

Increased plasma fatty acid concentrations after respiratory exacerbations are associated with elevated oxidative stress in cystic fibrosis patients¹⁻³

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ABSTRACT

Background: Oxidative stress and depleted antioxidant defenses occur in stable cystic fibrosis patients. During acute infection, the balance between oxidants and antioxidants may be further disturbed.

Objective: We examined the oxidative stress during acute infection in cystic fibrosis patients by measuring 8-*iso*-prostaglandin F_{2α} (8-*iso*-PGF_{2α}) and antioxidant defenses in relation to dietary intake, fatty acid status, immune function, and clinical status.

Design: Plasma concentrations of total 8-*iso*-PGF_{2α}, vitamins E and C, β-carotene, zinc, selenium, and copper; plasma fatty acid compositions; erythrocyte glutathione concentrations; glutathione peroxidase and superoxide dismutase activity; sputum glutathione and 8-*iso*-PGF_{2α} concentrations; lung function; clinical symptoms; and dietary intake were measured in 15 cystic fibrosis patients before and after 10–14 d of intravenous antibiotic treatment for a pulmonary exacerbation.

Results: After treatment, respiratory status improved (percentage of forced expiratory volume in 1 s: 60 ± 6% at baseline compared with 74 ± 7% after treatment, *P* = 0.01), quality of well-being improved (*P* = 0.001), and total plasma 8-*iso*-PGF_{2α} concentrations increased from 469 nmol/L at baseline (interquartile range: 373–554 nmol/L) to 565 nmol/L after treatment (interquartile range: 429–689 nmol/L; *P* = 0.008). Total energy, fat, carbohydrate, and protein intakes per kilogram body weight also increased; however, dietary antioxidant intake was unchanged. Plasma fatty acid concentrations increased after treatment, strongly correlating with plasma 8-*iso*-PGF_{2α} concentrations (*r* = 0.768, *P* = 0.001). There were no significant changes in white cell counts or plasma concentrations of vitamins E and C or β-carotene. Erythrocyte glutathione peroxidase activity was reduced after treatment, whereas there was no significant change in superoxide dismutase activity.

Conclusions: Oxidative stress increased after treatment for pulmonary exacerbations and was strongly linked to increased concentrations of plasma fatty acids. Although intravenous antibiotic therapy and physiotherapy improved lung function within 10–14 d of treatment, the biochemical effects of oxidation continued further. Thus, antioxidant intervention during treatment for and recovery from acute infection in cystic fibrosis should be considered. *Am J Clin Nutr* 2002;75:668–75.

KEY WORDS Cystic fibrosis, oxidative stress, exacerbation, antioxidants, isoprostanes, fatty acids, vitamin E, vitamin C, β-carotene, glutathione peroxidase, superoxide dismutase

INTRODUCTION

Cystic fibrosis patients are predisposed to oxidative stress (1–3). Factors such as neutrophil response to infection (4) and increased metabolic rate (5) increase oxidant burden, although cystic fibrosis patients have reduced antioxidant protection. Despite the administration of pancreatic enzyme supplements to 90% of cystic fibrosis patients with pancreatic insufficiency, residual steatorrhea and azotorrhea occur (6) with the potential for ongoing malabsorption of fat-soluble antioxidant vitamins, namely vitamin E and β-carotene. Malabsorption leads to energy losses of ≤10–20%, which, when considered with the increased resting energy expenditure of cystic fibrosis patients, produce an energy requirement that is 120–150% of the US recommended daily allowance (5). The high-fat diet recommended to meet this increased energy requirement may alter antioxidant protection, ie, although there may be an increased intake of fat-soluble antioxidants (eg, vitamin E), intake of antioxidants from foods such as fruit and vegetables (namely β-carotene and vitamin C) may be limited. Furthermore, the loss of permeability of glutathione in the cystic fibrosis transmembrane conductance regulator channel may result in the deficiency of glutathione in the fluid lining the respiratory tract (7). Consequently, many different and interacting factors can potentially lead to increased oxidative stress in cystic fibrosis patients.

There is much evidence confirming the occurrence of oxidative stress during cystic fibrosis (1, 2). Reported indexes include elevated plasma malondialdehyde (8–11), breath pentane and ethane (12), and plasma hydroperoxide concentrations (8, 11)

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and depletion of the major lipoperoxidation substrates, linoleic and arachidonic acid (8, 13). We (14) and Montuschi et al (15) have observed elevated 8-*iso*-prostaglandin F_{2α} (8-*iso*-PGF_{2α}) in stable cystic fibrosis patients. However, studies of oxidative stress during pulmonary exacerbation in cystic fibrosis are conflicting; various studies show that after treatment, oxidative stress increases [protein carbonyls (16)], decreases [lipid hydroperoxides (17)], or does not change [malondialdehyde (16, 17)], protein carbonyls (17), and sputum nitrate/nitrite (18)].

During a pulmonary exacerbation in cystic fibrosis, the oxidant-antioxidant balance may be further disturbed as a result of the influx of neutrophils, which characterize an acute infection (19), and increased resting energy expenditure (20). Furthermore, during acute infection, nutrient intake may decrease because of appetite loss, vomiting, or interactions with certain drugs (21). It is hypothesized that altered nutritional status (in particular antioxidant and fatty acid status) may contribute to changes in oxidative stress after treatment for pulmonary exacerbations.

To understand further the importance of oxidative stress in cystic fibrosis patients, we examined the changes in oxidative stress that occur after treatment for a pulmonary exacerbation by measuring 8-*iso*-PGF_{2α} and antioxidant defenses in relation to dietary intake, fatty acid status, immune function, and clinical status.

SUBJECTS AND METHODS

Study design

Fifteen cystic fibrosis patients aged 7–29 y were enrolled upon admission to the John Hunter Hospital for treatment of an acute pulmonary exacerbation. Patients were eligible for entry into the present study if they had a minimum of 4 out of 11 of the following characteristics: change in expectorated sputum (volume, color, or consistency); increased cough (change in character or frequency); new or increased hemoptysis; increased dyspnea during age-appropriate activities; malaise, fatigue, or lethargy; anorexia or weight loss; sinus pain or tenderness; change in nasal discharge; change in physical examination of chest; decrease in pulmonary function [forced expiratory volume in 1 s (FEV₁) or forced vital capacity (FVC)] by ≥10% from previous exam; or chest radiographic changes indicative of pulmonary infection. Thus, all patients entering the study were experiencing an exacerbation severe enough to warrant intravenous antibiotics. A common treatment protocol was established for all patients entering the study, which included the following: intravenous antibiotic treatment for ≥10–14 d; no change in vitamin intake; physiotherapy twice daily; cystic fibrosis–ward diet as advised by a dietitian; gastrostomy or intragastric feeds as clinically indicated; pancreatic enzyme supplements; bronchodilators (to be used twice daily before physiotherapy when an asthmatic component exists); systemic corticosteroids (not to be initiated as treatment and only used if the patient is taking a stable maintenance dose); or inhaled corticosteroids for asthma prophylaxis (same dose).

Blood and sputum samples were collected upon commencement of intravenous antibiotic treatment and again after 10–14 d of intravenous antibiotic treatment. On each occasion, 15 mL blood was collected and a spontaneous sputum sample was requested. The blood sample was drawn from nonfasting patients because it was considered unethical to add to the patients' burden by requiring a fasting period upon hospital admission. Other

investigators have shown that it is unlikely that plasma isoprostane, fatty acid, or total antioxidant status (including vitamin E) are altered by postprandial effects (22). Lung function and dietary intake were also recorded. The diagnosis of cystic fibrosis was previously confirmed by elevated sweat chloride concentrations in all cases. The exclusion criterion was age <5 y (unable to perform reproducible spirometry). Written, informed consent was obtained from the subjects or their guardians. Ethics approval was obtained from the Hunter Area Health Service Research and the University of Newcastle Human Research Ethics Committees.

Subject characteristics

Routine pulmonary function tests were performed in all subjects before and after 10–14 d of antibiotic treatment by using a spirometer (1085D Breeze cardiorespiratory diagnostic software, version 3.8, 1991; Medgraphics, St Paul, MN) with established normal values (23). FVC and FEV₁ were recorded and compared with predicted values. Height was measured with a Dyfed stadiometer (Holtain, Crymych, United Kingdom). Weight was recorded by using digital scales (model no. 824/890; GEC/Avery, Somerset, United Kingdom). Full blood counts were performed by using a COULTER GEN-S hematology analyzer (Beckman-Coulter, Fullerton, CA). Other markers of respiratory status were recorded, which included finger clubbing (bulbous swelling on the terminal phalanges of the fingers and toes) and chest auscultation (crackles or wheezing). Other characteristics recorded included genotype (ΔF508 zygosity), pancreatic sufficiency, other related conditions, medications used during treatment, history of antibiotic use and hospitalizations over the past 2 y for intravenous antibiotics, and missed school or work days in past 12 mo. Patient perception of well-being was quantified by using the quality-of-well-being questionnaire, which records physical activity, social activity, mobility, and clinical symptoms (24).

Vitamin and mineral analysis

Blood samples were drawn into EDTA-coated tubes and centrifuged at 3000 × *g* for 10 min at 4°C. Plasma was collected and frozen at –70°C within 30 min of blood collection. Vitamins A and E and β-carotene were separated on a reverse-phase HPLC column and measured by using a programmable wavelength ultraviolet-visible detector (25). Samples were thawed, mixed with ethanol (for the precipitation of proteins), and mixed by vortex before the addition of hexane. After mixing by vortex again, samples were centrifuged at 3000 × *g* for 10 min at 4°C and the hexane phase was removed and injected into an HPLC column [300 × 3.5 mm internal diameter, lab-packed Whatman ODS 3 (5 μm) (Whatman International, Kent, United Kingdom)] at a flow rate of 1 mL/min and a run time of 20 min at ambient temperature. At 0.01 min, vitamin A was measured at 310 nm; at 5.5 min, vitamin E was measured at 280 nm; and at 9.0 min, β-carotene was measured at 450 nm. Plasma vitamin C was separated on a reverse-phase HPLC column and measured by using an electrochemical detector (26). Samples were mixed with trichloroacetic acid (for the precipitation of proteins), mixed by vortex, and centrifuged at 3000 × *g* for 10 min at 4°C, and the supernatant fluid was injected into an HPLC column [150 × 3.5 mm internal diameter, lab-packed Whatman ODS 3 (5 μm) (Whatman International)] at a flow rate of 1 mL/min and a run time of 15 min at ambient temperature. Measurements were made with



an amperometric electrochemical detector with a potential of 0.6 V against an Ag/AgCl reference electrode (26). Plasma concentrations of zinc, selenium, and copper were analyzed by inductively coupled plasma mass spectrometry. Samples were diluted in an ammonium EDTA-based diluent in a quantitative application. Platinum and rhodium were used as internal standards in the diluent. Calibration was performed by use of a standard technique in a pooled plasma base.

Total (free and esterified) isoprostane assays

Sputum preparation

Sputum was collected and chilled, and mucus plugs were placed in a tube precoated with butylated hydroxytoluene (BHT; Sigma Chemical Company, St Louis). The mucus plugs were diluted 3-fold with phosphate-buffered saline (pH = 7.4) and mixed by vortex for 1 min. After centrifugation at $8000 \times g$ for 20 min at 4°C, the supernatant fluid was stored in tubes precoated with BHT at -70°C until analyzed.

Plasma preparation

Blood samples were collected in EDTA-coated tubes that contained reduced glutathione (Sigma Chemical Company) as an antioxidant. The samples were centrifuged at $3000 \times g$ for 10 min at 4°C. The plasma fraction was removed and stored at -70°C in tubes precoated with BHT (Sigma Chemical Company) for isoprostane analysis. Tritium-labeled prostaglandin (PGF_{2α}; Amersham, Arlington Heights, IL) was added to an aliquot of plasma for the determination of the recovery rate after purification. Ethanol was added for precipitation of the proteins. Potassium hydroxide was added to the resulting supernatant fluid and the solution was incubated to cleave esterified isoprostanes. The sample was acidified and purified by first passing through a Sep-Pak C-18 reverse-phase cartridge (Waters, Milford, MA) followed by further purification by use of a Sep-Pak Silica cartridge (Waters) (27).

Enzyme immunoassay

Purified plasma and sputum were analyzed with an 8-isoprostane enzyme immunoassay kit (Cayman Chemical, Ann Arbor, MI) (14). Absorbance values were measured by using a plate reader (Multiskan Ascent; Thermo Labsystems, Helsinki) that used a wavelength of 405 nm, with the raw data corrected for recovery. The assay was validated by adding a series of known amounts of pure 8-*iso*-PGF_{2α} standard to base volumes of purified plasma. The concentration of total 8-*iso*-PGF_{2α} in these samples was determined by enzyme immunoassay. A high correlation (0.99) was obtained between the known amounts of pure 8-*iso*-PGF_{2α} added and the concentration determined by enzyme immunoassay. The antiserum used in this assay has 100% cross-reactivity with 8-*iso*-PGF_{2α}; 0.2% each with PGF_{2α}, PGF_{3α}, PGE₁, and PGE₂ and 0.1% with 6-*keto*-PGF_{1α}. The detection limit of the assay is 4 ng/L. This kit has been used to measure 8-*iso*-PGF_{2α} concentrations in human plasma, bronchoalveolar lavage, and other fluids (14, 15, 28).

Plasma fatty acid analysis

Whole blood was collected into EDTA-coated tubes and was centrifuged at $3000 \times g$ for 10 min at 4°C. The plasma was separated and stored at -70°C until analysis. Total fatty acids were measured by using the method of Lepage and Roy (29). Two milliliters of a methanol-to-toluene mixture (4:1, by vol), containing

21:0 (0.02 g/L) and BHT (0.12 g/L) was added to 200 μL plasma. Fatty acids were methylated by adding 200 μL acetyl chloride drop by drop while mixing by vortex and heating the sample to 100°C for 1 h. After cooling, the reaction was stopped by adding 5 mL 6% K₂CO₃. The sample was centrifuged at $3000 \times g$ for 10 min at 4°C to facilitate the separation of layers. The upper toluene layer was used for gas chromatography analysis of the fatty acid methyl esters by use of a 30 m × 0.25 mm fused carbon-silica column (DB-225) that was coated with cyanopropylphenyl (J & W Scientific, Folsom, CA). Both the injector and detector port temperatures were set at 250°C. The oven temperature was initially set at 170°C for 2 min, was increased 10°C/min until the temperature of 190°C was reached and held for 1 min, at which point the temperature was then increased 3°C/min until the temperature of 220°C was reached and maintained, giving a total run time of 30 min. A split ratio of 10:1 and an injection volume of 5 mL were used. The chromatograph was equipped with a flame ionization detector, autosampler, and autodetector. Sample fatty acid methyl ester peaks were identified by comparing their retention times with those of a standard mixture of fatty acid methyl esters and were quantified by using a Hewlett Packard 6890 series gas chromatograph with chemstations (version A.04.02; Palo Alto, CA) for gas chromatographic analysis.

Glutathione peroxidase enzyme assay

Whole blood was collected into EDTA-coated tubes and centrifuged at $8500 \times g$ for 10 min at 4°C. Plasma was discarded and cells were washed with 10 volumes cold buffer, which consisted of 50 mmol tris-HCl/L [(Tris(hydroxymethyl)aminomethane hydrochloride; pH = 7.5) containing 5 mmol EDTA/L and 1 mmol dithiothreitol/L. Samples were centrifuged again at $8500 \times g$ for 10 min at 4°C and the supernatant fluid was discarded. Cells were then lysed by adding exactly 4 volumes of ice-cold deionized water. After centrifuging again at $8500 \times g$ for 10 min at 4°C, the supernatant fluid was collected and stored at -70°C for analysis. Erythrocyte glutathione peroxidase activities were measured by using a GPx-340 spectrophotometric assay kit (Bioxytech; OXIS International, Portland, OR) to obtain values in units per milliliter. The hemoglobin concentration of the samples was also measured by using kit no. 525 for total hemoglobin (Sigma) to obtain erythrocyte glutathione peroxidase activity values in units per gram hemoglobin.

Superoxide dismutase enzyme assay

Whole blood was collected into EDTA-coated tubes and centrifuged at $3000 \times g$ for 10 min at 4°C. The erythrocyte pellet was separated and stored at -70°C before analyzed, at which time it was thawed, resuspended in 4 vol ice-cold water, and mixed thoroughly by vortex. An ice-cold extraction reagent of ethanol:chloroform (62.5:37.5, by vol) was added to the erythrocyte suspension and the suspension was mixed by vortex for 30 s. After the samples were centrifuged at $3000 \times g$ for 10 min at 4°C, the upper phase was collected and stored at -70°C until analyzed. Erythrocyte Cu/Zn superoxide dismutase activities were measured by using a superoxide dismutase-525 spectrophotometric assay kit (Bioxytech; OXIS International) to obtain values in units per milliliter. The hemoglobin concentration of the samples was also measured by using kit no. 525 for total hemoglobin (Sigma) to obtain erythrocyte Cu/Zn superoxide dismutase activity values in units per milligram hemoglobin.



TABLE 1
Subject characteristics¹

Age (y)	15.9 ± 1.7 ²
< 12 (n)	4
12–18 (n)	7
> 18 (n)	4
Sex (n)	
Males	11
Females	4
Height (cm)	159 (128–169) ³
z Score for height	–1.1 ± 0.3
Weight (kg)	51 (29–55)
z Score for weight	–0.9 ± 0.2
BMI (kg/m ²)	18.9 ± 0.6
Shwachman score ⁴	62 ± 4
No. of hospital admissions in past 2 y	2.7 ± 0.5
No. of courses of intravenous antibiotics in past 2 y	2.6 ± 0.5
No. of courses of nebulized antibiotics in past 2 y	4.2 ± 0.8
No. of missed school days in past 12 mo	27 ± 6

¹n = 15.² $\bar{x} \pm \text{SEM}$.³Median; interquartile range in parentheses.⁴Range: 0–100.

Glutathione assay

Erythrocyte analysis

Whole blood was collected into EDTA-coated tubes and centrifuged at 3000 × g at 4°C for 10 min. The erythrocyte pellet was separated and stored at –70°C until analyzed (≤14 d), at which time it was thawed and resuspended in 4 volumes 5% ice-cold metaphosphoric acid. After the sample was mixed by vortex and centrifuged at 3000 × g for 10 min at 4°C, the upper aqueous layer was collected and assayed with the use of a GSH-400 spectrophotometric assay kit (Bioxytech; OXIS International). The hemoglobin concentrations of the samples were measured by using kit no. 525 for total hemoglobin (Sigma) to obtain erythrocyte glutathione concentration values in micromoles per milligram hemoglobin.

Sputum analysis

Sputum was collected and chilled, and mucus plugs were placed in a tube precoated with BHT. The mucus plugs were diluted 3-fold with phosphate-buffered saline (pH = 7.4) and mixed by vortex for 1 min. After the solution was centrifuged at 8000 × g for 20 min at 4°C, the supernatant fluid was stored in tubes precoated with BHT at –70°C until analyzed (≤14 d), at which time they were thawed and assayed with the use of a GSH-400 spectrophotometric assay kit (Bioxytech; OXIS International).

Dietary intake

Dietary intake was assessed by using 24-h dietary recalls completed upon admission to the hospital and again after treatment, just before discharge (30). Food records were analyzed by using the DIET/1 nutrient calculation software (version 4.0; Xyris Software, Highgate Hill, Australia), which is based on the 1992 Australian food tables and the composition of Australian manufactured foods (31). The mean nutrient intakes for each subject group were determined from these analyses.

Statistical analysis

Results were analyzed by using the MINITAB software, version 12 for WINDOWS (Minitab Inc, State College, PA). Data

were tested for normality by using the Anderson-Darling test. Statistical comparisons were performed by using the paired Student's *t* test for normally distributed data and the Wilcoxon test for non-normally distributed data. Normally distributed data are reported as means ± SEs and nonparametric data are reported as median and interquartile ranges (ie, quartile 1–quartile 3). Differences were considered significant when *P* < 0.05. Relations between variables were studied by linear regression using Pearson's product-moment correlation coefficient. Nonparametric data were normalized by using log and square root transformations (32).

RESULTS

Subject characteristics of the 15 cystic fibrosis patients in the present study are shown in **Table 1**. Medications used to treat the subjects included the following: pancreatic enzymes (*n* = 15, 100%), antibiotics (*n* = 15, 100%), bronchodilators (*n* = 11, 73%), inhaled corticosteroids (*n* = 6, 40%), oral steroids (*n* = 1, 7%), gut motility agents (*n* = 2, 13%), α-dornase or mucolytic agents (*n* = 5, 33%), laxatives (*n* = 2, 13%), insulin (*n* = 2, 13%), ursodeoxycholic acid (liver enzyme inducer) (*n* = 1, 7%), antiviral agents (*n* = 1, 7%), anticonvulsant (*n* = 1, 7%), and acetaminophen (*n* = 1, 7%).

Paired blood samples (before and after treatment) were collected from all 15 subjects. Paired spontaneous sputum samples could be produced by only 9 of the subjects.

Lung function (percentage of FEV₁) and quality-of-well-being score improved significantly after antibiotic treatment (**Table 2**). There were no significant changes in white cell counts after treatment (10.6 ± 0.9 × 10⁹/L compared with 9.2 ± 0.9 × 10⁹/L), including neutrophils, lymphocytes, monocytes, and eosinophils. Platelet counts decreased significantly after treatment (289 ± 22 × 10⁹/L compared with 264 ± 26 × 10⁹/L; *P* = 0.042).

Plasma 8-*iso*-PGF_{2α} concentrations increased after treatment, suggesting increased oxidative stress. Sputum 8-*iso*-PGF_{2α} concentrations did not change significantly after antibiotic therapy. No significant changes were observed in vitamin E, vitamin C, or β-carotene concentrations after treatment, whereas vitamin A concentrations increased. Plasma zinc and selenium concentrations did not change significantly, whereas plasma copper concentrations were reduced after antibiotic therapy (**Table 3**).

TABLE 2Changes in clinical markers after treatment¹

	Before treatment	After treatment
Percentage of FEV ₁ (%)	60 ± 6 ²	74 ± 7 ³
Percentage of FVC (%)	76 ± 5	87 ± 6
Quality-of-well-being score ⁴	0.53 (0.44–0.59)	0.65 (0.65–0.68) ⁵
Chest auscultation (n)		
Wheezing	1	3
Crackles	6	3
Reduced breath sounds	1	1
Bronchial breathing	1	3

¹n = 15. FEV₁, forced expiratory volume in 1 s; FVC, forced vital capacity.² $\bar{x} \pm \text{SEM}$.^{3,5}Significantly different from before treatment: ³*P* = 0.011, ⁵*P* = 0.001.⁴Median; interquartile range in parentheses. Quality-of-well-being questionnaire (24).

TABLE 3
Biochemical markers of oxidative stress¹

Parameter	Before treatment	After treatment
Plasma 8- <i>iso</i> -PGF _{2α} (pmol/L)	469 (373–554) ²	565 (429–689) ³
Sputum 8- <i>iso</i> -PGF _{2α} (pmol/L) ⁴	8246 ± 2246 ⁵	9893 ± 2540
Vitamin E (μmol/L)	14.5 ± 1.6	15.6 ± 2.0
Vitamin C (μmol/L)	52 ± 8	66 ± 9
β-Carotene (μmol/L)	0.1 (0.1–0.1)	0.1 (0.1–0.1)
Vitamin A (μmol/L)	1.1 ± 0.1	1.4 ± 0.2 ³
Zinc (μmol/L)	12 (9–13)	11 (10–14)
Selenium (μmol/L)	1.01 (0.87–1.22)	1.18 (1.01–1.22)
Copper (μmol/L)	16.9 (14.0–19.5)	15.1 (13.3–17.5) ⁶
Glutathione peroxidase (U/g Hb)	16.2 ± 1.1	13.8 ± 0.7 ⁶
Superoxide dismutase (U/mg Hb)	2.36 ± 0.07	2.37 ± 0.07
Erythrocyte glutathione (μmol/g Hb)	6.1 (5.8–8.7)	6.6 (5.2–7.8)
Sputum glutathione (μmol/L) ⁴	878 ± 188	521 ± 103

¹*n* = 15. 8-*iso*-PGF_{2α}, 8-*iso*-prostaglandin F_{2α}; Hb, hemoglobin.²Median; interquartile range in parentheses.^{3,6}Significantly different from before treatment: ³*P* < 0.01, ⁶*P* < 0.05.⁴*n* = 9.⁵ $\bar{x} \pm \text{SEM}$.

Total energy intake per kilogram body weight significantly increased after treatment for pulmonary exacerbation (**Table 4**). Total fat, saturated fat, monounsaturated fat, and polyunsaturated fat intakes rose significantly. Protein, iron, zinc, thiamine, riboflavin, niacin, sodium, magnesium, and phosphorus intakes per kilogram body weight also increased. However, despite this overall increase in food intake, the energy intake per kilogram body weight of vitamin A and of the dietary antioxidants β-carotene and vitamin C did not increase.

TABLE 4
Nutrient intake¹

Nutrient/kg body weight	Before treatment	After treatment
Energy (kJ)	205 (187–259) ²	280 (241–384) ³
Protein (g)	2.34 (1.39–2.53)	3.17 (2.36–3.38) ³
Fat (g)	2.24 (1.89–2.80)	3.00 (2.27–5.04) ³
Saturated fat (g)	1.09 (0.80–1.17)	1.32 (0.97–2.17) ⁴
Monounsaturated fat (g)	0.71 (0.58–0.92)	1.06 (0.87–1.36) ³
Polyunsaturated fat (g)	0.16 (0.12–0.24)	0.23 (0.18–0.28) ⁴
Carbohydrate (g)	6.56 ± 0.66 ⁵	7.97 ± 1.05
β-Carotene (μg)	35.4 (8.2–69.9)	17.0 (9.6–111.9)
Vitamin C (mg)	2.9 (1.7–4.2)	3.3 (2.6–6.6)
Vitamin A (μg)	11.1 (4.3–17.9)	19.8 (6.6–39.5)
Iron (mg)	0.30 ± 0.04	0.38 ± 0.03 ⁴
Zinc (mg)	0.29 (0.17–0.36)	0.35 (0.29–0.50) ⁴
Thiamine (mg)	0.034 ± 0.005	0.058 ± 0.007 ⁶
Riboflavin (mg)	0.057 ± 0.008	0.093 ± 0.013 ³
Niacin (mg)	0.49 ± 0.06	0.78 ± 0.09 ³
Sodium (mg)	53.5 ± 6.9	93.3 ± 9.3 ⁶
Potassium (mg)	75.8 (60–113)	90.1 (82–123)
Magnesium (mg)	6.5 (4.5–8.0)	8.5 (6.5–11.9) ⁴
Calcium (mg)	24.8 ± 4.0	37.1 ± 6.7
Phosphorus (mg)	28.5 (21.1–41.6)	46.1 (34.1–59.2) ³

¹*n* = 15.²Median; interquartile range in parentheses. Normally distributed.^{3,4,6}Significantly different from before treatment: ³*P* < 0.01, ⁴*P* ≤ 0.05,⁶*P* = 0.001.⁵ $\bar{x} \pm \text{SEM}$.

Concentrations of plasma total, saturated, and monounsaturated, and polyunsaturated fatty acids increased after the treatment period (**Table 5**). After treatment, plasma 8-*iso*-PGF_{2α} concentrations correlated strongly with total, saturated, and monounsaturated, but not polyunsaturated, fatty acid concentrations (**Figure 1**).

Erythrocyte glutathione peroxidase activity decreased after the treatment period (Table 3). No significant change was observed in erythrocyte superoxide dismutase activity. Additionally erythrocyte and sputum glutathione concentrations did not change significantly after treatment. Erythrocyte glutathione concentrations correlated positively with plasma 8-*iso*-PGF_{2α} (*r* = 0.399, *P* = 0.053), and sputum glutathione concentrations correlated with sputum 8-*iso*-PGF_{2α} concentrations (*r* = 0.507, *P* = 0.032).

Correlations were observed between plasma 8-*iso*-PGF_{2α} and copper concentrations (*r* = 0.466, *P* = 0.013) and platelet counts (*r* = 0.537, *P* = 0.003). A correlation was also observed between percentage of FEV₁ and plasma vitamin C concentrations (*r* = 0.364, *P* = 0.048). Percentage of FVC correlated with plasma vitamin A concentrations (*r* = 0.390, *P* = 0.033) and negatively with plasma copper concentrations (*r* = −0.462, *P* = 0.010).

DISCUSSION

In the present study, we sought to examine any changes in the clinical status, biochemical markers of oxidative stress, and dietary intake of cystic fibrosis patients after intravenous antibiotic treatment for an acute pulmonary exacerbation. Although lung function (percentage of FEV₁) and patient perception of well being improved significantly, antioxidant defenses showed no significant improvement and plasma 8-*iso*-PGF_{2α} concentrations increased, suggesting that there was increased oxidative stress. Plasma fatty acid concentrations also increased after treat-

TABLE 5
Plasma fatty acid composition¹

Fatty acid	Before treatment	After treatment
	<i>mg/L</i>	
14:0	16.3 (12.1–31) ²	24.7 (16.6–49.1) ³
16:0	333 (311–453)	400 (312–578) ³
16:1n–7	34.3 (24.9–65.7)	51.4 (29.7–73.8)
18:0	115 (108–134)	146 (102–172)
18:1n–9	390 (321–443)	417 (322–537) ³
18:1n–7	36.0 ± 3.0 ⁴	40.1 ± 4.3 ³
18:2n–6	367.7 ± 28.7	418 ± 38.7
18:3n–6	7.4 (5.6–9.7)	9.5 (7.6–12.7)
18:3n–3	8.0 ± 0.8	9.9 ± 1.1
20:3n–6	31.1 ± 1.9	34.5 ± 2.4
20:4n–6	93.7 ± 6.8	108.9 ± 9.9 ³
20:5n–3	16.8 ± 2.6	21.6 ± 2.8 ³
22:0	11.2 ± 0.7	11.7 ± 0.8
22:6n–3	9.7 ± 0.9	10.8 ± 1.1
24:1n–9	26.8 ± 1.3	26.0 ± 1.3
Total polyunsaturates	518 (461–663)	549 (498–737) ³
Total monounsaturates	481 (407–576)	554 (419–658) ³
Total saturates	483 (444–618)	601 (437–823) ³
Total fatty acids	1429 (1345–1666)	1621 (1347–2221) ³

¹*n* = 15.²Median; interquartile range in parentheses.³Significantly different from before treatment, *P* < 0.05.⁴ $\bar{x} \pm \text{SEM}$.

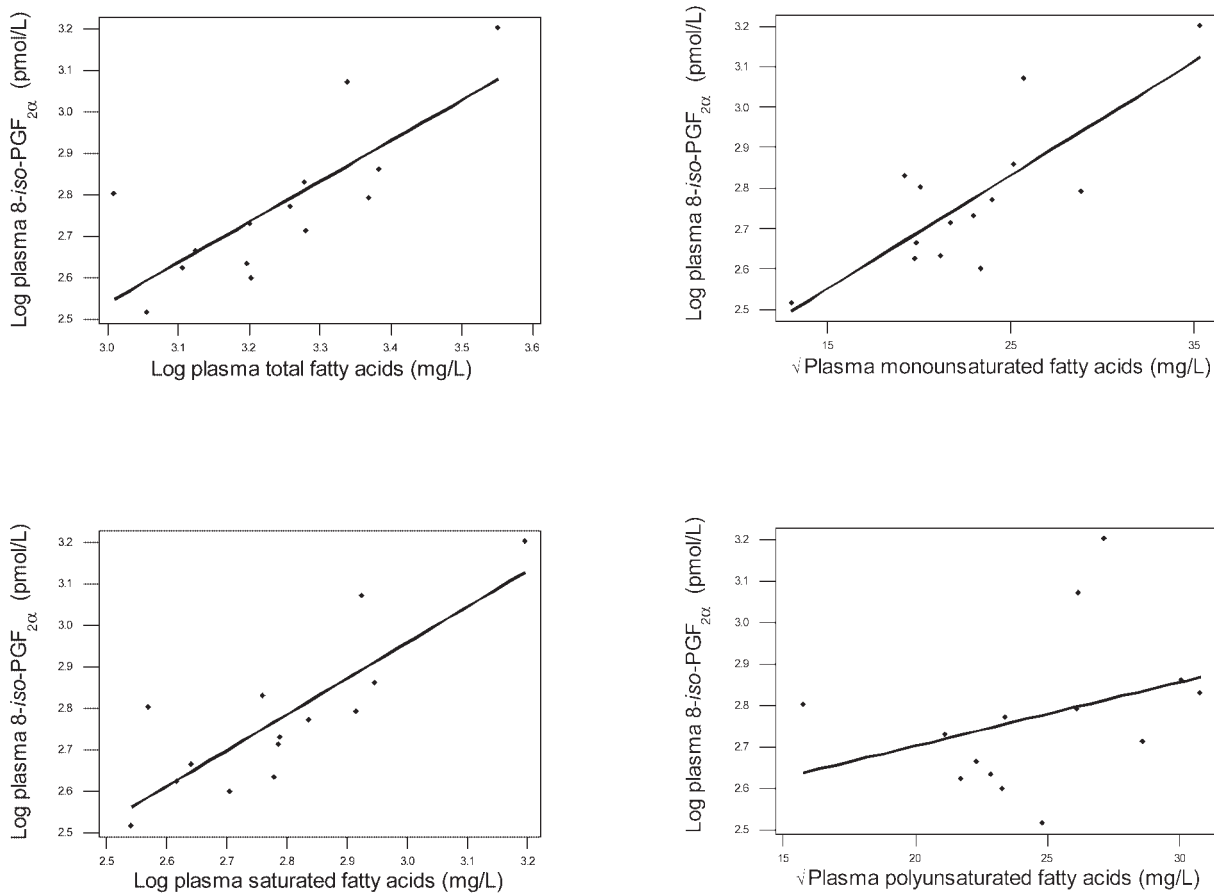


FIGURE 1. Plasma 8-*iso*-prostaglandin $F_{2\alpha}$ (8-*iso*-PGF $_{2\alpha}$) concentration compared with plasma concentration of total fatty acids ($r = 0.768$, $P = 0.001$), saturated fatty acids ($r = 0.824$, $P < 0.001$), monounsaturated fatty acids ($r = 0.789$, $P = 0.001$), and polyunsaturated fatty acids ($r = 0.332$, $P = 0.245$) after treatment for pulmonary exacerbations ($n = 15$). Data are transformed as indicated.

ment, correlating strongly with plasma 8-*iso*-PGF $_{2\alpha}$. This suggests that the increase in dietary fat intake, with resultant increases in plasma fatty acid concentrations, contributed strongly to the increase in oxidative stress observed.

We previously showed elevated oxidative stress in stable cystic fibrosis patients who had elevated plasma 8-*iso*-PGF $_{2\alpha}$ concentrations compared with control subjects (14). However, the changes that occur in 8-*iso*-PGF $_{2\alpha}$ after treatment for a pulmonary exacerbation have not been previously examined. Other studies of oxidative stress reported mixed patient responses to the treatment of acute infection in cystic fibrosis. McGrath et al (16) observed improved lung function (FEV $_1$ and FVC) and increased plasma concentrations of vitamins A and E, α -carotene, β -carotene, and lycopene but no significant change in plasma vitamin C concentrations after treatment. In addition, they observed a reduction in inflammatory markers, which included white cell counts. However, plasma malondialdehyde concentrations did not change significantly, suggesting that there was no improvement in oxidative stress, and protein carbonyls increased, suggesting that there was increased free radical damage to proteins. Range et al (17) observed improved lung function and increased plasma vitamin C, E, and A concentrations after treatment. Lipid hydroperoxide concentrations improved;

however, no change was observed in plasma malondialdehyde or protein carbonyl concentrations. Linnane et al (18) showed that although lung function improved after treatment, sputum nitrite and nitrate concentrations (stable oxidation products of nitric oxide) did not improve. Each of these reports suggests that oxidative stress was not resolved after 14 d of treatment.

In the present study, dietary intake, in particular fat intake, increased after treatment. This is most likely attributable to increased appetite as respiratory symptoms improve. The strong correlations between plasma fatty acid and 8-*iso*-PGF $_{2\alpha}$ concentrations suggest that the high-fat diet recommended for cystic fibrosis patients predisposes them to oxidative stress, probably in part because of the increased availability of unsaturated fatty acids, which act as a lipoperoxidation substrate. Diets rich in polyunsaturated fat have been shown to increase susceptibility to oxidative stress (33–35). Data also suggest that high-fat diets may amplify oxidative stress, irrespective of how saturated the fat that is being consumed (36). Free radical synthesis and increased oxidative stress were shown to be evoked by high saturated fat and low fiber consumption (37), and a diet high in saturated fat was shown to induce oxidative stress in humans (36) and pregnant rats (38). The link between high-fat diets and oxidative stress needs further investigation because it applies to

many conditions in which excessive quantities of oxidants are present, eg, atherosclerosis, diabetes, and cataracts (39). This link also suggests that if cystic fibrosis patients are to gain maximum benefit from a high-fat diet, antioxidant supplementation is needed to minimize the damaging effects of oxidation.

In the present study, intakes of vitamin C and β -carotene did not change significantly after treatment, despite an increase in total energy, fat, and protein intake per kilogram body weight. Although vitamin E intake is not available when using the DIET/1 nutrient calculation software, the food sources of vitamin E are similar to those for vitamin A, which did not change; thus, it is probable that vitamin E intake was also unchanged. This suggests that the foods selected by subjects as their appetites improved were not rich in antioxidants. Thus, although plasma lipid concentrations increased, dietary antioxidant supplies did not change. This is reflected by the plasma concentrations of vitamins E and C and β -carotene, which did not significantly change after treatment. Similarly, superoxide dismutase enzyme activity did not change, whereas glutathione peroxidase activity was reduced, suggesting glutathione peroxidase is being exhausted in the presence of increased oxidative stress. Although 24-h dietary recall data are reasonably well established for measuring average intakes of groups (30), we acknowledge that there are limitations of this method, which relies on subject recall and a limited time frame. Because of the poor health status of the patients, it was deemed unethical to add to the patients' burden by collecting additional dietary information.


In the present study, plasma vitamin A concentrations increased after treatment, agreeing with a previous study of cystic fibrosis (40). Infectious stresses were also observed as depressing plasma vitamin A concentrations in other situations (41, 42), with possible reasons including increased synthesis of retinol binding protein, reduced urinary losses, and reduced metabolic demands for the nutrient as the infection passes (40). Interestingly, the correlations between vitamin C and percentage of FEV₁, and vitamin A and percentage of FVC, suggest that these vitamin concentrations may have an influence on lung function, possibly because of an antioxidant effect. Other investigators have reported similar links between vitamin concentrations and lung function (43).

In addition to increased plasma fatty acid concentrations, other factors may have contributed to the increase in plasma 8-*iso*-PGF_{2 α} concentrations. Each patient was treated with ≤ 4 different antibiotics, some of which were previously shown to induce oxidative stress (eg, gentamicin) (44). Other medications used by some patients have also been reported as altering oxidative stress [eg, acetaminophen (45, 46), phenytoin (47–49), and beclomethosone (50)]. Furthermore, no change in white cell counts was observed after treatment. In particular, there was no reduction in neutrophil count, which was in the normal range at the start of treatment. This indicates that immune cell production of oxidants may not have changed significantly over the treatment period.

Although indexes of oxidative stress did not improve after treatment for pulmonary exacerbations, some markers of inflammation improved, such as blood platelets (51). The correlation between 8-*iso*-PGF_{2 α} and platelet count is evidence for the involvement of platelets in the inflammatory process. Similarly, the reduction in plasma copper concentrations after treatment indicates that inflammation was reduced. During inflammation, the proinflammatory cytokines interleukin 1, interleukin 1 α , and interleukin 1 β induce the production of plasma ceruloplasmin (which binds to copper), resulting in the release of copper into plasma (52). The reduction in

plasma copper concentrations with treatment and the correlations between plasma copper compared with the percentage of FVC and 8-*iso*-PGF_{2 α} suggest that ceruloplasmin concentrations abate as inflammation diminishes.

Although erythrocyte and sputum glutathione concentrations did not change significantly after antibiotic treatment, the correlation between erythrocyte glutathione compared with plasma 8-*iso*-PGF_{2 α} , and sputum glutathione compared with sputum 8-*iso*-PGF_{2 α} , suggests that in the presence of excess oxidants, glutathione synthesis or transport increased. Compensatory, but probably inadequate, increases in erythrocyte glutathione were seen before in cystic fibrosis patients (53) and in other conditions of oxidative stress (54, 55). The high sputum 8-*iso*-PGF_{2 α} concentrations are interesting. As isoprostanes are potent vasoconstrictory agents in rat lungs (56), one can speculate about the effect of these high sputum isoprostane concentrations on lung tissue and their effect on pulmonary function.

In conclusion, the present study showed that although hospitalization of cystic fibrosis patients for acute pulmonary exacerbation improved clinical symptoms and lung function, the antioxidant defenses did not improve and the damaging effects of oxidation continued after cessation of antibiotic treatment. The strong correlation between plasma fatty acids and 8-*iso*-PGF_{2 α} suggests that a high fat intake may perpetuate the high degree of oxidation, particularly in situations where excess oxidants are present. Thus, supplementation with antioxidants should be considered in cystic fibrosis patients who require a high-fat diet to meet their energy requirements. 

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Erratum

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