was assessed at baseline and after 12 months of supplementation with the NIH modified assessment system[24] and with the Bhalla scoring system (based on chest computed tomography).[25]

At each visit a sample of sputum was taken for a microbiological study which included seeding in general and selective media for CF habitual pathogens and bacterial counts. We analysed initial colonisation of CF habitual microorganisms and first appearance in sputum was considered (at least 3 samples of positive sputum) independently of persistence during the study. Respiratory exacerbations were registered prospectively, both during the year prior to supplementation (observation period) and during the year of supplementation. Exacerbations were considered as: a) slight-moderate: Increase of volume or purulence of sputum or an increase of dyspnoea not due to other causes, accompanied or not by other symptoms (cough, feverishness, asthenia, poor general condition, anorexia with weight loss, pleuritic chest pain, changes on respiratory exam, changes of chest X-ray indicating infection, or an increase of systemic inflammation markers —reactive protein C [PCR] or VSG— treated with oral antibiotics, or the presence of a positive culture for a microorganism at a dilution ≥ 10^{-5} and treated with oral antibiotics); b) severe: If this is also associated with significant clinical worsening (fever ≥ 38° C, tachypnoea, significant decrease of oxygen saturation or of respiratory function, hypercapnoea or presence of complications, such as pneumonia, acute respiratory failure, haemoptisis, pneumothorax, haemodynamic instability or worsening of cognitive status) and treatment with intravenous antibiotics.[26] The number of days with oral or parenteral antibiotic treatment during the year prior to supplementation and during the year of supplementation was also registered.

Nutritional Evaluation

The following anthropometric parameters were measured at the beginning and at the end of supplementation (baseline and at 12 months): weight and height (and body mass index calculated with these); skin folds (tricipital, abdominal, bicipital and subscapular) using a constant pressure Holtain skinfold calliper; arm circumference, measured with a non-extensible measuring tape and estimation of arm muscle circumference. The same researcher performed all anthropometric measurements, by triplicate, on the dominant limb and determined the average. Percentiles were estimated based on Spanish population[27] reference values. Lean body mass and body fat percentages were estimated according to Durnin[28] and Siri[29] formulae. Hand grip dynamometry was performed using an adult dynamometer (AS Medizintechnik, Tuttlingen, Germany) and 3 measurements of the dominant member were obtained with the dynamometer and the average was calculated. A prospective 4-day diet register was drawn up (including at least one Saturday or Sunday) according to a previously described protocol.[3,30] An experienced dietician gave the patients precise instructions on how to fill this in. The same dietician revised the questionnaires with the patients to resolve any possible doubts or errors and omissions. Patient intake was compared with estimated theoretical total energy expenditure using the formula proposed by the Cystic Fibrosis Foundation.[31]

Laboratory Parameters

At the baseline visit and after the end of supplementation analytical determinations were made (that coincided with the annual routine follow-up study) these includes a haemogram, biochemical parameters (using an autoanalyser), albumin, liposoluble vitamins A, D and E (using high performance liquid chromatography) and total immunoglobulins (Ig) (IgA, IgG, IgM and IgE). A 72 hour faeces sample was taken for quantitative determination of faecal fats and nitrogen using spectrophotometric techniques (near infrared reflectance analysis). A glycaemia curve was also determined (after oral overload with 75g of glucose) following WHO 1998 criteria with measurements of glucose, insulin and peptid C at baseline and at 2 hours. During baseline blood extraction, and also at 3, 6 and 12 months from beginning of supplementation, part of the serum and plasma samples was immediately separated and frozen at -70º C, and stored until they were thawed to carry out: Determination of the fatty acid composition of serum phospholipids (FASP) according to previously described methods;[3] b) tumour necrosis factor alpha (TNF-α) and its α soluble receptors 60 and 80 (TNF-60 and TNF-80) (BLK Diagnostics International, Barcelona, Spain), and c) anti-LDL antibodies oxidised with malonyl-dialdehyde of IgG and IgM (using the ELISA test described in previous studies).[32]

Quality of Life Study

Quality of Life was assessed before supplementation and at the end of this by means of the St George respiratory questionnaire. Validated by our group in adults with CF.[33]

Statistical Study

Data analysis was carried out using SPPS for Windows, version 12 (SPSS Inc., Chicago, Illinois). Results are expressed as averages and standard deviation. The Shapiro-Wilks test was used to examine the normal distribution of quantitative variables. The comparisons between paired variables (at baseline and at 12 months) were

performed using the t-test or the Wilcoxon test, depending on normality, and the chi square test was used for qualitative variables. For comparison between groups (CF vs. control) the t-test or the Mann-Whitney test were used, depending on normality, for quantitative values and the chi square test was used for qualitative variables. The ANOVA test for repeated measurements was used to assess the effects of supplementation with a mixture of EFA on the FA of serum phospholipids, spirometric parameters and inflammation and oxidation markers (comparing with baseline values). Only the data of the 17 patients that completed the study were analysed. It was considered that there were statistically significant differences with a two-tailed $p < .05$.

Results

During screening 38 patients with CF were assessed. Of these, 19 did not comply with all the inclusion criteria or presented exclusion criteria. Of the 19 remaining patients, 2 abandoned the study during the first week of treatment due to the appearance of side effects (belching, epigastralgias and gastroesophageal reflux). The characteristics of the 17 patients that completed the study can be seen in Table 1. At the beginning of supplementation, all patients presented normal levels of vitamin A (retinol) and vitamin E (tocopherol). Only one patient had a level of 25 hydroxyvitamin D (25-OH-D) below 20ng/ml. Twelve patients had carbohydrate metabolism alterations. Three patients had diabetes related to CF, 8 had carbohydrate intolerance and one had altered basal glycaemia. The patients with CF had the following combinations of genotypes: ΔF508/ΔF508 (n = 6), G542X/G542X (n = 1), ΔF508/Q890X (n = 1), ΔF508/R1066C (n = 1), R334W/R334W (n = 1), G551D/712-IG>T1890 (n = 1), ΔF508/G85E (n = 1), ΔF508/R709X (n = 1), R334W/621+1G->T (n = 1), N1303k/V232D (n = 1), N1303K/F508del (n = 1) and N1303K/N1303k (n = 1).

Pulmonary Variables

During the year of supplementation the total number of exacerbations seen was significantly reduced, as also the number of courses and days of antibiotic administration, in comparison with the previous year. Nine patients had some severe exacerbation during the observation year (53%) in comparison with only 5 (23%) during the supplementation year ($p < .05$; chi square) (Table 2).

Bahlla and NIF scores did not change (Table 2). With relation to baseline, there was a significant increase of FEV_1 (ml), as also FVC (ml) and FVC (%) at the end of the year of supplementation. FEV_1 (%) also improved, although it did not attain statistical significance. When we compared registered data of spirometries of previous years, there were no significant differences between FEV_1 (ml and %) and FVC (ml and %) at the end of the year of supplementation, and values registered 24 months before the beginning of supplementation with EFAs (year -2). However, both FEV_1 (ml and %) and FVC (ml and %) at baseline were significantly less than the values registered 24 months before (Table 3).

Nutritional Evaluation

After the year of supplementation statistically significant differences were found in lean body mass (kg), dynamometry and arm muscle circumference (Table 4). Although total calories in the diet were slightly lowered, no significant differences were seen in the percentage, in relation to total caloric content, of carbohydrates, lipids, proteins or intake of monounsaturated, saturated or polyunsaturated FAs (total, n-3 and n-6) (basal vs. 12 months).

Laboratory Values

No significant differences were found in these patients between baseline values and values after one year of treatment in basal levels of lipids, glucose, insulin and basal C peptide and after glucose overload in glycosylated Hb, somatomedin C,

Table 1
Clinical Characteristics

	n = 17	
	Mean	SD
Age (years)	26.4	10.6
Age at diagnosis	8.5	3.4
Males, n (%)	8 (47)	
Females, n (%)	9 (53)	
Genetics, %		
ΔF508/ΔF508	35%	
ΔF508/no ΔF508	35%	
no ΔF508/no ΔF508	30%	
Predominant disorder at diagnosis:		
Digestive, n (%)	7 (41)	
Respiratory, n (%)	8 (47)	
Heat Stroke, n (%)	1 (6)	
Failure to thrive, n (%)	1 (6)	
Pancreatic failure, n (%)	13 (77)	
Alterations of carbohydrate metabolism, n (%)	12 (70)	
First colonisation by pathogens, n (%)		
Pseudomonas	14 (82)	
Staphylococcus aureus	10 (59)	
Haemophilus influenzae	14 (82)	
Treatment with aerosol antibiotics, n (%)		
Tobramycin:	10 (59)	
Sodium colistimethate	6 (35)	
None	1 (6)	

SD: standard deviation.

Table 2
Lung Parameters (Clinical and Radiological) and Quality of Life

a) Evolution of Severity Scores (radiological and clinical-radiological) and Quality of Life Scores		
	Baseline	After 12 months of supplementation
NIH Score	75 ± 12	79 ± 11
BAHLLA Score (TACAR)	15 ± 2	15 ± 2
Quality of Life (St. George Test)		
Symptoms	35 ± 22	32 ± 19
Activity	27 ± 28	25 ± 25
Impact	17 ± 14	17 ± 16
Total	23 ± 18	22 ± 17
b) Evolution of Annual Exacerbations and Days of Antibiotic Treatment		
	During the observation period	During the 12 months of supplementation
Number of annual exacerbations		
Number of total annual exacerbations	3.1 ± 2.2	2.2 ± 1.4*
Number of severe annual exacerbations	0.7 ± 0.8	0.4 ± 0.6
Days of antibiotic treatment		
Days of oral treatment/year	39 ± 25	27 ± 21
Days of i.v. treatment/year	8 ± 12	4 ± 8
Total days (oral and i.v.)/year	47 ± 33	32 ± 22*

BAHLLA (TACAR): Bhalla score system (based on high resolution chest computed tomography): i.v.: intravenous; NIH: National Institutes of Health.
Data are expressed as mean ± standard deviation.
Wilcoxon or Student's test.
* $p < .05$; values are expressed as mean ± standard deviation.

Table 3
Evolution of Spirometry Parameters

	Year -2	Year -1	Baseline	6 months	Year 1	(p)[a]
FEV_1 (ml)	2,223 ± 981[b]	2,161 ± 1,037[b]	1,960 ± 913	2,014 ± 899	2,127 ± 1,065[b,*]	.02*
FEV_1 (%)	65 ± 20[b]	62 ± 21	57 ± 18	58 ± 18	59 ± 18	NS
FVC (ml)	3,116 ± 1,086[b]	3,071 ± 1,165	2,883 ± 1,144	3,130 ± 1,108[b]	3,037 ± 1,192**	.03**
FVC (%)	72.76 ± 13.79[b]	70.35 ± 14.88	67 ± 15	73 ± 14[b]	71 ± 15**	.03**

FEV_1: forced expiratory volume in one second (expressed in ml and in percentage with respect to the aforesaid [%]); FVC: forced volume capacity; NS: not significant.
Values are expressed as mean ± standard deviation.
* Linear model.
** Quadratic model.
[a] (p): ANOVA for repeated measurements, including: Baseline and at 6 and at 12 months from beginning of supplementation.
[b] $p < .05$ vs. baseline.

Table 4
Anthropometric and Nutritional Parameters

	Baseline		12 months	
	Mean	SD	Mean	SD
Anthropometry				
Weight, kg	56	7	57	9
Ideal weight %	89	11	91	12
BMI, kg/m^2	21.3	3	21.5	3
Lean mass, kg	43.5	8	44.7	8**
Lean mass, kg	12.8	5.1	12.7	5
Fat mass, kg	22	9	21	9
Dynamometry, kg	18	7	21	11*
Arm muscle circumference, cm	20	3	21	3**
Arm muscle circumference, percentile	37	25	54	26**
Nutritional parameters				
High energy content intake[a], % of estimated requirements	111	5	102	28
Fat absorption, %	90	6	91	6

SD: standard deviation; BMI: body mass index.
Wilcoxon test for paired data or Student's t test.
* $p < .05$.
** $p < .01$.
[a] The equation of the Cystic Fibrosis Foundation was used to determine the estimated high energy intake.

albumin, fat and nitrogen in faeces, percentage of fat absorption, liposoluble vitamins (A, D and E9 or total Ig (IgA, IgG, IgM and IgE). Baseline percentages of total palmitoleic, stearic, oleic and monounsaturated FA were significantly decreased in subjects with CF, in comparison with healthy controls. These differences disappeared after the year of diet supplementation, with the exception of stearic acid values. With reference to baseline there were significant changes in patient's fatty acid composition of serum phospholipids (FASP), with a linear increase in DHA, AG-n-3 and LIN levels (quadratic model), and with a linear decrease in oleic acid and monounsaturated FA levels and the arachidonic/DHA ratio, and following a quadratic model, palmitoleic and arachidonic acid (AA) (Table 5). At baseline, statistically significant differences were found in TNF-α and anti-LDL typo IgM antibodies (higher) and soluble receptor 60 (lower) in comparison with the control group, these differences disappeared after the year of supplementation. In the CF group (baseline vs. 12 months) a linear and significant decrease was seen in TNF-α levels and an increase (following a quadratic model) of TNF-α soluble receptors to 60 and 80. Anti-LDL oxidised typo IgM antibodies decreased linearly, and this decrease was of statistical significance, whereas Ig G type antibodies decreased, but this decrease did not achieve statistical significance (except at 6 months when these values were significant with reference to baseline values) (Table 6).

Quality of Life

A trend towards a decrease was seen in the St. George questionnaire score (improvement of quality of life) after supplementation, but this did not achieve statistical significance (Table 2).

Discussion

In this study we have observed an improvement in respiratory parameters (spirometry, decrease of exacerbations, and courses of antibiotics), nutritional parameters (increase of lean body mass and dynamometry) and of inflammation and oxidation markers, achieved with supplementation with a combination of low doses of n-3-FAs and n-6-FAs over 12 months. These changes took place at the same time as the serum phospholipid pattern of our adult patients with CF became normalised, moreover, the changes in phospholipid composition were detectable a few months after beginning supplementation. These data coincide with those of other studies carried out on healthy populations or patients with CF, in which there was seen to be an incorporation of FAs in the short term (even during the first month), both into cell membranes and into plasma phospholipids, after supplementation with low to medium doses of n-3-FAs[4,9,18,34] and in other studies with patients with CF that used higher doses.[10-12,16,17,19]

The increase of LIN and of DHA and the reduction of serum levels of AA (with an associated decrease of the AA/DHA ratio), all proposed as markers of disease severity in CF,[3,7] could explain the improvement in inflammatory, clinical and spirometric parameters seen in our study. The decrease of proinflammatory cytokines could reduce the amount of mucus, chaemotaxis and neutrophil activation and the inflammatory, vaso- and bronchoconstrictor[35] responses. N-3-FAs, that compete for the same elongases and desaturases as the n-6 series, favour the release of less proinflammatory eicosanoids.[1] Also inhibition of AA release by phospholipase A2 (that seems to be increased in patients with CF) could reduce the inflammatory response and accelerated replacement of n-6 series FAs seen in these patients.[35] Furthermore, the decrease of AA levels in serum and, possibly, in airways, could increase nitric oxide in the airways, which has low or decreased levels in CF and has antimicrobial properties.[36] Finally, the release of resolvins, docosatriennes and neuroprotectins that participate in the resolution phase of inflammation, which increases in patients with CF, could increase.

Concomitant administration of gamma linolenic acid (GLA) in our study could have acted synergistically (together with n-3 FA) by elevating the dihomo-gammalinolenic acids that would also compete with AA and would favour the release of less proinflammatory eicosanoids.[1] Our results with relation to lung improvement (FEV_1 in ml and FVC in ml and %, with fewer exacerbations and a decrease in antibiotic courses) had similar results to those of the studies published by De Vizia et al,[10] who used higher doses of EPA and DHA

Table 5
Evolution of the Fatty Acid Profile in Serum Phospholipids

Fatty acid composition of serum phospholipids, %	Controls		Cases								(p)
			Baseline		3 months		6 months		12 months		
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	
Myristic acid (14:0)	0.4	0.4	0.7	0.5	0.3	0.1[a]	0.3	0.1[a]	0.4	0.4	NS
Palmitic acid (16:0)	33.7	8.2	31.6	2.7	32.6	2.1	33.2	1.9	32.3	2.5	NS
Palmitoleic acid (16:1)	0.6	0,4[c]	1.4	1.4	0.7	0.3	0.7	0.2	0.7	0.4	0.05*
Stearic acid (18:0)	14.1	2.1[c,d]	17.4	2.4	17.1	1.6	17.1	1	17	1.6	NS
Oleic acid (18:1)	11.4	2,8[c]	13.5	1.9	12.3	1.8[b]	12.2	1.8[a]	11.7	2,0[b]	0.001*
LIN (18:2)	25.1	3.5[c]	21.1	3.3	23.3	3,4[b]	22.4	3.6	22.8	3.9	0.021**
AA (20:4)	9.8	2.5	10.5	2.1	9.2	1.9	9.2	1.7[a]	9.9	1.5	0.010**
EPA (20:5)	0.7	0.8	0.9	0.6	1.2	0.4[a]	1.2	0.4	1.2	0.7	NS
DHA (22:6)	4.1	1.7[c]	2.9	1.2	3.1	1.1	3.4	1.5	3.8	1.1[a]	0.012*
Saturated fatty acids	48.3	7.9	49.7	4.4	50	2.3	50.7	2.1	49.7	2.7	NS
Monounsaturated fatty acids	11.9	2,9[c]	15	3	13.0	1.5[a]	12.9	1,8[b]	12.4	2.1[b]	0.02*
Polyunsaturated fatty acids	39.7	6,1[c]	35.4	3.1	36.9	2.7	36.3	2.4	37.8	3.3[a]	NS
Omega-3-fatty acid	4.8	2.1	3.8	1.6	4.3	1.4	4.6	1.5	5.0	1.8[a]	0.014*
Omega-6-fatty acid	34.9	5,0[c]	31.6	2.8	32.5	2.6	31.6	2.6	32.7	3.4	NS
AA/DHA	2.7	1,2[c]	4.2	2.0	3.2	0.9	2.9	0.9[a]	2.8	0,8[b]	0.011*
Ole/lino	0.4	0.1[c]	0.7	0.1	0.5	0.1[b]	0.5	0.1[a]	0.5	0.2[b]	0.005*

AA: arachidonic acid; FA: fatty acid; FASP: fatty acid composition of serum phospholipids; SD: standard deviation; DHA: docosahexaenoic acid; EPA: eicosapentaenoic acid; LIN: linoleic acid; NS: not significant; Ole/lino: oleic acid/linoleic acid ratio; (p): ANOVA for repeated measurements.

*Linear model.

**Quadratic model.

Compared to baseline:

[a] $p < .05$.

[b] $p < .01$.

Mann Whitney or t-test: Cases vs. Controls at Baseline:

[c] $p < .05$.

Cases vs. controls at 12 months of supplementation:

[d] $p < .05$.

Table 6
Evolution of Inflammation and Oxidation Markers

	Controls		Cases								(p)
			Baseline		3 months		6 months		12 months		
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	
TNF-α, pg/ml	3.1	7.2[c]	14.1	12.6	6.7	5.2[a]	6.6	6.7[a]	5.8	6.9[a]	.003*
TNF-60, ng/ml	4.3	5.5[c]	1.3	0.6[d]	1.1	0.2	1.7	1.1	2.8	1.2[b]	.006**
TNF-80, ng/ml	7.2	5.5[e]	6.0	2.8	5.4	2.2	7.2	3.4	11.5	5.7[b]	.004**
Oxidised anti-LDL antibodies (udo) IgG	0.12	0.04	0.12	0.05	0.11	0.05	0.10	0.03[a]	0.10	0.03	NS
Oxidised anti-LDL antibodies (udo) IgM	0.10	0.03[c]	0.14	0.04	0.14	0.04	0.13	0.04	0.12	0.03	.014*

SD: standard deviation; Ig: immunoglobulin; LDL: low density lipoprotein; NS: not significant; (p): ANOVA for repeated measurements, TNF-α: *tumour necrosis factor* α; TNF-60 and TNF-80: *TNF-α soluble receptors*.

*Linear model.

**Quadratic model; compared to baseline:

[a] $p < .05$.

[b] $p < .01$.

Mann Whitney or t-test: Cases vs. Controls at Baseline:

[c] $p < .05$.

Cases vs. controls at 12 months of supplementation:

[d] $p < .05$.

[e] $p < .01$.

for 8 months. Lawerence[13] also supplemented with EPA for a short period, and found a decrease in the volume of sputum and an improvement of FEV_1 and the Shwachman score. These results would strengthen our observations and those of other groups[3,7] that related LIN levels with FEV_1.

In contrast, other studies did not find an effect on lung function, maybe because they were of shorter duration (4-24 weeks)[11,17-19] or because of the small numbers of patients assessed.[4,15] As well as improvement in lung parameters we observed a discrete weight increase, but at the expense of the lean body mass, with an increase in arm circumference, arm muscle circumference and hand grip dynamometry. Similarly to other studies published,[37, 38] we also saw that TNF-α levels were increased at baseline in stable patients in comparison with the control group. These differences disappeared after the year of supplementation. Both in patients with CF and COPD and bronchiectasia without CF, elevated plasma levels of TNF-α have been found (even in patients that were clinically stable) associated with a reduction of lean body mass, an increase of muscle proteolysis, an increase of respiratory exacerbations and to worse phenotypes with worse pulmonary function.[37-39] Reduction of inflammatory markers (TNF-α) and an increase in soluble receptors (that act as counter-regulating factors) seen in our study after supplementation may have increased the improvement of clinical parameters (fewer and less severe exacerbations and an increase in lean body mass) and spirometry. On the other hand, elevated levels of TNF-α do not seem to promote anorexia,[35] as was corroborated by the fact that patients achieved theoretical calorie requirements (according to the formula of the Cystic Fibrosis Foundation) and that diet intake was not modified,

and even decreased slightly after the year of supplementation in spite of observing a continuous and significant decrease in TNF-α levels from the 3rd month until the end of supplementation. In most of the studies that carried out supplementation with n-3-FAs in CF no significant modifications were found in children's weight or growth rate[11,16,18], possibly due to the short duration of these studies.

De Vizia observed a significant improvement in weight after the first 4 months, but this was not maintained at the end of the study.[10] Lepage et al did observe a significant weighted increase in the group supplemented with biscuits enriched with n-3-FA.[2] In contrast, Durieu et al, in a study in which they administered n-3-FAs by the intravenous route during 3 months, observed a significant weighted decrease in the 7 adults studied, but not in the 6 children assessed.[19]

Oxidative stress is increased in patients with CF, in spite of a normal intake of antioxidants in the diet; possibly the immune response could play an important role in its development.[40] A state of oxidative stress could favour an increase in antibodies to oxidised anti-LDL and could be involved in FA imbalances. In our study we have seen an increase in oxidised anti-LDL antibodies in patients in comparison with controls (IgM) that, after supplementation, decreased (both IgG and IgM). In the context of a decrease of other inflammatory variables and clinical improvement, the measurement of these antibodies could be useful as an oxidation-inflammation marker in these patients. A trend towards a decrease was seen in the St. George questionnaire score (improvement of quality of life) after supplementation, but this did not achieve statistical significance, for the items: "symptoms" (that includes aspects such as presence of dyspnoea, coughing, expectoration and the frequency of these), "activity" (where limitation of activity due to dyspnoea is assessed) and in total score. Maybe if the sample were larger, we could have seen more evident changes. Although no changes were made in treatment strategies during the study, as it was a non-controlled study, it is impossible to rule out the possibility that the slight improvement observed in quality of life could be due to other interventions affecting the treatment of these patients.

In contrast to other studies with supplementation at higher doses,[12,13] we did not observe significant side effects (diarrhoea, steatorrhoea, or a need for increasing the doses of pancreatic enzymes). However, 2 patients selected for the study abandoned treatment in the first week due to digestive intolerance (belching and regurgitation with a taste of fish). Maybe these effects could be minimised by the administration of other pharmaceutical presentations.

This study is not free of limitations: First, as it was not randomised or controlled with placebo, we cannot rule out a bias in favour of treatment. However, the global improvement of the variables assessed (clinical, analytical and spirometry) that would have foreseeably worsened (if the disease continued its normal course), support the results and make this effect improbable. Second, as the study was carried out in a single centre, and strict inclusion criteria were applied, a relatively small sample of patients was selected that, possibly, comply with treatment to a greater degree than an average population of patients. Furthermore, although the precision of the sample was sufficient for most of the relevant variables studied, the possibility of a type 1 error cannot be ruled out for some of the conclusions. Third, we have not measured FA composition in cell membranes in tissues; however, the measurement of FA in serum phospholipids seems to adequately reflect tissue levels, also, it serves as a marker of intake and treatment compliance.[3,34] Fourth, with this study design it is not possible to determine what components of the FA mix cause the effects seen, or which are the most appropriate doses. However, this study does have its strengths, such as a control group of similar characteristics to compare fatty acid composition of serum phospholipids and oxidation and inflammation markers, the long follow-up time and the simultaneous study of multiple effects (lung function tests, clinical, radiological, biochemical, and nutritional scores, number of exacerbations, duration of antibiotic courses, quality of life and levels of inflammation and oxidation mediators).

In summary, supplementation with a low dose combination of n-3-FAs and n-6-FAs maintained over 12 months makes it possible to normalise the pattern of fatty acid composition of serum phospholipids and improve clinical parameters (reduction of exacerbations and courses of antibiotics), nutritional (lean body mass and dynamometry), spirometry, and inflammation and oxidation markers. These results open the door to further multicentre, controlled, randomised studies with a sufficient number of patients to make it possible to obtain evidence based conclusions.[41]

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References

1. Christophe A, Robberecht E. Directed modification instead of normalization of fatty acid patterns in cystic fibrosis: an emerging concept. Curr Opin Clin Nutr Metab Care. 2001;4:111-13.
2. Lepage G, Yesair DW, Ronco N, Champagne J, Bureau N, Chemtob S, et al. Effect of an organized lipid matrix on lipid absorption and clinical outcomes in patients with cystic fibrosis. J Pediatr. 2002;141:178-85.
3. Olveira G, Dorado A, Olveira C, Padilla A, Rojo-Martínez G, García-Escobar E, et al. Serum phospholipid fatty acid profile and dietary intake in an adult Mediterranean population with cystic fibrosis. Br J Nutr. 2006;96:343-9.
4. Van Biervliet S, Devos M, Delhaye T, Van Biervliet JP, Robberecht E, Christophe A. Oral DHA supplementation in DeltaF508 homozygous cystic fibrosis patients. Prostaglandins Leukot Essent Fatty Acids. 2008;78:109-15.
5. Freedman SD, Katz MH, Parker EM, Laposata M, Urman MY, Alvarez JG. A membrane lipid imbalance plays a role in the phenotypic expression of cystic fibrosis in cftr (-/-) mice. Proc Natl Acad Sci USA. 1999;96:13995-4000.
6. Freedman SD, Blanco PG, Zaman MM, Shea JC, Ollero M, Hopper IK, et al. Association of cystic fibrosis with abnormalities in fatty acid metabolism. N Engl J Med. 2004;350:560-569.
7. Strandvik B, Gronowitz E, Enlund F, Martinsson T, Wahlström J. Essential fatty acid deficiency in relation to genotype in patients with cystic fibrosis. J Pediatr. 2001;138:650-55.
8. Christophe A, Robberecht E, Franckx H, De Baets F, van de Pas M. Effect of administration of gamma-linolenic acid on the fatty acid composition of serum phospholipids and cholesteryl esters in patients with cystic fibrosis. Ann Nutr Metab. 1994;38:40-7.
9. Christophe A, Robberecht E, De Baets F, Franckx H. Increase of long chain omega-3 fatty acids in the major serum lipid classes of patients with cystic fibrosis. Ann Nutr Metab 1992;36:304-12.
10. De Vizia B, Raia V, Spano C, Pavlidis C, Coruzzo A, Alessio M. Effect of an 8-month treatment with omega-3 fatty acids (eicosapentaenoic and docosahexaenoic) in patients with cystic fibrosis. J Parenter Enteral Nutr. 2003;27:52-7.
11. Katz DP, Manner T, Furst P, Askanazi J. The use of an intravenous fish oil emulsion enriched with omega-3 fatty acids in patients with cystic fibrosis. Nutrition. 1996;12:334-39.
12. Henderson WR, Astley SJ, McCready MM, Kushmerick P, Casey S, Becker JW, et al. Oral absorption of omega-3 fatty acids in patients with cystic fibrosis who have pancreatic insuficiency and in healthy control subjects. J Pediatr. 1994;124:400-8.
13. Lawrence RH, Sorrell TC. Eicosapentaenoic acid modulates neutrophil leukotriene B4 receptor expression in cystic fibrosis. Clin Exp Immunol. 1994;98:12-6.
14. Kurlandsky LE, Bennink MR, Webb PM, Ulrich PJ, Baer LJ. The absorption and effect of dietary supplementation with omega-3 fatty acids on serum leukotriene B4 in patients with cystic fibrosis. Pediatr Pulmonol. 1994;18:211-17.
15. Thies NH. The effect of 12 months' treatment with eicosapentaenoic acid in five children with cystic fibrosis. J Paediatr Child Health. 1997;33:349-51.
16. Lloyd-Still JD, Powers CA, Hoffman DR, Boyd-Trull K, Lester LA, Benisek DC, et al. Bioavailability and safety of a high dose of docosahexaenoic acid triacylglycerol of algal origin in cystic fibrosis patients: a randomized, controlled study. Nutrition. 2006;22:36-46.
17. Jumpsen JA, Brown NE, Thomson AB, Paul Man SF, Goh YK, Ma D, et al. Fatty acids in blood and intestine following docosahexaenoic acid supplementation in adults with cystic fibrosis. J Cyst Fibros. 2006;5:77-84.

18. Panchaud A, Sauty A, Kernen Y, Decosterd LA, Buclin T, Boulat O, et al. Biological effects of a dietary omega-3 polyunsaturated fatty acids supplementation in cystic fibrosis patients: a randomized, crossover placebo controlled trial. Clin Nutr. 2006;25:418-27.
19. Durieu I, Vericel E, Guichardant D, Roth H, Steghens JP, Drai J, et al. Fatty acids platelets and oxidative markers following intravenous n-3 fatty acids administration in cystic fibrosis: An open pilot observational study. J Cyst Fibros. 2007;6:320-6.
20. Lloyd-Still JD, Jonson SB, Holman RT. Essential fatty acid status and fluidity of plasma phospholipids in cystic fibrosis infants Am J Clin Nutr. 1991;54:1029-35.
21. Kerem E, Conway S, Elborn S, Heijerman H, Consensus Committee. Standards of care for patients with cystic fibrosis: a European consensus. J Cystic Fibros. 2005;4:7-26.
22. Soriguer F, Morcillo S, Cardona F, Rojo-Martínez G, de la Cruz Almaráz M, Ruiz de Adana M, Olveira G, et al. Pro12Ala polymorphism of the PPARG2 gene is associated with type 2 diabetes mellitus and peripheral insulin sensitivity in a population with a high intake of oleic acid. J Nutr. 2006;136:2325-30.
23. Roca J, Sanchis J, Agustí Vidal A, Segarra F, Navajas D, Rodriguez-Roisin R, et al. Spirometric reference values for a Mediterranean population. Bull Eur Physiopathol Respir. 1986;18:101-2.
24. Sockrider MM, Swank PR, Seilheimer DK, Schidlow DW. Measuring clinical status in cystic fibrosis: internal validity and reliability of a modified NIH score. Pediatr Pulmonol. 1994;17:86-96.
25. Bhalla M, Turcios N, Aponte V, Jenkins M, Leitman BS, McCauley DI, et al. Cystic Fibrosis: Scoring System with thin-section CT. Radiology. 1991;179:783-88.
26. Vendrell M, De Gracia J, Olveira C, Martinez MA, Girón R, Máiz L, et al. Normativa SEPAR: diagnóstico y tratamiento de las bronquiectasias. Arch Bronconeumol. 2008;44:629-40.
27. Alastrué A, Sitges A, Jaurrieta M, Puig P, Abad JM, Sitges-Creus A. Valoración antropométrica del estado de nutrición: normas y criterios de desnutrición y obesidad. Med Clín (Barc). 1983;80:691-99.
28. Durnin JV, Womersley J. Body fat assessed from total body density and its estimation from skinfold thickness: measurements on 481 men and women aged from 16 to 72 years. Br J Nutr 1974;32:77-97.
29. Siri WE. Body composition from fluid spaces and density: analysis of methods. En: Brozeck J y Henschel A, editores. Techniques for measuring body composition. Washington DC: National Academy of Sciences; 1961. p.223-44.
30. Olveira G, Olveira C, Casado-Miranda E, Padilla A, Dorado A, Rojo G, et al. Markers for the validation of reported dietary intake in adults with cystic fibrosis. J Am Diet Assoc. 2009;109:1704-11.
31. Ramsey BW, Farrel PM, Pencharz P. Nutritional assessment and management in cystic fibrosis: a consensus report. The Consensus Committee. Am J Clin Nutr. 1992;55;108-6.
32. Tinahones FJ, Gomez-Zumaquero J M, Garrido-Sanchez L, García-Fuentes E, Rojo-Martínez G, Esteva I, et al. Influence of age and sex on levels of anti-oxidized LDL antibodies and anti-LDL immune complexes in the general population. J Lipid Res. 2005;46:452-7.
33. Padilla A, Olveira G, Olveira C, Dorado A, Plata AJ, Gaspar I, et al. Validity and reliability of the St George's Respiratory Questionnaire in adults with cystic fibrosis. Arch Bronconeumol. 2007;43:205-11.
34. Harris WS, Pottala JV, Sands SA, Jones PG. Comparison of the effects of fish and fish-oil capsules on the n 3 fatty acid content of blood cells and plasma phospholipids. Am J Clin Nutr. 2007;86:1621-5.
35. Van Biervliet S, Van Biervliet JP, Robberecht E, Christophe A. Docosahexaenoic acid trials in cystic fibrosis: a review of the rationale behind the clinical trials. J Cyst Fibros. 2005;4:27-34.
36. Keen C, Olin AC, Edentoft A, Strandvik B. Airway nitric oxide in patients with cystic fibrosis is associated with pancreatic function, Pseudomonas infection, and polyunsaturated fatty acids. Chest. 2007;131:1857-64.
37. Schmitt-Grohé S, Hippe V, Igel M, Von Bergmann K, Posselt HG, Krahl A, et al. Serum leptin and cytokines in whole blood in relation to clinical and nutritional status in cystic fibrosis. J Pediatr Gastroenterol Nutr. 2006;43:228-33.
38. Ionescu AA, Nixon LS, Luzio S, Lewis-Jenkins V, Evans WD, Stone MD, et al. Pulmonary function, body composition, and protein catabolism in adults with cystic fibrosis. Am J Respir Crit Care Med. 2002;165:495-500.
39. Martínez-García MA, Perpiñá-Tordera M, Román-Sánchez P, Soler-Cataluña JJ, Carratalá A, Yago M, et al. The association between bronchiectasis, sistemic inflammation and Tumor Necrosis Factor alpha. Arch Bronconeumol. 2008;44:8-14.
40. Wood LG, Fitgerald DA, Gibson PG, Cooper DM, Collins CE, Garg ML. Oxidative stress in cystic fibrosis: dietary and metabolic factors. J Am Coll Nutr. 2001;20:157-65.
41. McKarney C, Everard M, N'Diaye T. Omega-3 fatty acids (from fish oils) for cystic fibrosis. Cochrane Database of Systematic Reviews 2007 Issue 4, Art. No.: CD002201. DOI: 10.1002/14651858.CD002201.pub2.