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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 60 mg/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

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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 W (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 (>240 mg%); (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 300 mg of ω-3 triglycerides from fish oil, providing 100 mg of DHA and 150 mg of EPA. The total daily ω-3 dose corresponded to 60 mg/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.