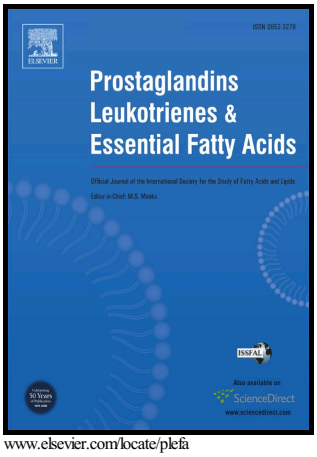

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The clinical benefits of long-term supplementation with omega-3 fatty acids in cystic fibrosis patients - a pilot study

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Abstract

Effectiveness of omega-3 supplementation in cystic fibrosis (CF) remains controversial. This study sought to evaluate clinical status, exercise tolerance, inflammatory parameters, and erythrocyte fatty acid profile after 1 year of oral omega-3 supplementation in CF patients. Fifteen ΔF508-homozygous patients undergoing chronic azithromycin were randomized to receive omega-3 fish oil supplementation at a dose of 60mg/Kg/day or placebo. In

comparison with the previous year, in the supplemented group, the number of pulmonary exacerbations decreased at 12 months (1.7 vs. 3.0, p <0.01), as did the duration of antibiotic therapy (26.5 days vs. 60.0 days, p <0.025). Supplementation significantly increased the levels of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) as early as <3 months of administration, with concomitant decreases in arachidonic acid (AA) levels. This pilot study suggests that long-term omega-3 supplementation offers several clinical benefits as to the number of exacerbations and duration of antibiotic therapy in CF patients.

1. Introduction

Cystic fibrosis (CF) has been associated with disturbances in lipid metabolism. Increased release of arachidonic acid (AA) from cell membrane phospholipids and essential fatty acid (EFA) deficiency, including low concentrations of linoleic acid (LA) and docosahexaenoic acid (DHA), have also been documented [1-3]. Due to its strong association with pancreatic insufficiency [4-5], EFA deficiency has been presumed to be secondary to fat malabsorption. As this latter deficiency was also reported in pancreatic-sufficient patients, Peretti et al. described the involvement of other mechanisms, such as the excessive oxidation of EFA as an energy source, increased production of eicosanoids linked to inflammatory responses, higher turnover rates of lipids in cell membranes, defective incorporation in the plasma membrane, and down-regulation of desaturase and lipid peroxidation activities [6-8]. Ceramide deficiency has also been associated with EFA alterations [1,9]. EFA deficiency is more marked in severe CF genotypes, suggesting an association between EFA metabolism disturbances and the basic CF defect [3,10,11]. Another concern is the role plaid by arachidonic acid-derived mediators in the pulmonary inflammatory response of CF patients. Pulmonary inflammation is responsible for the progressive lung destruction observed in CF, and polymorphonuclear neutrophils exert a major influence [12]. Leukotriene (LT) B4, an

eicosanoid derived from AA metabolism in neutrophils and alveolar macrophages, has been implicated in the enhanced lung inflammatory response seen in CF cases [3,13-15].

Supplementing omega-3 polyunsaturated fatty acids (ω-3 PUFA) could serve to down-regulate the production of inflammatory mediators and thus improve clinical outcomes [16-18]. Both eicosapentaenoic acid (EPA) and DHA can, in fact, competitively inhibit proinflammatory mediator formation derived from AA, thereby reducing immune cell activities. On the other hand, LTB5, a 5-lipoxygenase product of EPA, displays little chemotactic effect, compared to LTB4. Moreover, EPA and DHA give rise to anti-inflammatory and inflammation-resolving mediators, called resolvins, protectins, and maresins [19]. Consequently, ω-3 PUFA supplementation in CF patients may reduce inflammation and provide certain clinical benefits [14,18,20-22]. Nevertheless, its effectiveness remains controversial and it is not yet recommended for routine use [20,23].

This study was a pilot trial, seeking to evaluate clinical status, lung function, exercise tolerance, inflammatory parameters, and erythrocyte fatty acid profile after 1 year of oral ω-3 PUFA supplementation in CF patients, as well as the safety of the proposed supplementation scheme.

2. Patients and methods

2.1. Study design

This study used a randomized, double-blind, placebo-controlled clinical trial design. After informed consent, each patient was randomly assigned to either ω-3 PUFA supplementation or placebo (ratio 1:1). The randomization was centralized by the pharmacist according to a pre-defined blocked list, stratified by patient's weight at the time of inclusion. The compliance was assessed at each visit using diary, pill counts and information provided by the caregiver or the patient himself. Clinical and lung function parameters were evaluated for each patient at baseline and every 3 months for a total duration of 12 months. Clinical status

comprised the cumulative number of pulmonary exacerbations (PEs) and the duration of antibiotic therapy, measured in days at each visit and during the last 12 months preceding supplementation initiation. PE was defined as an acute episode requiring antibiotic therapy by any means of administration during the study period [24]. Nutritional status was expressed as the body mass index z-score (BMI z-score), adjusted for age and gender according to the Centers for Disease Control and Prevention (CDC) guidelines [25]. Lung function tests were performed according to the American Thoracic Society (ATS) and European Respiratory Society (ERS) guidelines [26]. Forced vital capacity (FVC), forced expiratory volume in one second (FEV_1), and forced expiratory flow 25-75% (FEF_{25-75}) were expressed as percentages of the normal predicted values for age, gender, and height. Exercise tolerance was evaluated by measuring the maximal oxygen uptake (VO_2 max) before and at the end of the study using standardized techniques [27], involving an electronically-braked cycle ergometer. The initial exercise workload was between 10 and 20 Watts (W), incrementally increased by 10-20 W/minute at the discretion of the operator according to disease severity. The same conditions were used for each exercise test for each patient. At the baseline visit and 3, 6, and 12 months after supplementation initiation we conducted biochemical blood analyses of erythrocyte membrane EFA profiles, expressed as percentages of total fatty acids (total ω-3, α-linolenic acid [ALA], EPA, DHA, total ω-6, LA, AA, ω-6/ω-3 ratio, and AA/DHA ratio) and inflammatory markers (white blood cell [WBC], neutrophils, C-reactive protein [CRP], erythrocyte sedimentation rate [ESR], and immunoglobulins G [IgG]). EFA profiles were quantified by means of gas chromatography (HP6890; Hewlett-Packard-Agilent) and mass spectrometry (MS 5973; Agilent Technologies) [5]. At baseline and every 6 months thereafter, blood was drawn for safety testing, involving complete blood count, standard liver enzymes (aspartate transaminase, alanine transaminase, γ-glutamyltransferase, total and direct bilirubin) and coagulation analysis (prothrombin and partial thromboplastin time, fibrinogen).

Chest X-ray or computed tomography (CT) scan were performed at the baseline visit in order to exclude any acute pulmonary illness, along with abdominal ultrasound in order to exclude liver abnormalities.

2.2. Study population

We included patients from two CF care units that implement similar standard CF care protocols: Hôpital Universitaire des Enfants Reine Fabiola (HUDERF), Université Libre de Bruxelles and Universitair Ziekenhuis Brussel (UZ Brussel), Vrij Universiteit Brussel, Belgium. All patients included into the study were clinically stable, homozygous for the ΔF508 mutation, over 5 years of age, already undergoing azithromycin treatment for at least 3 months, able to perform lung function tests and swallow capsules, and invited to participate between October 2008 and April 2010. The exclusion criteria were: 1) upper or lower respiratory infection within 2 weeks before baseline evaluation or abnormalities on chest X-ray or CT scan; 2) undergoing any chronic (>1 week daily) oral or intravenous anti-inflammatory treatment other than azithromycin within 3 months before study initiation; 3) active bleeding or increased risk of bleeding; 4) coagulation alterations and/or platelets < 50.000 /mm^3; 5) diabetes; 6) FEV_1 < 40%; 7) significant liver disease, defined as elevated liver function values 2-fold higher than the upper normal range or abnormal ultrasound (Williams score > 5 [28]) ; 8) hypercholesterolemia (>240mg%); 9) participating in another study; 10) pregnancy. Sixteen patients agreed to participate but one patient was excluded on account of incident diabetes revealed at the baseline visit. The major objections to not participating in the study were the study's duration, the daily intake of capsules, the possible placebo intake, or the participation in another study.

2.3. Supplementation: ω-3 PUFA versus placebo

Omega-3 PUFA supplementation was administered over 12 months, with Omega 3 Premium® capsules (Laboratoires Ponroy, Boufféré, France) used. Each capsule contained

300mg of ω-3 triglycerides from fish oil, providing 100mg of DHA and 150mg of EPA. The total daily ω-3 dose corresponded to 60mg/Kg body weight. The daily number of capsules ranged from 3 to 9 capsules, depending on the patient's weight. The supplementation was taken at mealtimes along with pancreatic enzymes increased by at least 10% daily so as to avoid gastro-intestinal disorders. The placebo capsules contained medium-chain triglycerides (Laboratoires Ponroy, Boufféré, France) and looked identical to the active ones.

2.4. Safety

To evaluate the supplementation's safety and tolerance, all adverse events were assessed by means of consultation, physical examination, and data collected in diaries, which were checked at each visit, in addition to laboratory tests to evaluate liver enzymes, platelets and coagulation, along with Williams score at abdominal ultrasound.

2.5. Statistical analysis

The statistical analysis was performed using Analyse-it® 3.90.7 software for Microsoft Excel (Analyse-it Software, Ltd., Leeds, United Kingdom), according to the manufacturer's instructions and on the intention-to-treat principle.

We performed the Mann-Whitney U-test as non-parametric assessment for intergroup comparisons, along with the Wilcoxon test for paired observations of groups in intragroup repeated measures.

Parametric tests were carried out following log transformations in order to stabilize the variances. These included paired t-tests, with one-way analysis of variance for repeated measures (ANOVA), as indicated.

2.6. Ethical Committee

This study was approved by the university hospital's ethical committee (HUDERF) (N°EudraCT: 2006-004155-38) and was registered on the clinicaltrials.gov website (NCT00959010) prior to enrollment of the first patient.

3. Results

3.1. Population

We enrolled 15 patients, who were randomized to receive ω-3 PUFA supplementation (n=7) or placebo (n=8). Their baseline characteristics are shown in Table 1. All patients were suffering from pancreatic insufficiency. No significant difference was observed between the two groups in terms of baseline characteristics. Thirteen patients completed the study and 2 dropped out: one patient assigned to the ω-3 PUFA supplementation out after 1 month due to complaints of fishy breath, and the second one assigned to the placebo after 1 week due to the daily intake of capsules.

3.2. Efficacy

Clinical status

Compared to the placebo group, the cumulative intravenous duration of antibiotic therapy was significantly lower at 12 months (total days of intravenous therapy: 6 vs. 121; total oral days: 172 vs. 402, $p < 0.01$) in the supplemented group. In comparison with the previous year, the cumulative number of pulmonary exacerbations at 12 months was decreased in the supplemented group (1.7 [1-3] vs. 3.0 [1-6], $p < 0.01$), as was the cumulative duration of antibiotic therapy (median: 26.5 days [0-56] vs. 60.0 [0-122], Mann-Whitney: $p < 0.025$) (Fig.1). Compared to the placebo group, the cumulative duration of antibiotic therapy by any way of administration in the supplemented group was decreased at 9 months (median: 24.5

days [0-43] vs. 46.0 [15-92]; Mann-Whitney: $p < 0.02$) and 12 months (median: 26.5 days [0-56] vs. 65.0 [15-113]; Mann-Whitney: $p < 0.025$) (Fig.2).

No difference in BMI was observed between the groups throughout the study, with all patients exhibiting low BMI z-scores at the baseline visit (Table 1) and these scores remaining stable throughout the study.

Lung function and exercise tolerance

Both groups exhibited median FEV_1 values >65% of predicted values. No between-group difference was observed in either lung function parameters (FEV_1%, FVC%, and FEF_{25-75}%) or VO_2 max values at baseline visit. As expected in CF, lung function and VO_2 max decreased slightly over time in both groups, yet with no difference revealed between the two.

Inflammatory parameters

No differences in WBC count, neutrophil count, CRP levels, ESR or IgG levels were observed between the groups at baseline visit. No significant change in inflammatory parameters emerged between the start and end values.

EFA profiles

When analyzing the erythrocyte membranes, we found no between-group difference in the EFA profile, consisting of total ω-3, ALA, EPA, DHA, total ω-6, LA, AA, ω-6/ω-3 ratio, and AA/DHA ratio values, at the start of the study. In the placebo group, the erythrocyte membrane profile remained stable throughout the study length. Compared to the initial values, supplementation increased the erythrocyte levels of total ω-3 by 1.7-fold ($p < 0.01$), EPA by 4.2-fold ($p < 0.025$), and DHA by 1.6-fold ($p < 0.05$) after 3 months, as well as by 1.9-fold ($p < 0.05$), 4.2-fold ($p < 0.02$), and 1.9-fold ($p < 0.05$) after 6 months, respectively, with no further increase observed after 12 months. This increase was accompanied by a progressive decrease in AA by 1.2-fold ($p < 0.05$) after 3 months, by 1.3-fold ($p < 0.01$) after 6 months, and by 3.5-

fold ($p < 0.01$) after 12 months. The AA levels were within normal ranges at baseline, yet then progressively decreased and became significantly lower than normal values at 6 months ($p < 0.01$). The changes in EPA, DHA, and AA/DHA levels observed in the erythrocyte membrane at the different visits are shown in Fig.3.

3.3. Safety

Supplementation was well tolerated. No serious clinical adverse events occurred, with only one patient dropping out after the first month due to complaints of fishy breath. None of the patients suffered from any symptoms like abdominal pain or steatorrhea. There were no differences in the platelets, liver enzymes or standard coagulation tests between the groups, nor were in the Williams scores.

4. Discussion and conclusions

EFA deficiency has commonly been reported to occur in CF [1-3], particularly in patients with severe mutations like the ΔF508 mutation [10,11]. For this reason, our study included only those who were homozygous for the ΔF508 mutation. Given that azithromycin is now a well-validated anti-inflammatory treatment in CF [29], we opted to include solely CF patients undergoing chronic azithromycin treatment. To the best of our knowledge, this was the first randomized, double-blind, placebo-controlled clinical trial involving CF patients homozygous for the ΔF508 mutation treated with azithromycin.

Correcting EFA deficiency could be beneficial to clinical symptoms and lung function in CF patients [14,18,20-22]. However, the effectiveness of ω-3 PUFA supplementation remains controversial, especially due to the small-sized study populations tested so far, yet also due to variability in the type, form, and duration of supplementation evaluated [14,16-18,30-34]. Our study suggests this supplementation capable of offering several clinical benefit in terms of the

cumulative number of exacerbations and duration of antibiotic therapy, factors that have a clear impact on CF patients' quality of live, as well as on the overall cost of care [35]. Only very few studies have to date evaluated the effect of ω-3 PUFA supplementation on the number of exacerbations and duration of antibiotic therapy, with currently-available results being controversial. For example, Alicandro et al. observed no difference in the number of pulmonary exacerbations after 1 year of high-dose DHA supplementation, probably due to their population including young patients aged 6 to 12 years old [36]. Similarly, Van Biervliet et al. also reported there to be no difference in the number of infections after 1 year of DHA algal oil supplementation [30] in their patients. In contrast, De Vizia et al. reported a decreased need for antibiotics after 8 months of EPA-DHA fish oil supplementation compared with the previous 8 months [14]. In our study, while the number of exacerbations and duration of antibiotic therapy were comparable in both groups prior to treatment, both parameters decreased significantly after 12 months of supplementation. Furthermore, supplementation significantly decreased the number of intravenous treatments required. As shown in the EFA profiles, the supplementation we administered contained high doses of EPA and DHA, which were associated with improved erythrocyte membrane concentrations. This had, in fact, already occurred after just 3 months of supplementation, with concomitant decreases in AA and ARA/DHA levels. While this change in the balance between ω-6 and ω-3 was expected to influence the CF-related systemic inflammation, no change was observed in the standard inflammatory parameters, probably due to these markers' poor sensitivity. However, the positive effect observed in the number of exacerbations could be explained by the immunomodulatory properties of ω-3 PUFA, primarily in their effects on polymorphonuclear cells. Interestingly, these clinical benefits occurred progressively, reaching statistical significance after 9 months of supplementation, suggesting them to be induced by slow mechanisms. Yet a larger study cohort could have allowed us to detect earlier effects. In

contrast, a decrease in EPA and DHA concentrations was reported at the 12 month visit, in comparison with the 6 month analysis. This decline could be accounted for by a drop in patient compliance, though this was not clearly detected through our survey procedure, or alternatively to either study duration or interval between the two last visits being perhaps too long. Nevertheless, a persistent beneficial impact of the supplementation is still plausible, given the long-term clinical effects observed despite the decreased EFA levels. We hypothesize that the EFA replenishment of cells involved in the reduction of lung infection remains functionally sufficient in spite of lower or discontinued supplementation. This, in turn, could indicate that the monocyte lineage plays a pivotal role, both in its longer persistent lifetime or renewal [37] in the body, in addition to its crucial influence on the containment of lung infections.

In our study, no change in lung function was detected between the groups. As expected in CF, lung function decreased slightly during the study period in both groups. Lung function is typically the primary parameter used for assessing the clinical benefits of ω-3 PUFA supplementation, yet the results of this have also been controversial. De Vizia et al., for example, reported significant improvement in FEV_1 [14] values with supplementation, yet this effect was not confirmed in other studies [30,31,33,36,38,39]. No change in exercise tolerance was observed between the groups in our study. Also, just as lung function worsened, VO_2 max values were found to decrease in both groups during the study period. To the best of our knowledge, our study was the first to assess the effect of ω-3 PUFA supplementation on exercise tolerance. The absence of differences in lung function and exercise tolerance between the groups could be explained by the duration of this study, probably too short to influence such parameters, with any clinical effects only significantly manifesting after 9 months.

The supplementation was well tolerated. Although all patients exhibited low BMI z-scores at baseline, they maintained these scores throughout the study, suggesting that the ω-3 PUFA supplementation did not adversely affect growth. Also, despite the ω-3 PUFA supplementation being administered at high doses, it did not modify the platelets, liver enzymes, and standard coagulation tests and abdominal ultrasound results all remained unchanged. The most common adverse event of ω-3 PUFA supplementation is steatorrhea [18,32]. Additional pancreatic enzymes are recommended [20] in these cases. The absence of steatorrhea in our study could be related to our 10% increased daily dose of pancreatic enzyme replacement therapy. Thus we suggest that the dose of pancreatic enzymes should be increased for future studies at the start of fish oil supplementation.

Lastly, as no further increase of ω-3 profile was observed after 12 months, we suggest as hypothesis that patient compliance began to fail in the final semester. Shorter time-spacing between visit intervals for double-blind studies appears preferable, as the care load for swallowing nine capsules daily seems to be difficult to accept, especially when patients know they could, in fact, be simply receiving the placebo. Moreover, the fact that supplementation discontinuation did not alter the reduction of lung infections could lead to considerations of sequential boosting periods of supplementation in order to facilitate comfort and compliance.

The major limitation to our study is that it's a pilot study with a small sample size related to the inclusion and exclusion criteria, the study's duration and the daily intake of capsules. A second limitation of this study is the poor compliance that may be related to the study's duration and the long time-spacing between visit intervals as well as the daily intake of capsules. The last limitation is that the primary endpoint of the study as cited on the ClinicalTrials.gov website was the change of LTB4/LTB5 ratio but the analysis of LTB5 requires sophisticated techniques and it was not finally performed.

In conclusion, this pilot study shows that ω-3 PUFA to be absorbed and incorporated into erythrocyte membranes after 3 months of EPA and DHA supplementation, producing delayed yet significant clinical benefits in terms of the number of exacerbations and duration of antibiotic therapy. Additional studies with larger patient populations are now required to confirm these encouraging results of this pilot study, possibly modifying the supplementation agenda so as to increase comfort and convenience for the patients.

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This study was funded by the Belgian Cystic Fibrosis Association. The Ponroy laboratories provided ω-3 PUFA and placebo supplementation, both free of charge; they had no role in study design, data collection or analysis, or in the drafting of the article. Conflicts of interest: The authors state that they have no conflicts of interest to declare.

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References

1. B. Strandvik, Fatty acid metabolism in cystic fibrosis, Prostaglandins Leukot Essent Fatty Acids 83 (2010) 121-9.

2. T.C. Coste, M. Armand, J. Lebacq, P. Lebecq, P. Wallemacq, T. Leal, An overview of monitoring and supplementation of omega 3 fatty acids in cystic fibrosis, Clinical Biochemistry 40 (2007) 511-20.

3. S.D. Freedman, P.G. Blanco, M.M. Zaman, et al., Association of cystic fibrosis with abnormalities in fatty acid metabolism, N Engl J Med 350 (2004) 560-69.

4. A. Maqbool, J.I. Schall, P.R. Gallagher, B.S. Zemel, B. Strandvik, V.A. Stallings, Relation between dietary fat intake and serum fatty acid status in children with cystic fibrosis, J Pediatr Gastroenterol Nutr 55 (2012) 605-11.

5. T.C. Coste, G. Deumer, G. Reychler, P. Lebecq, P. Wallemacq, T. Leal, Influence of pancreatic status and sex on polyunsaturated fatty acid profiles in cystic fibrosis, Clin Chem 54 (2008) 388-95.

6. N. Peretti, V. Marcil, E. Drouin, E. Levy, Mechanisms of lipid malabsorption in cystic fibrosis: the impact of essential fatty acids deficiency, Nut Metab (Lond) 2 (2005) 11.

7. N. Peretti, C.C. Roy, E. Drouin, et al., Abnormal intracellular lipid processing contributes to fat malabsorption in cystic fibrosis, Am J Physiol Gastrointest Liver Physiol 290 (2006) G609-15.

8. S.W. Njoroge, M. Laposata, W. Katrangi, A.C. Seegmiller, DHA and EPA reverse cystic fibrosis-related FA abnormalities by suppressing FA desaturase expression and activity, J Lipid Res 53 (2012) 257-65.

9. C. Guibault, G. Wojewodka, Z. Saeed et al., Cystic fibrosis fatty acid imbalance is linked to ceramide deficiency and corrected by fenretinide, Am J Respir Cell Mol Biol 41 (2009) 100-6.

10. B. Strandvik, E. Gronowitz, F. Enlund, T. Martinsson, J. Wahlström, Essential fatty acid deficiency in relation to genotype in patients with cystic fibrosis, J Pediatr 139 (2001) 650-5.

11. Van Biervliet S, Vanbillemont G, Van Biervliet JP, Declercq D, Robberecht E, Christophe A, Relation between fatty acid composition and clinical status or genotype in cystic fibrosis patients, Ann Nutr Metab. 51 (2007) 541-9.

12. M.S. Zach, Lung disease in cystic fibrosis-an updated approach, Pediatr Pulmonol 8 (1990) 188-202.

13. R.A. Lewis, K.F. Austen, R.J. Soberman, Leukotrienes and other products of the 5-lipoxygenase pathway, Biochemistry and relation to pathobiology in human diseases, N Engl J Med 323 (1990) 645-55.

14. B. De Vizia, V. Raia, C. Spano, C. Pavlidis, A. Coruzzo, M. Alessio, Effect of an 8-month treatment with omega-3 fatty acids (eicosapentaenoic and docosahexaenoic) in patients with cystic fibrosis, JPEN J Parenter Enteral Nutr 27 (2003) 52-57.

15. B. Strandvik, Fatty acid metabolism in cystic fibrosis, N Engl J Med 350 (2004) 605-7.

16. L.E. Kurlandsky, M.R. Bennink, P.M. Webb, P.J. Ulrich, L.J. Baer, The absorption and effect of dietary supplementation with omega-3 fatty acids on serum leukotriene B4 in patients with cystic fibrosis, Pediatr Pulmonol 18 (1994) 211-7.

17. A. Panchaud, A. Sauty, Y. Kernen, et al., Biological effects of a dietary omega 3 polyunsaturated fatty acids supplementation in cystic fibrosis patients: a randomized crossover placebo-controlled trial, Clinical Nutrition 25 (2006) 418-27.

18. R. Lawrence, T. Sorrell, Eicosapentaenoic acid in cystic fibrosis: evidence of a pathogenic role for leukotriene B_4, Lancet 342 (1993) 465-9.

19. P. Calder, Marine omega-3 fatty acids and inflammatory processes: effects, mechanisms and clinical relevance, Biochim Biophys Acta 1851 (2015) 469-84.

20. C. Oliver, H. Watson, Omega-3 fatty acids for cystic fibrosis, Cochrane Database Syst Rev 11 (2013) CD002201.

21. G. Steinkamp, H. Demmelmair, I. Ruhl-Bagheri, H. von der Hardt, B. Koletzko, Energy supplements rich in linoleic acid improve body weight and essential fatty acid status of cystic fibrosis patients, J Pediatr Gastroenterol Nutr 31 (2000) 418-23.

22. A.W. van Egmond, M.R. Kosorok, R. Koscik, A. Laxova, P.M. Farrell, Effect of linoleic acid intake on growth of infants with cystic fibrosis, Am J Clin Nutr 63 (1996) 746-52.

23. M.R. Al-Turkmani, S.D. Feedman, M. Laposata, Fatty acids alterations and n-3 fatty acids supplementation in cystic fibrosis, Prostaglandins Leukot Essent Fatty Acids 77 (2007) 309-18.

24. L. Saiman L, B.C. Marshall, N. Mayer-Hamblett, et al., Azithromycin in patients with cystic fibrosis chronically infected with Pseudomonas aeruginosa: a randomized controlled trial, JAMA 290 (2003) 1749-56.

25. R.J. Kuczmarski, C.L. Ogden, S.S. Guo, et al., 2000 CDC Growth Charts for the United States: methods and development, Vital Health Stat 11. 246 (2002) 1-190.

26. M.R. Miller, J. Hankinson, V. Brusasco, et al., ATS/ERS Task Force. Standardisation of spirometry, Eur Resp J 26 (2005) 319-38.

27. American Thoracic Society, American College of Chest Physicians. ATS/ACCP Statement on cardiopulmonary exercise testing, Am J Respir Crit Care Med 167 (2003) 211-77.

28. S.G. Williams, J.E. Evanson, N. Barrett, M.E. Hodson, J.E. Boultbee, D. Westaby, An ultrasound scoring system for the diagnosis of liver disease in cystic fibrosis, J. Hepatol. 22 (1995) 513-21.

29. K.W. Southern, P.M. Barker, A. Solis-Moya, L. Patel, Macrolide antibiotics for cystic fibrosis, Cochrane Database Syst Rev 11 (2012) CD002203.

30. S. Van Biervliet, M. Devos, T. Delhaye, J.P. Van Biervliet, E. Robberecht, A. Christophe, Oral DHA supplementation in DeltaF508 homozygous cystic fibrosis patients, Prostaglandins Leukot Essent Fatty Acids 78 (2008) 109-15.

31. J.D. Lloyd-Still, C.A. Powers, D.R. Hoffman, 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 22 (2006) 36-46.

32. W.R. Henderson Jr, S.J. Astley, M.M. McCready, et al., Oral absorption of omega-3 fatty acids in patients with cystic fibrosis who have pancreatic insufficiency and in healthy control subjects, J Pediatr 124 (1994) 400-8.

33. N.H. Thies, The effect of 12 months' treatment with eicosapentaenoic acid in five children with cystic fibrosis, J Paediatr Child Health 33 (1997) 349-51.

34. E. Leggieri, R.V. De Biase, D. Savi, S. Zullo, I. Halili, S. Quattrucci, Clinical effects of diet supplementation with DHA in pediatric patients suffering from cystic fibrosis, Minerva Pediatr 65 (2013) 389-98.

35. P.A. Flume, P.J. Mogayzel Jr, K.A. Robinson, et al., Cystic fibrosis pulmonary guidelines: treatment of pulmonary exacerbations Am J Respir Crit Care Med 180 (2009) 802-8.

36. G. Alicandro, N. Faelli, R. Gagliardini R, et al., A randomized placebo-controlled study on high-dose oral algal docosahexaenoic acid supplementation in children with cystic fibrosis, Prostaglandins Leukot Essent Fatty Acids 88 (2013) 163-9.

37. R. Gentek, K. Molawi, M.H. Sieweke, Tissue macrophage identity and self-renewal, Immunological Reviews 262 (2014) 56–73.

38. D.P. Katz DP, T. Manner, P. Furst, J. Askanazi, The use of an intravenous fish oil emulsion enriched with omega-3 fatty acids in patients with cystic fibrosis, Nutrition 12 (1996) 334-39.

39. J.A. Jumpsen, N.E. Brown, A.B. Thomson AB, et al., Fatty acids in blood and intestine following docosahexanoic acid supplementation in adults with cystic fibrosis, J Cyst Fibros 5 (2006) 77-84.

What is known about this subject?

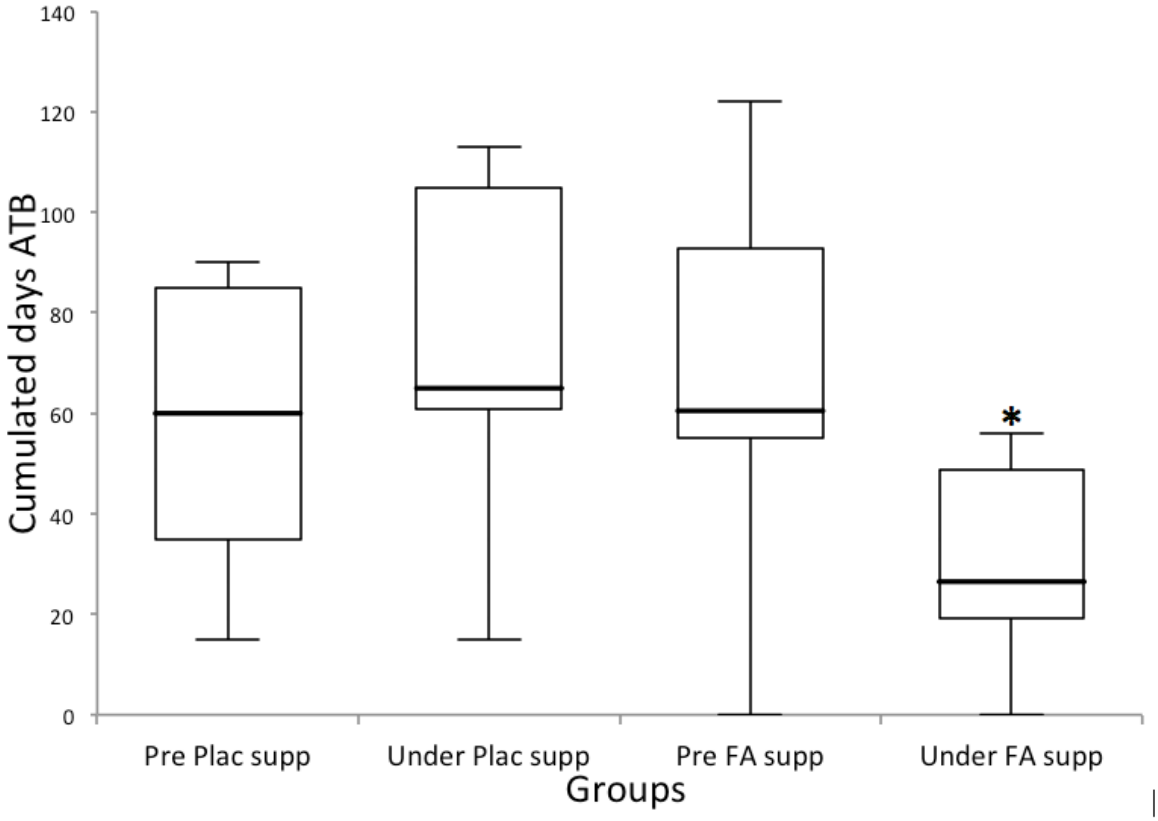
Highlights

- Essential fatty acid deficiency has been reported to occur in cystic fibrosis (CF) patients.
- Eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) exert anti-inflammatory properties.
- The clinical benefits of omega-3 supplementation in CF remain controversial.

What are the new findings and/or what is the impact on clinical practice?

- An omega-3 fish oil supplementation of 60mg/Kg/day was found to increase the erythrocyte levels of EPA and DHA, with concomitant decreases in those of arachidonic acid (AA).
- This supplementation has shown beneficial in terms of duration of antibiotic therapy and number of exacerbations.
- Omega-3 supplementation should be considered as treatment for CF, particularly in patients with severe CF genotypes.

Fig. 1. Evolution of 12-month antibiotic therapy before and after ω-3 PUFA supplementation

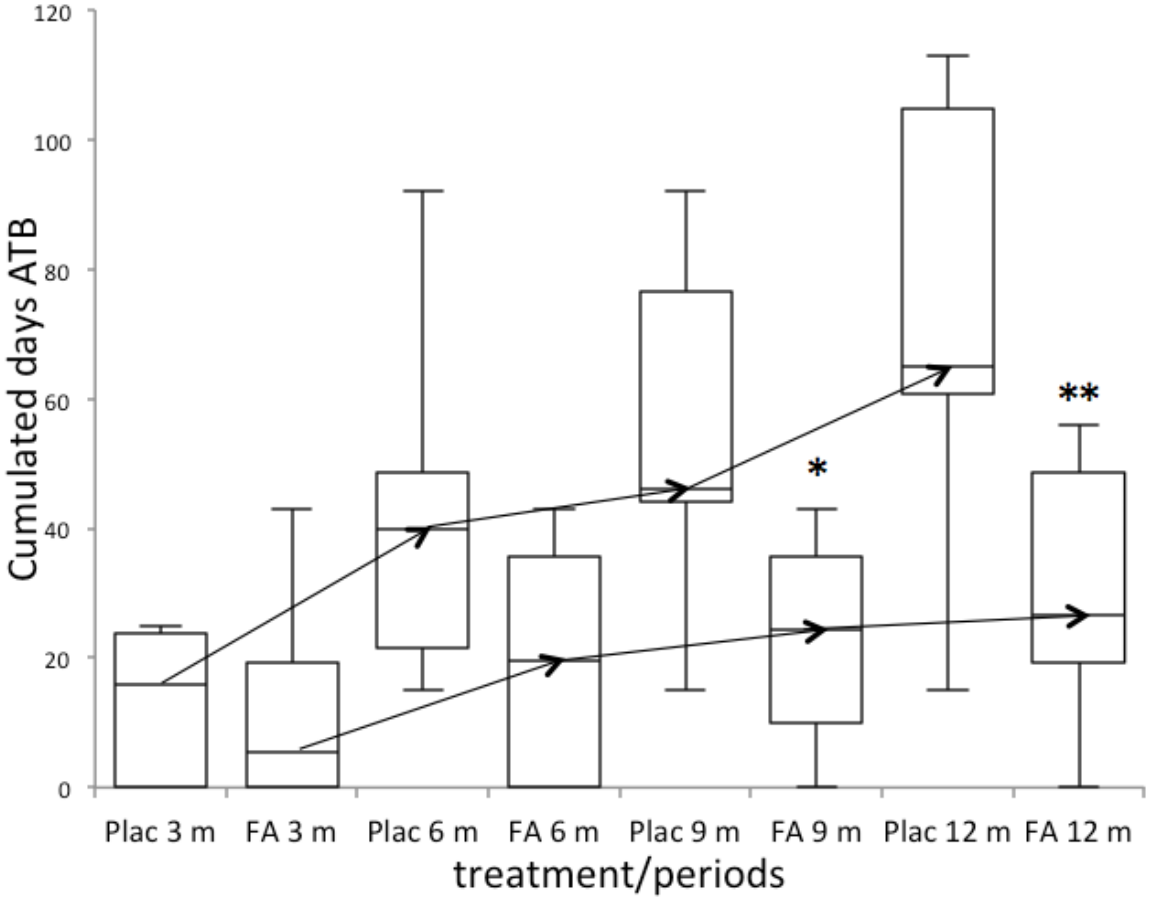


ATB: antibiotic therapy; Plac: placebo; FA: fatty acid

Legend

Boxes contain the median and are limited by the 25th and 75th percentiles. Outside limits define 5th and 95th percentiles. Comparison of cumulated days of antibiotic therapy during 12 months, before and after supplementation, in two groups of cystic fibrosis (CF) patients (supplemented group [FA] and placebo group [Plac]). Medians of cumulated days of antibiotic therapy (ATB) during the pre-study (Pre) observations were identical in both groups, significantly decreasing from 60.0 to 26.5 days at 12 months after initiating ω-3 PUFA supplementation (FA vs. Plac; Mann-Whitney U-test: $p < 0.025$*) while remaining at the same level (60.0/65.0 days) in the Plac group.

Fig. 2. Evolution of antibiotic therapy duration every 3 months during the study

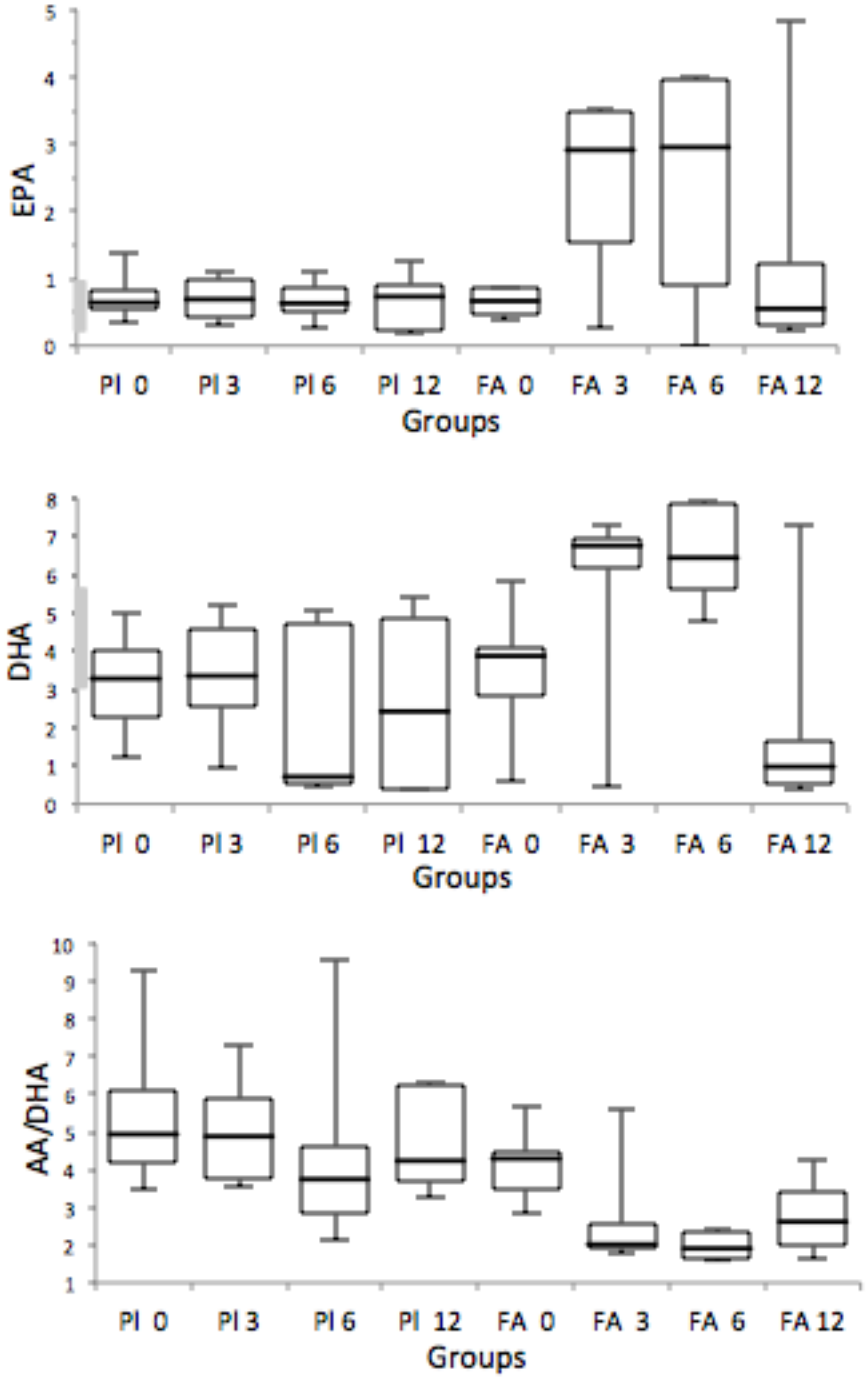


ATB: antibiotic therapy; plac: placebo; FA: fatty acid; m: months

Legend:

Boxes contain the median and are limited by the 25th and 75th percentiles. Outside limits define the 5th and 95th percentiles. Medians of cumulated days of antibiotic therapy (ATB) increased less over time in the supplemented group (FA) compared to the placebo group (Plac) (trends joining group medians: →), becoming significant at 9 months when reaching about half the level of the Plac group, with a median of 24.5 days in the FA group versus 46.0 days in the Plac group (Mann-Whitney U-test: p <0.02*). This benefit was confirmed at 12 months (Mann-Whitney U-test: 0.025**).

Fig. 3. Periodic evolution of erythrocyte-relative eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA), and arachidonic acid (AA)/DHA concentrations (% total fatty acids [FA]) during the study



EPA: eicosapentaenoic acid; Pl: placebo; FA: fatty acid (supplement); DHA: docosahexaenoic acid; AA: arachidonic acid

Legend:

EPA, DHA, and AA/DHA profiles are expressed as percentages of total fatty acids (% total FA) in the placebo group (Plac) and supplemented group (FA). Normal p25-p75 limits for erythrocyte EPA and DHA (% of total FA) are presented by

In the Plac group, median levels of EPA, DHA, and AA/DHA stabilized with time (0, 3, 6, and 12 months)

In the FA group, the median level of EPA increased significantly at 3 and 6 months, then returned to the initial level after 12 months. Parametric tests were performed after log transformation of values due to extended distributions: ANOVA repeated measures on the FA group: $p < 0.002$. Paired t-tests: FA 0 / FA 3: $p < 0.025$; FA 0 / FA 6: $p < 0.020$; FA 6 / FA 12: $p = 0.035$. The median level of DHA increased significantly at 3 and 6 months and returned to the initial level after 12 months. Parametric tests were performed after log transformation of values due to extended distributions. ANOVA repeated measures on FA groups: $p < 0.002$. Paired t-tests: FA 0 / FA 3: $p < 0.05$; FA 0 / FA 6: $p < 0.05$; FA 6 / FA 12: $p = 0.025$. The median level of AA/DHA only decreased significantly at 6 months. ANOVA repeated measures on FA groups: $p = 0.0022$. Paired t-tests: FA 0 / FA 6: $p < 0.02$.

Table 1 Baseline characteristics of patients assigned to ω-3 PUFA or placebo (Intention to treat n=15)

	Supplemented group (n=7)	Placebo group (n=8)	P value*
Age, years	14.0 (5.2-26.0)	17.5 (4.6-30.4)	0.26
Sex ratio (M:F)	4:3	7:1	0.45
FEV1%	83 (64-121)	65 (47-104)	0.34
FVC%	87 (76-110)	82 (66-104)	0.67
BMI z-score	-1.3 (-2.2-0.2)	-0.4 (-2.4-1.0)	0.43
Chronic PA, %	42.9	25.0	0.46
n°exacerbations last year	3.0 (1-6)	2 (0-4)	0.07
Days of ATB last year	60.0 (0-122)	60.0 (0-90)	0.73
VO2 max, ml/min/Kg	46 (38-56)	41 (30-63)	0.55
WBC, 10^3 /mm^3	8.7 (5.1-15.9)	7.9 (5.8-15.6)	0.64
ESR, mm/h	25 (3-52)	11 (3-24)	0.23
IgG, g/L	10.4 (6.3-28.4)	13.5 (6.3-20.2)	0.76

Abbreviations: Sex ratio (M:F): Male:Female; FEV1%: forced expiratory volume as a percentage of predicted value; FVC%: forced vital capacity as a percentage of predicted value; BMI: body mass index; PA: Pseudomonas Aeruginosa; n°exacerbations: number of exacerbations; ATB: antibiotics; VO2 max: maximal oxygen uptake; WBC: white blood cells; ESR: erythrocyte sedimentation rate; Ig: immunoglobulins

Data are expressed as median and range between brackets

* a P value of ≤ 0.05 was considered significant