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Elevated prostaglandin E metabolites and abnormal plasma fatty acids at baseline in pediatric cystic fibrosis patients: a pilot study

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ABSTRACT

Background: Airway inflammation is a significant contributor to the morbidity of cystic fibrosis (CF) disease. One feature of this inflammation is the production of oxygenated metabolites, such as prostaglandins. Individuals with CF are known to have abnormal metabolism of fatty acids, typically resulting in reduced levels of linoleic acid (LA) and docosahexaenoic acid (DHA).

Methods: This is a randomized, double-blind, cross-over clinical trial of DHA supplementation with endpoints of plasma fatty acid levels and prostaglandin E metabolite (PGE-M) levels. Patients with CF age 6–18 years with pancreatic insufficiency were recruited. Each participant completed 3 four-week study periods: DHA at two different doses (high dose and low dose) and placebo with a minimum 4 week wash-out between each period. Blood, urine, and exhaled breath condensate (EBC) were collected at baseline and after each study period for measurement of plasma fatty acids as well as prostaglandin E metabolites.

Results: Seventeen participants were enrolled, and 12 participants completed all 3 study periods. Overall, DHA supplementation was well tolerated without significant adverse events. There was a significant increase in plasma DHA levels with supplementation, but no significant change in arachidonic acid (AA) or LA levels. However, at baseline, AA levels were lower and LA levels were higher than previously reported for individuals with CF. Urine PGE-M levels were elevated in the majority of participants at baseline, and while levels decreased with DHA supplementation, they also decreased with placebo.

Conclusions: Urine PGE-M levels are elevated at baseline in this cohort of pediatric CF patients, but there was no significant change in these levels with DHA supplementation compared to placebo. In addition, baseline plasma fatty acid levels for this cohort showed some difference to prior reports, including higher levels of LA and lower levels of AA, which may reflect changes in clinical care, and consequently warrants further investigation.

1. Introduction

Airway inflammation is a major contributor to poor clinical outcomes in individuals with cystic fibrosis(CF) [1]. Several studies have shown that markers of inflammation are elevated in the sputum of individuals with CF, including neutrophil elastase and IL-8 [2,3]; while markers of inflammation resolution, such as lipoxin A4, are decreased [4]. Individuals with CF also display abnormalities of fatty acid (FA) metabolism, most notably characterized by decreased linoleic acid (LA) and docosahexaenoic acid (DHA) levels [5,6]. Research completed in both animals and humans has shown that these FA abnormalities may be related to increased inflammation through increased prostaglandin production [7,8]. In cell culture and animal models, DHA supplementation has been shown to reverse abnormal PUFA metabolism and some aspects of CF pathology, but results in human trials have been less clear [7,9–11].

This short-communication briefly describes a double-blind, placebo controlled, cross-over clinical trial to detect changes in prostaglandin metabolites in pediatric patients with CF who were receiving DHA supplementation.

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2. Methods

2.1. Study design

This was a prospective, randomized, double-blinded, placebo-controlled, cross-over trial of DHA supplementation. There were 3 study periods, each of 4 weeks duration: high-dose DHA (35 mg/kg/day), low-dose DHA (25 mg/kg/day), and placebo. A minimum 4-week wash-out period occurred between each of the three study periods. Study capsules were provided by Martek Bioscience Corp., USA. The DHA supplement was in triglyceride form from a microalgal source and the placebo was high oleic acid sunflower oil. Participants were randomized to a specific sequence of the three study periods and participants were instructed to take the study capsules as a single daily dose.

2.2. Participants

Individuals with CF greater than or equal to 6 years of age and less than 18 years of age were eligible. Individuals also needed to have a baseline forced expiratory volume in one second (FEV1) of greater than 40% predicted. Exclusion criteria included: CF-related diabetes, CF-related liver disease, consideration for lung transplantation, active pulmonary exacerbation, history of fish allergy, daily chronic use of non-steroidal anti-inflammatory drugs (NSAIDs), use of anticoagulant drugs, or chronic use of glucocorticoids.

Participants were recruited from a single pediatric CF center. All pediatric participants gave informed assent and parents gave informed consent prior to enrollment. The study was approved by the institutional review board (#081363) and the study is listed in clinicaltrials.gov (NCT00924547).

2.3. Study period measurements

Endpoints included plasma fatty acid levels, urine prostaglandin-E metabolite (PGE-M), and prostaglandin E2 (PGE2) measured in exhaled breath condensate (EBC). These were collected at baseline and after each of the three study periods. Weight and height were obtained at each study visit as a measure of patient safety, not a clinical endpoint.

Plasma fatty acids were obtained from lipid extraction and methylation by methods previously described [12]. Fatty acids were identified and quantified by gas chromatography/mass spectrometry (GC/MS) and reported as %mole. Urine PGE-M was quantified in the Vanderbilt Eicosanoid Core Laboratory by methods previously described [8]. Urine PGE-M levels were reported as ng/mg Cr. EBC was collected using RTube™ (Respiratory Research, Inc.) with patients being instructed to do tidal breathing for 5–7 min.

2.4. Statistical considerations

Sample size and power estimates were made using the PS program (Nashville, TN). Using a sample size of 13 patients and cross-over study design, the study has a 90% power to detect a 15 point difference in mean 8-isoprostane PGF2α between groups. To account for drop-out the goal enrollment was set at 18 patients.

3. Results

There were 17 participants enrolled in the study, 12 of which completed all study periods. Two participants withdrew before the completion of the first study period due to dislike of capsule taste, one withdrew after being admitted for constipation following a single dose of study capsule, and one withdrew after completion of the first study period due to diagnosis of CF-related diabetes on a routine oral glucose tolerance test. The participants were at their clinical baseline at time of enrollment (Table 1).

Table 1
Participant Demographics (n = 17).

Female (n, %)	6, 35%
Age (years)	10 [7,13]
Genotype (n, %)	
ΔF508 homozygous	10, 59%
ΔF508 heterozygous	6, 35%
FEV1% predicted at enrollment	101 [92,107]
BMI percentile at enrollment	54 [40,63]
Inpatient pulmonary exacerbations in year prior to enrollment	1 [0,2]
Outpatient antibiotic courses for pulmonary exacerbations in year prior to enrollment	1 [0,2]

Patient demographics (n = 17 individuals). Continuous variables are expressed as median with inter-quartile range (IQR). Inpatient and outpatient pulmonary exacerbations were determined by chart review documentation.

At baseline, study participants had elevated urine PGE-M levels with a median value of 25 ng/mg Cr (normal previously reported as less than 10 ng/mg Cr [8]). There was a decrease in urine PGE-M with both high and low doses of DHA supplementation (median decreases of 8 ng/mg Cr and 14 ng/mg Cr, respectively). However, there was also a decrease in PGE-M levels by a median value of 8 ng/mg Cr with the placebo (Fig. 1).

Plasma FA levels were reported as mole percent (Table 2). At baseline, participants had low plasma DHA levels (median 0.33 mol%). After DHA supplementation, participants in both the high-dose and low-dose DHA supplementation arms displayed significant increases in plasma DHA compared to baseline. There was not a significant difference in plasma DHA between low and high-dose supplementation. Baseline LA levels were higher than previously reported for individuals with CF [5] (median 25.73 mol%) and there was no significant change in LA with DHA supplementation. In addition, there was no significant change in arachidonic acid (AA) or mead acid levels with DHA supplementation. AA levels were lower at baseline than previously reported [5]. The triene/tetraene ratio (mead acid/arachidonic acid) was normal at baseline [13] and did not change with DHA supplementation.

Several fatty acids showed small absolute changes which reached statistical significance (eicosanoic acid, 20:1 n-9, baseline vs. placebo; eicosadienoic acid, 20:2 n-6, low-dose vs. high dose;

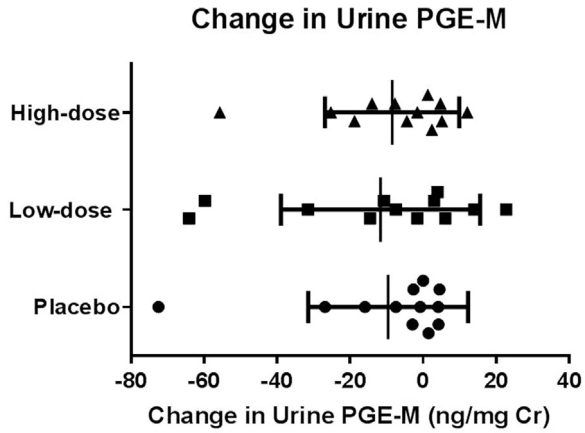

Fig. 1. Change in urine prostaglandin E metabolite (PGE-M). Data are displayed for the 12 participants who completed all study arms as individual change in PGE-M from baseline measurements. Study groups are displayed as high-dose DHA supplementation, low-dose DHA supplementation, and placebo. Statistical testing by Friedman Test. No statistical significance in the change in urine PGE-M was observed between the three treatment groups.