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Increased Δ5- and Δ6-desaturase, cyclooxygenase-2, and lipoxygenase-5 expression and activity are associated with fatty acid and eicosanoid changes in cystic fibrosis

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ABSTRACT

Patients with cystic fibrosis consistently demonstrate selective abnormalities in essential fatty acid concentrations, including decreased linoleate (LA) and docosahexaenoate (DHA), with variably increased arachidonate (AA). These changes appear important for the pathophysiology of the disease. However, the mechanisms of these changes are not clearly understood. The current study demonstrates that metabolism of LA and alpha linolenate (LNA) to AA and eicosapentaenoate (EPA), respectively, are significantly increased in two different cell culture models of cystic fibrosis. These changes correlated with increased expression of fatty acid Δ5- and Δ6-desaturases, key enzymes in this metabolic pathway. In contrast, cystic fibrosis cells showed decreased metabolism of AA and EPA to docosapentaenoate (DPA) and docosahexaenoate (DHA), respectively, although metabolism of 22:5n-3 to DHA was relatively unchanged. In addition, the expression and activity of both cyclooxygenase-2 and lipoxygenase-5 was markedly increased in these cells. Taken together, these findings are consistent with the conclusion that the diminished LA and increased AA in cystic fibrosis result from increased metabolism of LA, while the observed decrease in DHA is at least partly due to decreased elongation and desaturation beyond EPA.

1. Introduction

Cystic fibrosis (CF) is an autosomal recessive disease resulting from mutations in the gene for the cystic fibrosis transmembrane conductance regulator (CFTR) [1]. These genetic alterations result in decreased chloride conductance across the apical membrane of epithelial cells. The most common mutation in the CFTR gene is the ΔF508 mutation, resulting in the loss of a phenylalanine codon at position 508, representing approximately 70% of CF cases [2]. To date, more than 1500 CFTR mutations have been described, most of which are rare [2]. The incidence of CF is highest in Caucasians, with 1 in 30 acting as carriers of a CFTR gene mutation and a disease incidence of approximately 1 in 3000, and is much lower in those of African or Asian descent [3,4]. Clinical manifestations of CF vary in presentation and severity, and may include pancreatic insufficiency, malabsorption, small intestinal obstruction, frequent pulmonary infections, progressive lung disease, and infertility in male patients [2].

More than four decades ago, alterations in essential fatty acid (EFA) composition were identified in patients with CF [5]. Since then, numerous studies have confirmed these findings in CF patients [6–16]. Similar changes have been observed in *cftr*$^{-/-}$ knockout mice [17–19] and two independent respiratory epithelial cell culture models [20,21]. From these studies, several reproducible fatty acid changes have emerged as dominant. First, there is a consistent decrease in the level of 18:2n-6 (linoleate, LA). Second, there is a consistent reduction in the amount of 22:6n-3 (docosahexaenoate, DHA). Third, there are variable increases in 20:4n-6 (arachidonate, AA), although this finding is not entirely consistent. In most reports, there is an increase in AA in association with a decrease in LA, a precursor fatty acid for AA. Other studies show little or no increase in the amount of AA, but do show increases in other downstream n-6 fatty acids such as 20:3n-6 (see Fig. 1).

These EFA alterations drew significant attention following a report describing a mouse model with cystic fibrosis and the fatty acid changes described above [17]. Treatment of these mice with large amounts of daily DHA supplementation resulted in increased DHA and decreased AA concentrations in pancreas and lung tissue. The treated animals also exhibited reversal of CF-related pathology, including relief of pancreatic duct obstruction, a reduction in ileal hypertrophy, and a decrease in stimulated neutrophil accumulation into bronchoalveolar lavage fluid.

The above findings led to investigations that focused on the mechanism for the decrease in LA. A possible explanation is alterations in EFA metabolism. Several studies have demonstrated increased release of AA from CF cells [22–25] that contributes to airway pathology in CF [26]. Furthermore, cultured respiratory and pancreatic epithelial cells lacking CFTR expression show increased activity of Δ6-desaturase, the rate-limiting enzyme in the desaturation and elongation of fatty acids in the n-6 and n-3 pathways [27], in the conversion of LA to AA

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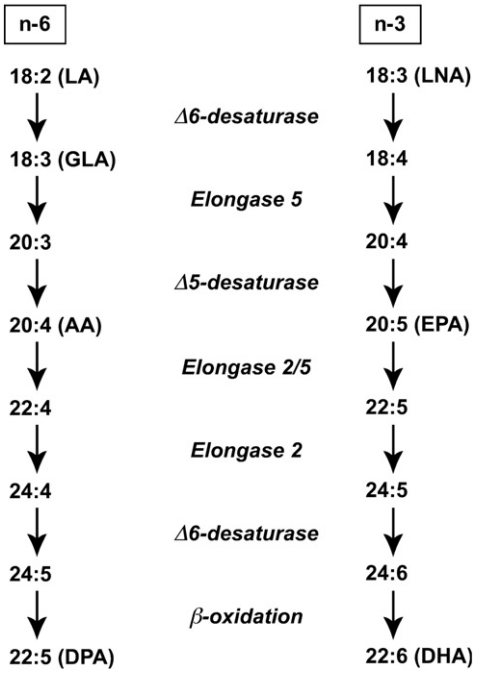

Fig. 1. Essential fatty acid metabolism through the n-6 (left) and n-3 (right) pathways. LA, linoleate; GLA, gamma linolenate; AA, arachidonate; DPA, docosapentaenoate; LNA, linolenate; EPA, eicosapentaenoate; DHA, docosahexaenoate.

[20,21,28]. This induction of Δ6-desaturase activity was reversible following incubation with DHA.

These results raise additional questions. The n-3 and n-6 fatty acids share a common metabolic pathway (detailed in Fig. 1). Consequently, induction of the n-6 pathway in CFTR-negative cells would presumably increase metabolism of the n-3 pathway as well. However, levels of DHA, the ultimate product of this pathway, are consistently decreased in CF. In fact, metabolism of 20:5n-3 (eicosapentaenoic acid, EPA) to DHA is reduced in these cells [21].

The goal of this study is to better understand the mechanisms of these fatty acid changes. To this end, a systematic parallel comparison of the n-3 and n-6 metabolic pathways was performed in two cell culture models of CF. The findings were correlated with mRNA expression of the corresponding metabolic enzymes. There was a consistent increase in the metabolism of both LA to AA and 18:3n-3 (alpha-linolenate, LNA) to EPA in CFTR-negative cells, which correlated with increased expression of both Δ5- and Δ6-desaturases. In contrast, there was decreased conversion of both AA and EPA to their downstream metabolites, including DHA. We hypothesize that this latter finding is due to increased metabolism of a specific pool of AA and EPA to oxygenated products. In support of this hypothesis, both cyclooxygenase-2 and lipoxygenase-5 expression and prostaglandin E_2 (PGE_2) and leukotriene B_4 (LTB_4) production were markedly increased in CFTR-negative cells.

2. Materials and methods

2.1. Materials

[1-^{14}C]LA (55 mCi/mmol), [1-^{14}C]LNA (55 mCi/mmol), [1-^{14}C]AA (55 mCi/mmol), [1-^{14}C]EPA (55 mCi/mmol) and [1-^{14}C]7,10,13,16,19-docosapentaenoic acid (22:5n-3) (55 mCi/mmol) were purchased from American Radiolabeled Chemicals, Inc (St. Louis, MO). All fatty acid methyl ester (FAME) standards for HPLC were purchased from NuChek Prep (Elysian, MN) with the exception of 24:5n-3 and 24:6n-3, which were purchased from Larodan Fine Chemicals (Malmö, Sweden). HPLC grade solvents were purchased from Fisher Scientific (Pittsburgh, PA) and liquid scintillation cocktail (IN-flow 2:1) was purchased from IN/US Systems, Inc. (Tampa, FL). Polyclonal anti-FADS2 (Δ6-desaturase) antibody was purchased from Santa Cruz Biotechnology (Santa Cruz, CA) and polyclonal anti-calnexin antibody was purchased from StressGen (Ann Arbor, MI). $CFTR_{inh}$-172 was purchased from Sigma-Aldrich (St. Louis, MO).

2.2. Cell culture

16HBEo$^-$ sense and antisense cells were a gift from Dr. Pamela Davis (Case Western Reserve University School of Medicine, Cleveland, OH). IB3 and C38 cells were obtained from ATCC (Manassas, VA). Cell culture was performed as previously described [20,21]. Briefly, cells were grown in tissue culture flasks pre-coated with LHC Basal media (Invitrogen, Carlsbad, CA) containing 0.1 mg/ml BSA (Sigma-Aldrich, St. Louis, MO), 10 μg/ml human fibronectin (Sigma-Aldrich), and 3 μg/ml vitrogen (Angiotech Biomaterials, Palo Alto, CA). Complete culture medium was comprised of minimum essential medium + glutamax (Invitrogen) supplemented with 100 μg/ml streptomycin, 100 U/ml penicillin, and 10% horse serum (Atlanta Biologicals, Lawrenceville, GA). The lipid composition of the horse serum is given in Table 1. Cells were grown at 37 °C in a 5% CO_2 humidified incubator. Medium was changed three times weekly. For experiments, sense and antisense cells were seeded in 6 well plates at 3×10^5 and 1×10^5 cells/well, respectively, and grown until 2 days post-confluence (7 days). IB3 and C38 cells were seeded in 6 well plates at 1×10^5 cells/well and grown until 2 days post-confluence (8 days).

2.3. Fatty acid composition analysis

Cells were cultured as above until 2 days post-confluence, after which they were washed twice in ice-cold PBS, scraped, and transferred to a glass tube. The saturated fatty acid 17:0 (10 μg) was added as an internal standard. To extract lipids, a modified method of Folch, Lees, and Sloane Stanley was used [29]. The cells were washed twice in cold phosphate-buffered saline (Invitrogen), scraped on ice, and pelleted by centrifugation (100*g* for 8 min). Six volumes of chloroform-methanol (2:1 v/v) were then added to the cells, and they were incubated on ice for 10 min, vortexed, and centrifuged (1100*g* for 10 min). The lower phase was then transferred to a new glass tube and dried down completely under nitrogen. Fatty acids were methylated using boron trifluoride (BF_3; 14% in methanol; Sigma-Aldrich) and a methanolic-base reagent [30] as follows: 0.5 ml 0.5 N methanolic NaOH (Acros Organics, Geel, Belgium) was added to the sample, which was then vortexed and heated at 100 °C for 3 min, followed by addition of 0.5 ml BF_3 at 100 °C for 1 min. To extract the FAMEs, 1 ml of hexane was added to the mixture, followed by 6.5 ml of a saturated NaCl solution, and the sample vortexed and centrifuged (500*g* for 4 min) to allow for separation of both liquid phases. The upper hexane layer was used for

Table 1
Fatty acid composition of horse serum.

Fatty acid	Mol%[a]	Fatty acid	Mol%[a]
Saturated		n-3	
14:0	0.79 ± 0.03	18:3n-3	3.00 ± 0.04
15:0	0.21 ± 0.01	20:3n-3	0.23 ± 0.01
16:0	16.53 ± 0.13	20:5n-3	0.18 ± 0.01
18:0	17.63 ± 0.13	22:5n-3	0.23 ± 0.01
20:0	0.32 ± 0.00	22:6n-3	0.13 ± 0.01
22:0	0.03 ± 0.00	n-7	
n-6		16:1n-7	1.75 ± 0.01
18:2n-6	42.87 ± 0.43	18:1n-7	1.72 ± 0.02
18:3n-6	0.39 ± 0.01	n-9	
20:3n-6	0.30 ± 0.00	18:1n-9	12.15 ± 0.08
20:4n-6	1.23 ± 0.02	20:1n-9	0.31 ± 0.00

Data are given as mean ± SEM of three replicates.
[a] Mol% = molar percentage of total fatty acids. No measurable levels of 19:0, 23:0, 24:0, 24:1, 20:2n-6, 22:4n-6, 22:5n-6, 20:1n-7, 20:3n-9, or 22:1n-9 were observed.