

ORIGINAL ARTICLE

Association of Cystic Fibrosis with Abnormalities in Fatty Acid Metabolism

Steven D. Freedman, M.D., Ph.D., Paola G. Blanco, M.D., Munir M. Zaman, M.D., Julie C. Shea, B.A., Mario Ollero, D.V.M., Ph.D., Isabel K. Hopper, R.N., Deborah A. Weed, R.N., Andres Gelrud, M.D., Meredith M. Regan, Sc.D., Michael Laposata, M.D., Ph.D., Juan G. Alvarez, M.D., Ph.D., and Brian P. O'Sullivan, M.D.

ABSTRACT

BACKGROUND

From the Departments of Medicine (S.D.F., P.G.B., M.M.Z., J.C.S., M.O., I.K.H., D.A.W., A.G., M.M.R.) and Obstetrics and Gynecology (M.O.), Beth Israel Deaconess Medical Center and Harvard Medical School, Boston; the Division of Laboratory Medicine, Massachusetts General Hospital and Harvard Medical School, Boston (M.L.); Instituto de Infertilidad Masculina, Centro de Infertilidad Masculina Androgen, Hospital San Rafael, La Coruña, Spain (J.G.A.); and the Department of Pediatrics, UMass Memorial Health Care, University of Massachusetts Medical School, Worcester (B.P.O.). Address reprint requests to Dr. Freedman at Beth Israel Deaconess Medical Center, Dana 552, 330 Brookline Ave., Boston, MA 02215, or at sfreedma@caregroup.harvard.edu.

Patients with cystic fibrosis have altered levels of plasma fatty acids. We previously demonstrated that arachidonic acid levels are increased and docosahexaenoic acid levels are decreased in affected tissues from cystic fibrosis–knockout mice. In this study we determined whether humans with mutations in the cystic fibrosis transmembrane conductance regulator (CFTR) gene have a similar fatty acid defect in tissues expressing CFTR.

METHODS

Fatty acids from nasal- and rectal-biopsy specimens, nasal epithelial scrapings, and plasma were analyzed from 38 subjects with cystic fibrosis and compared with results in 13 obligate heterozygotes, 24 healthy controls, 11 subjects with inflammatory bowel disease, 9 subjects with upper respiratory tract infection, and 16 subjects with asthma.

RESULTS

The ratio of arachidonic to docosahexaenoic acid was increased in mucosal and submucosal nasal-biopsy specimens ($P < 0.001$) and rectal-biopsy specimens ($P = 0.009$) from subjects with cystic fibrosis and pancreatic sufficiency and subjects with cystic fibrosis and pancreatic insufficiency, as compared with values in healthy control subjects. In nasal tissue, this change reflected an increase in arachidonic acid levels and a decrease in docosahexaenoic acid levels. In cells from nasal mucosa, the ratio of arachidonic to docosahexaenoic acid was increased in subjects with cystic fibrosis ($P < 0.001$), as compared with healthy controls, with values in obligate heterozygotes intermediate between these two groups ($P < 0.001$). The ratio was not increased in subjects with inflammatory bowel disease. Subjects with asthma and those with upper respiratory tract infection had values intermediate between those in subjects with cystic fibrosis and those in healthy control subjects.

CONCLUSIONS

These data indicate that alterations in fatty acids similar to those in cystic fibrosis–knockout mice are present in CFTR-expressing tissue from subjects with cystic fibrosis.

N Engl J Med 2004;350:560-9.

Copyright © 2004 Massachusetts Medical Society.

THE MECHANISMS BY WHICH MUTATIONS in the gene encoding the cystic fibrosis transmembrane conductance regulator (CFTR) protein lead to the expression of cystic fibrosis are unclear. Explanations must take into account the increased viscosity of ductal fluids as well as the excessive host inflammatory response.¹⁻⁶ Alterations in the metabolism of fatty acids may have an important role.⁷ A deficiency of essential fatty acids has been described in patients with cystic fibrosis⁸ that is characterized by a decrease in the plasma levels of linoleic acid (18:2n-6) and docosahexaenoic acid (22:6n-3) with a compensatory increase in the levels of eicosatrienoic acid (20:3n-9)⁹⁻¹¹ (Fig. 1). Similar changes have been observed in well-nourished patients with cystic fibrosis^{14,15} and obligate heterozygotes for mutant CFTR,¹⁶ suggesting that this abnormality is due not to nutritional deficiencies but to CFTR dysfunction.¹⁴ The results of studies of fatty acids in erythrocytes from patients with cystic fibrosis have been inconsistent.^{12,17} Therefore, we examined whether alterations in arachidonic acid (20:4n-6), docosahexaenoic acid, and other fatty acids are present in CFTR-regulated tissues from patients with cystic fibrosis and whether such abnormalities are specific to cystic fibrosis or are present in other inflammatory disorders.

METHODS

ENROLLMENT OF SUBJECTS

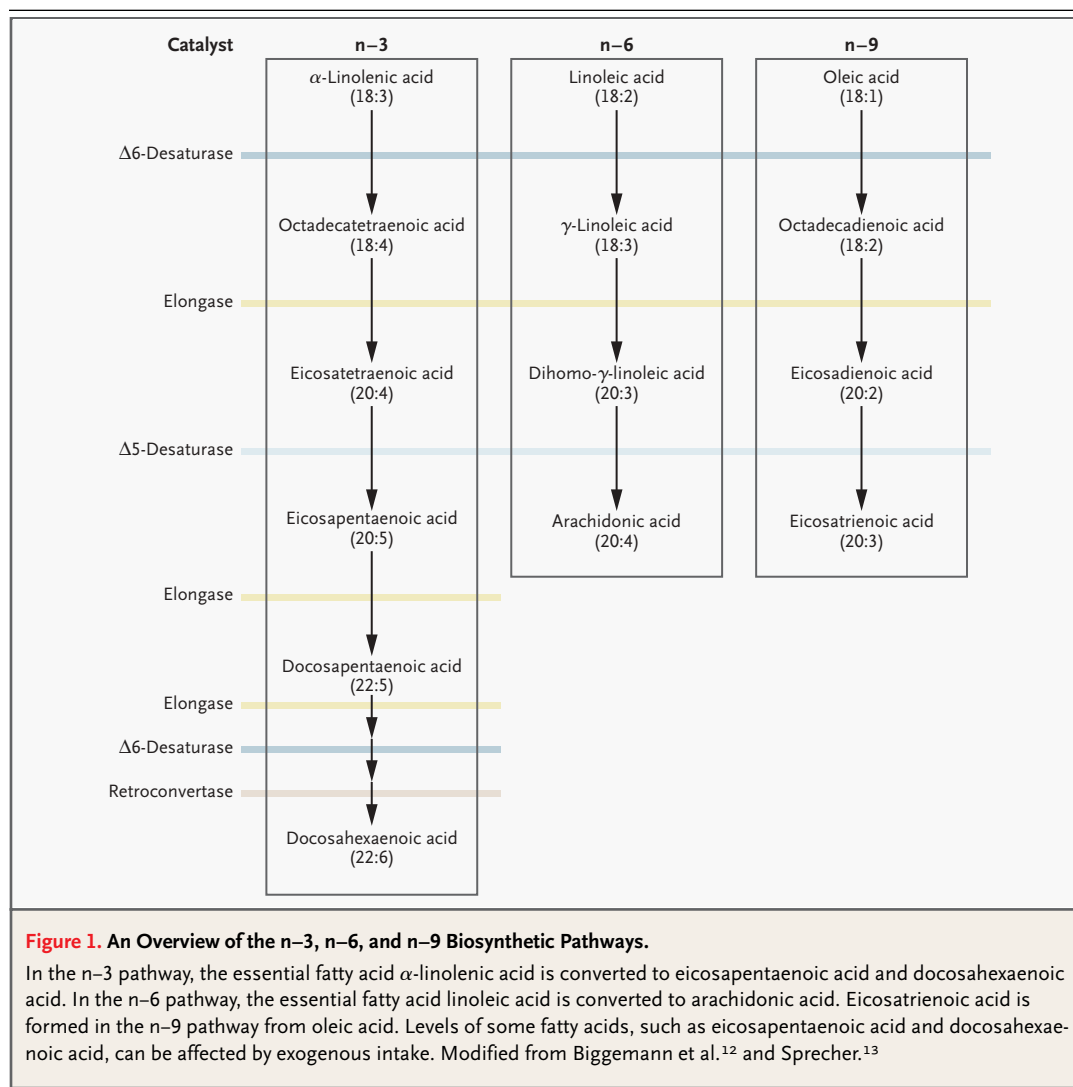
The study was conducted from October 2000 to September 2003. All subjects provided written informed consent before enrollment. The institutional review board at the University of Massachusetts Medical School (Worcester) and Beth Israel Deaconess Medical Center (Boston) approved the protocols. Subjects with cystic fibrosis met the consensus-statement requirements for the diagnosis of this disease¹⁸ and were followed at the UMass Memorial Cystic Fibrosis Center (Worcester). A total of 38 subjects with cystic fibrosis were enrolled (31 with pancreatic insufficiency and 7 with pancreatic sufficiency), all of whom had pulmonary disease. Pancreatic insufficiency was defined by the requirement for exogenous pancreatic enzymes for the treatment of clinically diagnosed steatorrhea. Forced expiratory volume in one second as a percentage of predicted values, body-mass index (the weight in kilograms divided by the square of the height in meters), and the National Institutes of Health prognostic score,

as defined by Taussig et al.,¹⁹ were obtained. All subjects were encouraged to eat high-fat (35 to 40 percent of calories), high-calorie diets and had regular visits with a nutritionist, in accordance with the guidelines of the Cystic Fibrosis Foundation.²⁰

We also evaluated five groups of control subjects. We studied 13 obligate heterozygotes: 12 were the mothers of subjects who were homozygous for the $\Delta F508$ CFTR mutation, and 1 was a father with an identified $\Delta F508$ mutation. We studied 11 subjects with inflammatory bowel disease (8 with Crohn's disease and 3 with ulcerative colitis), which was diagnosed endoscopically in the proper clinical context of chronic diarrhea and the exclusion of other causes. The diagnosis of acute upper respiratory tract infection in nine control subjects, which was presumed to be viral in nature, was based on the presence of a self-limited acute pharyngitis and rhinitis in the absence of an identified bacterial cause in otherwise healthy subjects. Twenty-five control subjects with physician-diagnosed asthma were enrolled. Twenty-three used daily inhaled corticosteroid therapy to control their symptoms, and the other two used beta-agonists. Blood from all 25 subjects with asthma was sent to Ambry Genetics for CFTR gene analysis. All CFTR exons, at least 20 bases 5' and 3' into each intron, as well as select portions of introns associated with specific mutations, were analyzed. Only subjects with asthma who had no known CFTR mutations (other than V470) were included in the analysis. A total of 24 healthy controls were recruited at both sites after a review of their medical history. Exclusion criteria for all control subjects included a family history of cystic fibrosis or features consistent with the presence of cystic fibrosis (chronic lung disease, male infertility, chronic sinusitis, and chronic pancreatitis) or the use of drugs that affect fatty acids (systemic corticosteroids, isotretinoin, and ursodiol).

TISSUE PROCUREMENT

A single physician obtained nasal-biopsy specimens (approximately 3 mm) from the inferior turbinate after the submucosal injection of 1 percent lidocaine. The inferior turbinate was chosen because this is where CFTR function is directly examined in patients with cystic fibrosis by means of testing of nasal potential difference.²¹ Rectal tissue, which also expresses CFTR,^{22,23} was obtained with the use of a suction-biopsy catheter placed 5 cm from the anal verge. All biopsy specimens were immediately



placed into a glass vial containing 1.2 ml of chloroform-methanol (2:1 vol/vol) containing 30 μ l of heptadecanoic acid (1 mg per milliliter) (17:0) in chloroform-methanol (1:1 vol/vol) as an internal standard. No complications occurred.

Scrapings of nasal mucosa from the inferior turbinate were obtained with a plastic Rhinoprobe (Arlington Scientific). A 2-cm row of cells was scraped from the inferior turbinate as described previously for the purification of CFTR messenger RNA,²⁴ and this procedure was repeated five to eight times to obtain sufficient tissue. Scrapings were placed in 10 ml of sterile normal saline and centrifuged at 400 \times g for five minutes at room temperature; the cell pellet was washed once and then processed for fatty-acid analysis after extraction with chloroform-methanol.

BLOOD AND LIPID ANALYSIS

Peripheral venous blood was obtained from subjects at the time of biopsy or nasal mucosal scraping, collected in a heparin-treated tube, and centrifuged at 400 \times g for 15 minutes at room temperature. The plasma was then removed, and the lipids were extracted by the addition of chloroform-methanol (2:1 vol/vol). The lipid samples were then vortexed, sonicated, and centrifuged at 400 \times g.²⁵ The organic phase of each sample was removed, and fatty acids were methylated.²⁵ Fatty acid methyl esters were quantified by gas chromatography-mass spectrometry with the use of a gas chromatograph (HP5890 Series II, Hewlett-Packard) equipped with a Supelcowax SP-10 capillary column (Supelco) attached to a mass spectrometer (HP-5971, Hewlett-Packard).

Total ion monitoring was performed, encompassing mass ranges from 50 to 550 atomic mass units. The mass of fatty acid methyl esters was determined by comparing areas of unknown fatty acid methyl esters with a fixed concentration of internal standard (equivalent to 30 µg of heptadecanoic acid for nasal-biopsy and rectal-biopsy specimens and 15 µg for nasal mucosa scrapings and plasma). All specimens were analyzed in a blinded fashion. In addition, samples from different groups of subjects were mixed in with the assay batches.

STATISTICAL ANALYSIS

Demographic characteristics are summarized as means (\pm SD) and compared between groups with the use of analysis of variance. Data on fatty acids are summarized as medians and interquartile ranges. Exclusions based on lipid analyses were made before statistical analyses were performed. The levels of fatty acids in nasal- and rectal-biopsy specimens were compared among three groups of subjects with use of the nonparametric Jonckheere–Terpstra test²⁶ for a priori ordered groups. The fatty acids from nasal-mucosa scrapings and plasma lipids were compared among groups with the use of Kruskal–Wallis tests for unordered groups. If the result of the global test was statistically significant, then Wilcoxon rank-sum tests were used to compare groups in a pairwise fashion and exact two-sided P values were reported. The analysis used SAS software, version 8.0 (SAS Institute), and StatXact software, version 5 (Cytel Software).

RESULTS

FATTY ACIDS IN NASAL-BIOPSY AND RECTAL-BIOPSY SPECIMENS

The demographic characteristics of the subjects with cystic fibrosis and the healthy control subjects who underwent a biopsy are shown in Table 1. No subject had a weight loss of more than 5 percent in the month before biopsy collection. For technical reasons, data could not be analyzed from one subject with cystic fibrosis and pancreatic insufficiency and four healthy control subjects who underwent nasal biopsy and from three subjects with cystic fibrosis and pancreatic insufficiency and two healthy control subjects who underwent rectal biopsy.

Table 2 shows the mole percentages of each fatty acid in nasal-biopsy specimens. There were significant differences between subjects with cystic fibrosis and pancreatic insufficiency and healthy control

subjects only for linoleic acid ($P=0.01$), arachidonic acid ($P=0.008$), and docosahexaenoic acid ($P=0.01$).

Since the biologic effects of fatty acids are dependent not only on the absolute levels of a particular fatty acid but also on the ratio of n–6 to n–3 fatty acids,²⁸ Figure 2 shows the individual data points for arachidonic acid and docosahexaenoic acid, as well as for the ratio of arachidonic to docosahexaenoic acid. Arachidonic acid levels were increased, with reciprocal changes in docosahexaenoic acid, in subjects with cystic fibrosis and pancreatic insufficiency, as compared with healthy control subjects (Fig. 2). The median ratio of arachidonic to docosahexaenoic acid in the subjects with cystic fibrosis

Table 1. Demographic Characteristics of Subjects Who Underwent Rectal and Nasal Biopsies.*

Group	Total No.	Sex	Age	Body-Mass Index	FEV ₁
		M/F	yr		%
Subjects with cystic fibrosis					
Pancreatic insufficiency	10	6/4	21.2 \pm 8.4	22.0 \pm 4.1	67.7 \pm 19.4
Pancreatic sufficiency	7	4/3	24.3 \pm 11.4	25.0 \pm 4.7	76.6 \pm 23.6
Healthy control subjects	11	4/7	26.5 \pm 7.2	NA	NA

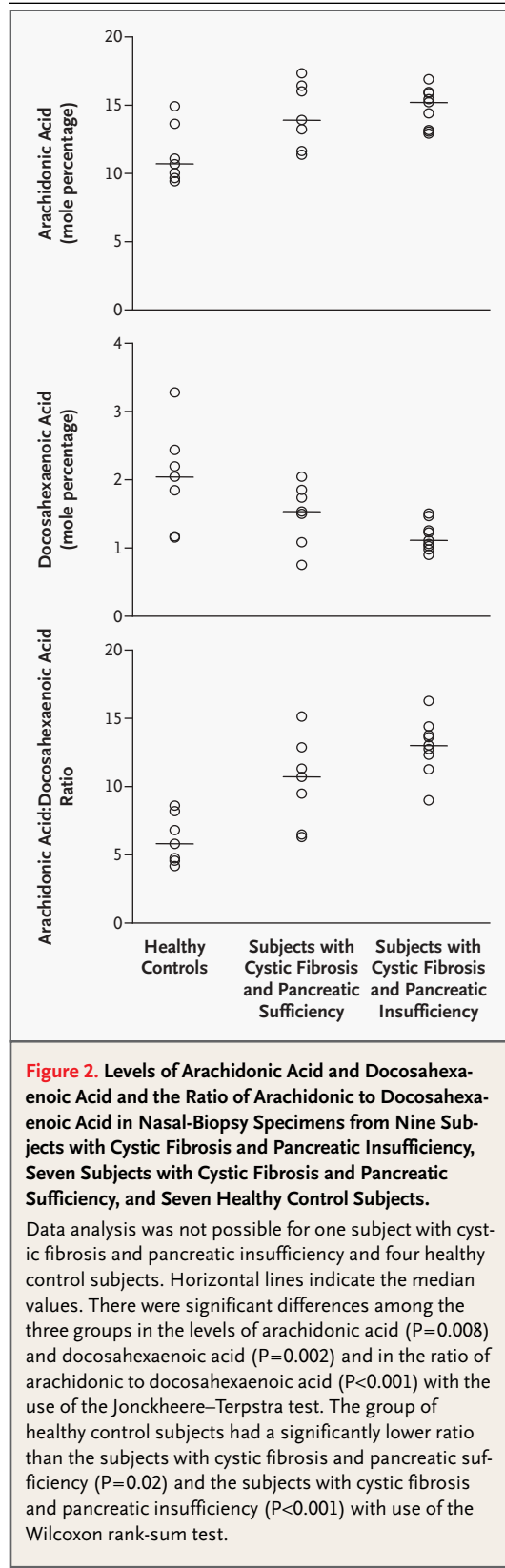
* Plus–minus values are means \pm SD. NA denotes not analyzed. Age, forced expiratory volume in one second (FEV₁) (normal >80 percent²⁷), and body-mass index (normal, 19 to 25²⁷) did not differ significantly among the groups.

Table 2. Complete Fatty Acid Analysis of Nasal-Biopsy Tissues from Subjects with Cystic Fibrosis and Pancreatic Insufficiency and Healthy Control Subjects.

Fatty Acid	Healthy Controls (N=7)*	Subjects with Cystic Fibrosis and Pancreatic Insufficiency (N=9)*	P Value†
	median (interquartile range)		
16:0	26.3 (22.4–27.6)	28.5 (26.4–29.2)	0.09
18:0n–6	19.4 (14.6–20.4)	21.2 (20.6–22.7)	0.06
Oleic acid	17.7 (16.6–26.5)	17.7 (16.8–18.5)	0.80
Linoleic acid	18.0 (13.6–18.6)	11.4 (10.2–13.0)	0.01
Dihomo- γ -linolenic acid	2.10 (1.67–3.45)	2.41 (2.28–2.52)	0.90
Arachidonic acid	10.7 (9.7–13.6)	15.2 (13.2–15.9)	0.008
22:4n–6	0.71 (0.42–0.85)	0.94 (0.78–0.99)	0.06
Docosahexaenoic acid	2.04 (1.17–2.44)	1.11 (1.02–1.25)	0.01

* Values are expressed as mole percentages.

† P values were calculated with the use of the Wilcoxon rank-sum test.



was 13.0, as compared with 5.8 in healthy control subjects ($P<0.001$). No overlap in the individual data points was observed. Although approximately 25 percent of the subjects with cystic fibrosis were having an acute exacerbation of their pulmonary disease at the time of tissue procurement, the range of values for the ratio of arachidonic to docosahexaenoic acid was similar in subjects with an acute pulmonary flare (median, 12.3; range, 9.0 to 13.0) and those without a flare (median, 13.7; range, 11.2 to 16.3). Similarly, there was no significant difference in these values when male subjects (median, 13.0; range, 12.3 to 16.3) were compared with female subjects (median, 12.4; range, 9.0 to 14.4).

Subjects with cystic fibrosis and pancreatic sufficiency were also studied, since they may have unique fatty acid profiles owing either to less severe CFTR dysfunction than subjects with pancreatic insufficiency²⁹ or to improved nutritional status. The median ratio of arachidonic to docosahexaenoic acid in subjects with cystic fibrosis and pancreatic sufficiency was 10.7, which was intermediate between the values in subjects with cystic fibrosis and pancreatic insufficiency and healthy control subjects ($P<0.001$ by the Jonckheere–Terpstra test).

In rectal tissue, the median docosahexaenoic acid level was lower ($P=0.05$) in subjects with cystic fibrosis and pancreatic insufficiency than in healthy controls, with no significant differences in arachidonic acid levels (Fig. 3). The median ratio of arachidonic to docosahexaenoic acid of 12.0 in rectal-biopsy specimens from subjects with cystic fibrosis and pancreatic insufficiency was similar to the ratio in nasal mucosal biopsy specimens and was significantly higher than the median value of 5.6 in healthy controls ($P=0.02$). There was no significant difference between subjects with pancreatic sufficiency and those with pancreatic insufficiency.

FATTY ACID LEVELS IN SCRAPINGS OF NASAL EPITHELIA MUCOSA

The demographic characteristics of the subjects from whom nasal epithelial scrapings were obtained are shown in Table 3. The individual data points for arachidonic and docosahexaenoic acids and the ratio of these two fatty acids are shown in Figure 4. Docosahexaenoic acid levels in nasal mucosal scrapings were similar to those in nasal-biopsy specimens. The range of arachidonic acid values was larger in nasal mucosal scrapings than in biopsy samples. The median ratio of arachidonic to docosahexaenoic acid in nasal mucosal cells was 9.1 in

subjects with cystic fibrosis and pancreatic insufficiency and 5.5 in obligate heterozygotes, and values in both groups were significantly different from the median value of 3.2 in healthy controls ($P < 0.001$ for each comparison).

Other inflammatory diseases were also examined. Eight of the 11 subjects with inflammatory bowel disease (7 with Crohn's disease and 1 with ulcerative colitis) had mild to moderately active disease requiring therapy, with a weight loss of no more than 5 percent in the month before sample collection. The median ratio of arachidonic to docosahexaenoic acid was lower in subjects with inflammatory bowel disease than in healthy control subjects and subjects with cystic fibrosis ($P = 0.03$ and $P < 0.001$, respectively) (Fig. 4). This difference was mostly due to the increase in docosahexaenoic acid levels.

Upper respiratory tract infection in otherwise healthy subjects was associated with a greater range of values, with the median ratio of arachidonic to docosahexaenoic acid intermediate between that observed in subjects with cystic fibrosis ($P < 0.001$) and healthy control subjects. As a control for chronic airway inflammation, nasal scrapings from 25 subjects with asthma were obtained. Nine of these subjects were excluded from the analysis owing to the presence of known *CFTR* mutations, novel variants of unknown functional significance, or inadequate samples. Of the remaining 16 subjects, 11 (69 percent) had two copies of the V470 polymorphism, 4 had one copy (25 percent), and 1 had no copies (6 percent). In total, 26 of 32 alleles (81 percent) had the V470 variant. As shown in Figure 4, arachidonic acid levels were higher in the subjects with asthma than in the subjects with cystic fibrosis ($P = 0.01$) and healthy control subjects ($P = 0.04$), whereas levels of docosahexaenoic acid were intermediate between these two groups ($P < 0.001$ for the comparison of healthy controls and subjects with cystic fibrosis). The median ratio of arachidonic to docosahexaenoic acid in subjects with asthma was 6.9, which was intermediate between that in subjects with cystic fibrosis (9.1, $P < 0.001$) and healthy control subjects (3.2, $P < 0.001$).

FATTY ACID PROFILES IN PLASMA

The fatty acid profile in plasma was examined to determine whether there were alterations in the levels of fatty acids similar to those in the tissues (Table 4). Linoleic acid and eicosatrienoic acid were also examined to determine whether an essential fatty acid-like deficiency was present in our subjects, like that

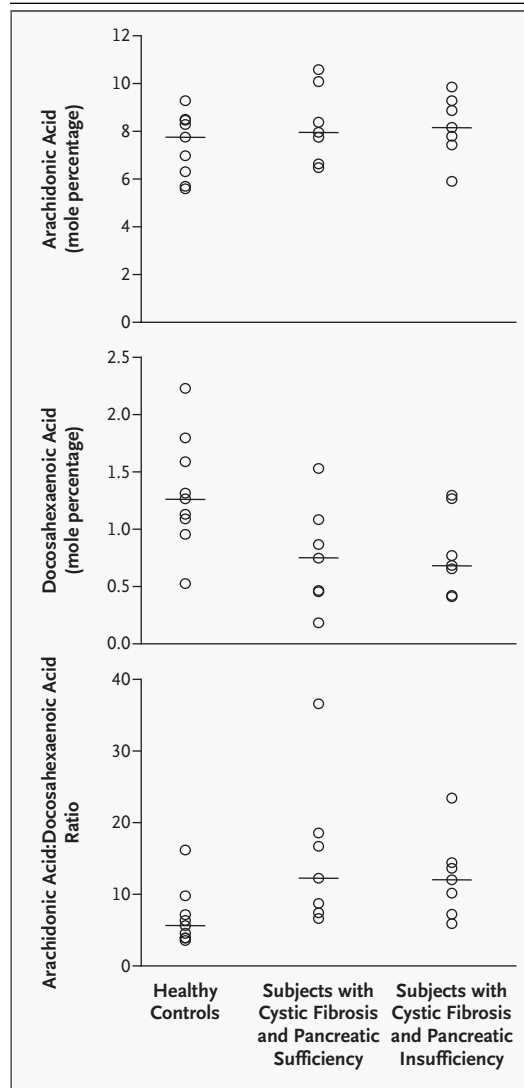


Figure 3. Levels of Arachidonic Acid and Docosahexaenoic Acid and the Ratio of Arachidonic to Docosahexaenoic Acid in Rectal-Biopsy Specimens from Seven Subjects with Cystic Fibrosis and Pancreatic Insufficiency, Seven Subjects with Cystic Fibrosis and Pancreatic Sufficiency, and Nine Healthy Control Subjects.

Data analysis was not possible for three subjects with cystic fibrosis and pancreatic insufficiency and two healthy control subjects. Horizontal lines indicate the median values. There were significant differences among the three groups in the levels of docosahexaenoic acid ($P = 0.01$) and in the ratio of arachidonic to docosahexaenoic acid ($P = 0.009$) but not in the levels of arachidonic acid ($P = 0.17$) with the use of the Jonckheere–Terpstra test. The ratio in the group of healthy control subjects differed significantly from the ratio in the group with cystic fibrosis and pancreatic sufficiency ($P = 0.01$) and in the group with cystic fibrosis and pancreatic insufficiency ($P = 0.02$) with use of the Wilcoxon rank-sum test.

Table 3. Demographic Characteristics of Subjects Who Underwent Nasal Mucosal Scraping.*

Group	Total No.	Sex	Age
		M/F	yr
Subjects with cystic fibrosis and pancreatic insufficiency	21	13/8	18.6±6.6
Subjects with asthma	16	9/7	19.1±6.0
Subjects with upper respiratory tract infection	9	3/6	28.5±6.4
Obligate heterozygotes	13	1/12	43.0±7.8
Subjects with inflammatory bowel disease	11	5/6	39.3±12.1
Healthy control subjects	16	7/9	28.8±3.6

* Ages are expressed as means (±SD) and differed significantly among the groups (P<0.001 by analysis of variance). All but three of the healthy control subjects are different from the ones who underwent nasal or rectal biopsy.

described in other reports.⁹⁻¹¹ Docosahexaenoic acid levels were significantly lower in the plasma of subjects with cystic fibrosis than in healthy control subjects (P<0.001), with intermediate values observed for obligate heterozygotes (P<0.001 for the comparison with subjects with cystic fibrosis and P=0.13 for the comparison with healthy control subjects). In contrast, arachidonic acid levels did not differ significantly among the groups (P=0.56). There was no significant difference in linoleic acid or eicosatrienoic acid levels between subjects with cystic fibrosis and healthy control subjects.

DISCUSSION

Our data demonstrate an altered ratio of arachidonic to docosahexaenoic acid in CFTR-expressing tissues in subjects with cystic fibrosis. The biologic effects of fatty acids depend not only on the absolute levels of a particular fatty acid but also on the ratio of n-6 to n-3 fatty acids.²⁸ The magnitude of these alterations and the fatty acids involved are similar to our earlier results in cystic fibrosis-knockout mice.³⁰ These data suggest that CFTR may have a role in cellular fatty acid metabolism and that abnormalities in fatty acids may affect the cystic fibrosis phenotype.

To determine whether acute or chronic inflammation can modify fatty acids, we studied three control groups with inflammatory disease. Inflammatory bowel disease was associated with very low ratios of arachidonic to docosahexaenoic acid. Subjects with asthma and acute upper respiratory tract infec-

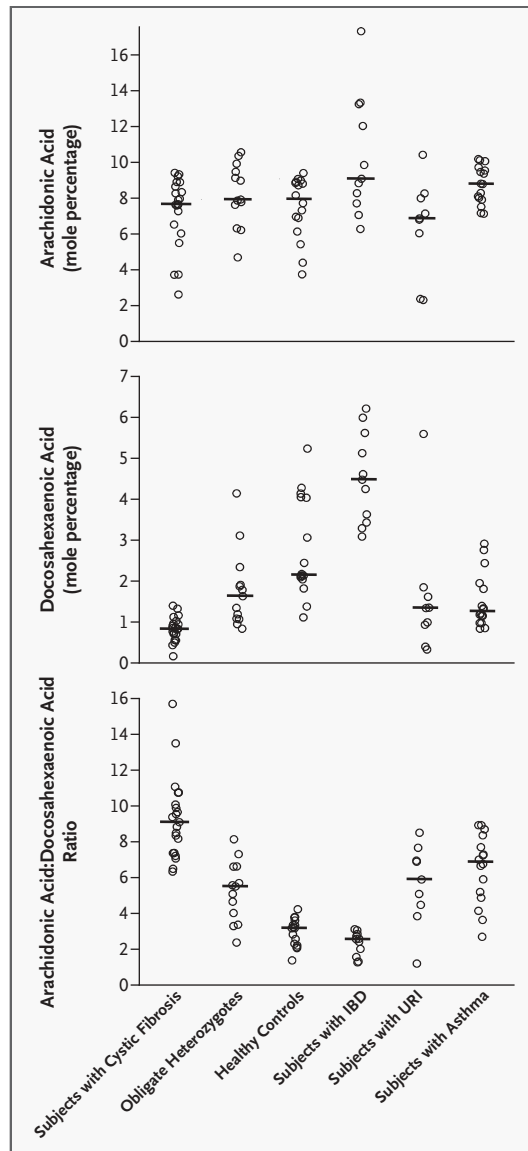


Figure 4. Levels of Arachidonic Acid and Docosahexaenoic Acid and the Ratio of Arachidonic to Docosahexaenoic Acid in Nasal Mucosal Scrapings from 21 Subjects with Cystic Fibrosis and Pancreatic Insufficiency, 13 Obligate Heterozygotes, 16 Healthy Control Subjects, 11 Subjects with Inflammatory Bowel Disease (IBD), 9 Subjects with Upper Respiratory Tract Infection (URI), and 16 Subjects with Asthma.

Horizontal lines indicate the median values. Overall, levels of arachidonic acid (P=0.01) and docosahexaenoic acid (P<0.001) and the ratios of arachidonic to docosahexaenoic acid (P<0.001) differed significantly among the six groups of subjects when analyzed by means of the Kruskal-Wallis test.

Table 4. Profile of Plasma Fatty Acids.*

Fatty Acid	Healthy Controls (N=14)	Obligate Heterozygotes (N=13)	Subjects with Cystic Fibrosis and Pancreatic Insufficiency (N=13)	P Value†‡
	<i>median (interquartile range)</i>			
Linoleic acid	25.6 (22.5–30.9)	28.9 (26.1–31.2)‡	19.9 (19.1–25.9)	0.01
Eicosatrienoic acid	0.10 (0.05–1.05)	0.00 (0–0.17)§	0.55 (0.24–0.84)	0.01
Arachidonic acid	7.0 (5.5–8.3)	7.7 (6.9–8.1)	7.7 (5.4–8.3)	0.56
Docosahexaenoic acid	1.72 (1.35–2.12)	1.26 (1.07–1.74)	0.60 (0.45–0.92)¶	<0.001

* Values are expressed as mole percentages.

† P values were calculated with the use of the Kruskal–Wallis test.

‡ P=0.002 for the comparison with the subjects with cystic fibrosis and pancreatic insufficiency by means of the Wilcoxon rank-sum test.

§ P=0.005 for the comparison with the subjects with cystic fibrosis and pancreatic insufficiency by means of the Wilcoxon rank-sum test.

¶ P<0.001 for the comparison with healthy controls and obligate heterozygotes by means of the Wilcoxon rank-sum test.

tion had a median value intermediate between that in subjects with cystic fibrosis and healthy control subjects. On the basis of the finding of an increased prevalence of CFTR gene mutations in subjects with asthma,³¹ we performed CFTR sequencing in all subjects with asthma, and those with mutations or variants were excluded. A large proportion of our subjects with asthma had the M470V allele, which may cause decreased CFTR function.³² The fatty acid abnormality in this group could be due to CFTR dysfunction, and such a possibility would be supported by the finding that obligate heterozygotes have fatty acid changes of similar magnitude. However, the fact that subjects with acute upper respiratory tract infection had fatty acid levels similar to those of the subjects with asthma suggests that inflammation may also explain this degree of fatty acid abnormality. The much greater change in the levels in subjects with cystic fibrosis indicates that inflammation alone is unlikely to explain all the changes in the arachidonic and docosahexaenoic acid levels.

Two lines of evidence support the suggestion that this fatty acid abnormality is caused by abnormal or mutant CFTR and not by abnormal intestinal absorption of fat due to pancreatic insufficiency. First, the ratio of arachidonic to docosahexaenoic acid was abnormal in nasal and rectal tissue from subjects with cystic fibrosis and pancreatic sufficiency, who have normal assimilation of micronutrients and macronutrients. Second, the ratio in nasal mucosal scrapings from obligate heterozygotes was intermediate between values in subjects with cystic fibrosis and healthy control subjects. This finding is

in agreement with previous reports that CFTR function is not normal in obligate heterozygotes^{33,34} and is associated with the development of cystic fibrosis-related diseases in humans such as sinusitis³⁵ and chronic pancreatitis.^{36,37}

The abnormal tissue levels of docosahexaenoic acid cannot solely be due to the low plasma levels, since fatty acid metabolism is cell-specific and the fatty acids in cells of CFTR-regulated tissues may not reflect the levels of fatty acids in the circulation.^{38–41} The findings in cystic fibrosis–knockout mice that the fatty acid abnormality is present only in affected tissues and that plasma fatty acids were the same as those in their wild-type littermates³⁰ indicates that the fatty acid defect can occur in the absence of alterations in plasma fatty acids. Although future studies should strictly control the dietary intake of fatty acids, especially eicosapentaenoic acid and docosahexaenoic acid, diet alone does not fully explain the fatty acid abnormality we identified.

Although the mechanism by which CFTR regulates fatty acid metabolism is unknown, the low docosahexaenoic acid levels may be important in the excessive host inflammatory response in cystic fibrosis. Docosahexaenoic acid is converted to docosatrienes and 17S series resolvins, which are potent antiinflammatory mediators that are normally generated during the resolution of inflammation.^{42,43} Therefore, the low docosahexaenoic acid levels in patients with cystic fibrosis may explain, at least in part, the inflammatory state associated with this disease and may have important therapeutic implications. We have previously shown that oral adminis-

tration of high doses of docosahexaenoic acid to cystic fibrosis—knockout mice corrects the fatty acid abnormality, reverses the histologic changes in the pancreas and ileum, and decreases neutrophil levels in mice with pseudomonas lipopolysaccharide-induced pneumonia.^{30,44} It will be important to determine whether correction of this fatty acid defect represents a treatment for cystic fibrosis.

Funded by grants from the Cystic Fibrosis Foundation and Genzyme Corporation (to Drs. Freedman and Alvarez) and supported in part by a grant (RR 01032, to the Beth Israel Deaconess Medical Center General Clinical Research Center) from the National Institutes of Health.

We are indebted to Joanne Cluette-Brown for her analysis of the lipid samples by gas chromatography–mass spectrometry; to Maynard Hansen, M.D. (Ear, Nose, and Throat), and Stephen Hardy, M.D. (Pediatric Gastroenterology), for procuring nasal- and rectal-biopsy samples, respectively; and to Rhoda Spaulding, M.S.N., F.N.P., for the enrollment of subjects at UMass Memorial Health Care.

REFERENCES

- Balough K, McCubbin M, Weinberger M, Smits W, Ahrens R, Fick R. The relationship between infection and inflammation in the early stages of lung disease from cystic fibrosis. *Pediatr Pulmonol* 1995;20:63-70.
- Khan TZ, Wagener JS, Bost T, Martinez J, Accurso FJ, Riches DW. Early pulmonary inflammation in infants with cystic fibrosis. *Am J Respir Crit Care Med* 1995;151:1075-82.
- Muhlebach MS, Stewart PW, Leigh MW, Noah TL. Quantitation of inflammatory responses to bacteria in young cystic fibrosis and control patients. *Am J Respir Crit Care Med* 1999;160:186-91.
- Noah TL, Black HR, Cheng PW, Wood RE, Leigh MW. Nasal and bronchoalveolar lavage fluid cytokines in early cystic fibrosis. *J Infect Dis* 1997;175:638-47.
- Kammouni W, Figarella C, Marchand S, Merten M. Altered cytokine production by cystic fibrosis tracheal gland serous cells. *Infect Immun* 1997;65:5176-83.
- Tabary O, Zahm JM, Hinrasky J, et al. Selective up-regulation of chemokine IL-8 expression in cystic fibrosis bronchial gland cells in vivo and in vitro. *Am J Pathol* 1998;153:921-30.
- Carlstedt-Duke J, Bronnegard M, Strandvik B. Pathological regulation of arachidonic acid release in cystic fibrosis: the putative basic defect. *Proc Natl Acad Sci U S A* 1986;83:9202-6.
- Kuo PT, Huang NN, Bassett DR. The fatty acid composition of the serum chylomicrons and adipose tissue of children with cystic fibrosis of the pancreas. *J Pediatr* 1962;60:394-403.
- Farrell PM, Mischler EH, Engle MJ, Brown DJ, Lau SM. Fatty acid abnormalities in cystic fibrosis. *Pediatr Res* 1985;19:104-9.
- Hubbard VS, Dunn GD, di Sant'Agnese PA. Abnormal fatty acid composition of plasma-lipids in cystic fibrosis: a primary and secondary defect? *Lancet* 1977;2:1302-4.
- Lloyd-Still JD, Johnson SB, Holman RT. Essential fatty acid status in cystic fibrosis and the effects of safflower oil supplementation. *Am J Clin Nutr* 1981;34:1-7.
- Biggemann B, Laryea MD, Schuster A, Griese M, Reinhardt D, Bremer HJ. Status of plasma and erythrocyte fatty acids and vitamin A and E in young children with cystic fibrosis. *Scand J Gastroenterol Suppl* 1988;143:135-41.
- Sprecher H. Metabolism of highly unsaturated n-3 and n-6 fatty acids. *Biochim Biophys Acta* 2000;1486:219-31.
- Roulet M, Frascarolo P, Rappaz I, Pilet M. Essential fatty acid deficiency in well nourished young cystic fibrosis patients. *Eur J Pediatr* 1997;156:952-6.
- Strandvik B, Gronowitz E, Enlund F, Martinsson T, Wahlstrom J. Essential fatty acid deficiency in relation to genotype in patients with cystic fibrosis. *J Pediatr* 2001;139:650-5.
- Christophe AB, Warwick WJ, Holman RT. Serum fatty acid profiles in cystic fibrosis patients and their parents. *Lipids* 1994;29:569-75.
- Kutty KM, Guha AK, Chandra RK, Vaze D. A study of erythrocyte fatty acids, adenosinetriphosphatase and acetylcholinesterase in cystic fibrosis. *Clin Biochem* 1979;12:98-9.
- Rosenstein BJ, Cutting GR. The diagnosis of cystic fibrosis: a consensus statement. *J Pediatr* 1998;132:589-95.
- Taussig LM, Katwinkel J, Friedewald WT, Di Sant'Agnese PA. A new prognostic score and clinical evaluation system for cystic fibrosis. *J Pediatr* 1973;82:380-90.
- Borowitz D, Baker RD, Stallings V. Consensus report on nutrition for pediatric patients with cystic fibrosis. *J Pediatr Gastroenterol Nutr* 2002;35:246-59.
- Knowles M, Gatz J, Boucher R. Increased bioelectric potential difference across respiratory epithelia in cystic fibrosis. *N Engl J Med* 1981;305:1489-95.
- Goldstein JL, Nash NT, al-Bazzaz F, Layden TJ, Rao MC. Rectum has abnormal ion transport but normal cAMP-binding proteins in cystic fibrosis. *Am J Physiol* 1988;254:C719-C724.
- Veeze HJ, Sinaasappel M, Bijman J, Bouquet J, de Jonge HR. Ion transport abnormalities in rectal suction biopsies from children with cystic fibrosis. *Gastroenterology* 1991;101:398-403.
- Knowles MR, Hohneker KW, Zhou Z, et al. A controlled study of adenoviral-vector-mediated gene transfer in the nasal epithelium of patients with cystic fibrosis. *N Engl J Med* 1995;333:823-31.
- Alvarez JG, Storey BT. Differential incorporation of fatty acids into and peroxidative loss of fatty acids from phospholipids of human spermatozoa. *Mol Reprod Dev* 1995;42:334-46.
- Hollander M, Wolfe DA. Nonparametric statistical methods. 2nd ed. New York: John Wiley, 1999:202-12.
- Willett WC, Dietz WH, Colditz GA. Guidelines for healthy weight. *N Engl J Med* 1999;341:427-34.
- Rubin D, Laposata M. Cellular interactions between n-6 and n-3 fatty acids: a mass analysis of fatty acid elongation/desaturation, distribution among complex lipids, and conversion to eicosanoids. *J Lipid Res* 1992;33:1431-40.
- Durno C, Corey M, Zielenski J, Tullis E, Tsui L-C, Durie P. Genotype and phenotype correlations in patients with cystic fibrosis and pancreatitis. *Gastroenterology* 2002;123:1857-64.
- 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 U S A* 1999;96:13995-4000.
- Tzietis M, Efthymiadou A, Strofalis S, et al. CFTR gene mutations — including three novel nucleotide substitutions — and haplotype background in patients with asthma, disseminated bronchiectasis and chronic obstructive pulmonary disease. *Hum Genet* 2001;108:216-21.
- Cuppens H, Lin W, Jaspers M, et al. Polyvariant mutant cystic fibrosis transmembrane conductance regulator genes: the polymorphic (Tg)m locus explains the partial penetrance of the T5 polymorphism as a disease mutation. *J Clin Invest* 1998;101:487-96.
- Farrell PM, Kosciak RE. Sweat chloride concentrations in infants homozygous or heterozygous for F508 cystic fibrosis. *Pediatrics* 1996;97:524-8.
- Gabriel SE, Brigman KN, Koller BH, Boucher RC, Stutts MJ. Cystic fibrosis heterozygote resistance to cholera toxin in the cystic fibrosis mouse model. *Science* 1994;266:107-9.
- Wang X, Moylan B, Leopold DA, et al. Mutation in the gene responsible for cystic

- fibrosis and predisposition to chronic rhinosinusitis in the general population. *JAMA* 2000;284:1814-9.
36. Cohn JA, Friedman KJ, Noone PG, Knowles MR, Silverman LM, Jowell PS. Relation between mutations of the cystic fibrosis gene and idiopathic pancreatitis. *N Engl J Med* 1998;339:653-8.
37. Sharer N, Schwarz M, Malone G, et al. Mutations of the cystic fibrosis gene in patients with chronic pancreatitis. *N Engl J Med* 1998;339:645-52.
38. Helge JW, Wu BJ, Willer M, Daugaard JR, Storlien LH, Kiens B. Training affects muscle phospholipid fatty acid composition in humans. *J Appl Physiol* 2001;90:670-7.
39. Laposata M. Fatty acids: biochemistry to clinical significance. *Am J Clin Pathol* 1995; 104:172-9.
40. London SJ, Sacks FM, Caesar J, Stampfer MJ, Siguel E, Willett WC. Fatty acid composition of subcutaneous adipose tissue and diet in postmenopausal US women. *Am J Clin Nutr* 1991;54:340-5.
41. Alasnier C, Berdeaux O, Chardigny J-M, Sebedio J-L. Fatty acid composition and conjugated linoleic acid content of different tissues in rats fed individual conjugated linoleic acid isomers given as triacylglycerols small star, filled. *J Nutr Biochem* 2002;13: 337-45.
42. Hong S, Gronert K, Devchand PR, Moussignac R-L, Serhan CN. Novel docosatrienes and 17S-resolvins generated from docosahexaenoic acid in murine brain, human blood, and glial cells: autacoids in anti-inflammation. *J Biol Chem* 2003;278: 14677-87.
43. Serhan CN, Hong S, Gronert K, et al. Resolvins: a family of bioactive products of omega-3 fatty acid transformation circuits initiated by aspirin treatment that counter proinflammation signals. *J Exp Med* 2002; 196:1025-37.
44. Freedman SD, Weinstein D, Blanco PG, et al. Characterization of LPS-induced lung inflammation in *cftr*^{-/-} mice and the effect of docosahexaenoic acid. *J Appl Physiol* 2002;92:2169-76.

Copyright © 2004 Massachusetts Medical Society.

FULL TEXT OF ALL JOURNAL ARTICLES ON THE WORLD WIDE WEB

Access to the complete text of the *Journal* on the Internet is free to all subscribers. To use this Web site, subscribers should go to the *Journal*'s home page (www.nejm.org) and register by entering their names and subscriber numbers as they appear on their mailing labels. After this one-time registration, subscribers can use their passwords to log on for electronic access to the entire *Journal* from any computer that is connected to the Internet. Features include a library of all issues since January 1993 and abstracts since January 1975, a full-text search capacity, and a personal archive for saving articles and search results of interest. All articles can be printed in a format that is virtually identical to that of the typeset pages. Beginning six months after publication, the full text of all Original Articles and Special Articles is available free to nonsubscribers who have completed a brief registration.