Improved antioxidant and fatty acid status of patients with cystic fibrosis after antioxidant supplementation is linked to improved lung function[1–3]

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ABSTRACT

Background: Oxidative stress, as measured by 8-*iso*-prostaglandin $F_{2\alpha}$ (8-*iso*-$PGF_{2\alpha}$), and depleted antioxidant defenses were shown in stable cystic fibrosis (CF) patients. The plasma fatty acid status of CF patients was linked to oxidative stress after respiratory exacerbations.

Objective: We examined changes in plasma 8-*iso*-$PGF_{2\alpha}$, antioxidant defenses, plasma fatty acid status, and clinical markers resulting from short-term antioxidant supplementation.

Design: Forty-six CF patients were randomly assigned to either group A [low dose of supplement (10 mg vitamin E and 500 µg vitamin A)] or group B [high dose of supplement (200 mg vitamin E, 300 mg vitamin C, 25 mg β-carotene, 90 µg Se, and 500 µg vitamin A)]. Plasma concentrations of 8-*iso*-$PGF_{2\alpha}$, vitamins E and C, β-carotene, zinc, selenium, and copper; plasma fatty acid composition; erythrocyte glutathione peroxidase (EC 1.11.1.9) and superoxide dismutase (EC 1.15.1.1) activities; lung function; and dietary intake were measured before and after 8 wk of supplementation.

Results: Antioxidant defenses in group B improved, whereas those in group A did not: in groups B and A, the mean (±SEM) changes (Δ) in vitamin E were 10.6 ± 1.5 and -1.9 ± 0.9 µmol/L, respectively ($P < 0.001$), Δβ-carotene were 0.1 ± 0.04 and -0.01 ± 0.02 µmol/L, respectively ($P = 0.007$), Δselenium were 0.51 ± 0.10 and -0.09 ± 0.04 µmol/L, respectively ($P < 0.001$), and Δglutathione peroxidase activity were 1.3 ± 0.3 and -0.3 ± 0.6 U/g hemoglobin, respectively ($P = 0.016$). There were no significant differences between the groups in Δ8-*iso*-$PGF_{2\alpha}$, Δvitamin C, Δfatty acid composition, Δsuperoxide dismutase activity, Δlung function, or Δwhite cell count. Within group B, Δβ-carotene correlated with Δpercentage of forced vital capacity ($r = 0.586$, $P = 0.005$), Δselenium correlated with Δpercentage of forced expiratory volume in 1 s ($r = 0.440$, $P = 0.046$), and Δplasma fatty acid concentrations correlated with Δpercentage of forced expiratory volume in 1 s ($r = 0.583$, $P = 0.006$) and Δ8-*iso*-$PGF_{2\alpha}$ ($r = 0.538$, $P = 0.010$).

Conclusions: Whereas increased β-carotene, selenium, and fatty acid concentrations are linked to improved lung function, increased plasma fatty acid concentrations are linked to oxidative stress. If oxidative stress is deemed to be important to the clinical outcome of CF patients, means of reducing oxidative stress while maintaining a high-fat, high-energy diet must be investigated.   *Am J Clin Nutr* 2003;77:150–9.

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

INTRODUCTION

Evidence supporting the occurrence of oxidative stress in cystic fibrosis (CF) is extensive (1–3). Lipoperoxidation is indicated by elevated concentrations of plasma malondialdehyde (3–7), breath pentane and ethane (8), and plasma hydroperoxides (3, 5) and by depletion of the major lipoperoxidation substrates, such as linoleic and arachidonic acids (5, 9). Increased oxidative damage to DNA was shown by elevated urinary concentrations of 8-hydroxyguanosine (10). We (11, 12) and others (13) observed elevated concentrations of 8-*iso*-prostaglandin $F_{2\alpha}$ (8-*iso*-$PGF_{2\alpha}$; the most well known isoprostane isomer) in stable CF patients. Isoprostanes are regarded as the most accurate and reliable biomarkers of oxidative stress because they are structurally stable, are produced in vivo, and are present in relatively high concentrations (14). Furthermore, isoprostane concentrations were shown to increase in experimental models of injury and can be suppressed by using antioxidants (15–17).

Many factors predispose CF patients to oxidative stress (1, 2). An increased oxidant burden results from activated neutrophils that colonize the lungs (18) and from an increased metabolic rate (19). Patients also have decreased antioxidant protection. Despite the administration of pancreatic enzyme supplements, residual steatorrhea and azotorrhea occur (20), resulting in malabsorption of fat-soluble antioxidants, namely vitamin E and β-carotene. The high-fat diet that is recommended to meet the increased energy requirements of CF patients (19) may also increase their susceptibility to oxidative stress, because we found a strong correlation between plasma fatty acid concentrations and 8-*iso*-$PGF_{2\alpha}$ concentrations (21).

Recent investigations in our laboratory confirmed previous reports (4, 5, 7, 22–26) that the antioxidant defenses (ie, vitamins E

and C and β-carotene) of stable CF patients are lower than those of healthy control subjects (11). This result occurred despite normal dietary intakes of antioxidant vitamins and the use of pancreatic enzyme supplements where required. Decreased activity of erythrocyte glutathione peroxidase (GSHPx, an antioxidant enzyme; EC 1.11.1.9) was also shown in CF patients (7). We recently reported that in CF patients, the activity of glutathione peroxidase may be diminished during times of increased oxidative stress, eg, after treatment of an acute infection, further contributing to decreased antioxidant defenses (21). Thus, it is hypothesized that supplementation with antioxidants, including vitamin E, vitamin C, β-carotene, and selenium (to stimulate GSHPx activity), may boost antioxidant defenses, decrease oxidative stress, and potentially lead to improved clinical outcome by reducing the rate of lung deterioration. The aim of the present study was to examine oxidative stress, as measured by 8-*iso*-PGF$_{2\alpha}$ concentrations, and antioxidant defenses in CF patients after antioxidant supplementation in relation to fatty acid status, dietary intake, and clinical status.

SUBJECTS AND METHODS

Subjects

Forty-six CF subjects from the CF clinic at the Children's Hospital at Westmead, Sydney, Australia, completed the study. The diagnosis of CF had been confirmed previously by elevated sweat chloride concentrations in all cases. Diets were not controlled during the study. During the study period, the therapeutic regimen of the patients was not changed except for intermittent antibiotic treatment of acute pulmonary exacerbations. All vitamin and mineral supplements other than the trial supplements were ceased for the duration of the study. The exclusion criteria were age < 5 y (unable to perform reproducible spirometry) and an abnormal vitamin E concentration (< 8 μmol/L) at baseline (27). Informed written consent was obtained from the subjects or their guardians. Ethics approval was obtained from the University of Newcastle Human Research Ethics Committees and the Children's Hospital at Westmead Ethics Committee.

Study design

All subjects underwent a run-in period of 4 wk during which they received a low-dose supplement [10 mg vitamin E (as *RRR*-α-tocopherol) and 500 μg vitamin A (as retinyl palmitate) in oil]. Thus, in addition to their dietary intakes of vitamins A and E, the patients received doses of vitamins A and E that were equivalent to the recommended daily intakes. This run-in period was necessary to wash out any supplements that patients had been taking before the commencement of the study. It was deemed unethical to cease vitamin A and E supplementation altogether because such supplementation forms part of routine treatment for some patients. After 4 wk, the first (baseline) blood samples and dietary and clinical data were collected. Vitamin E concentrations were assessed at baseline. Subjects were then randomly assigned to either group A, who continued to receive the low-dose supplement and thereby effectively served as a control group, or group B, who commenced to receive a high-dose supplement [200 mg vitamin E (as *RRR*-α-tocopherol), 300 mg vitamin C (as sodium ascorbate), 25 mg β-carotene (*all-trans* isomer), 90 μg Se (as selenomethionine), and 500 μg vitamin A (as retinyl palmitate) in oil], for an additional 8 wk. After the subjects had received these supplements for 8 wk, blood samples and dietary and clinical data were collected a second time. Supplements were taken with breakfast, using the usual dose of pancreatic enzymes. Supplements were obtained from RP Scherer Pty Ltd (Melbourne) and dispensed by the pharmacy at the Children's Hospital at Westmead. Subjects were randomly assigned from a centralized pool, which was derived by using a random-numbers computer program. Compliance was measured with diary cards that were used to record daily intakes and by the counting of pills.

Blood samples were collected from nonfasting patients because it was considered unethical to add to the patients' burden by requiring a fasting period before the blood tests. Other researchers showed that it is unlikely that plasma fatty acids, total cholesterol, triacylglycerol, vitamin E, and total antioxidant status are altered by postprandial effects (28).

Subject characteristics

Routine pulmonary function tests were conducted by using a spirometer (1085D Breeze cardiorespiratory diagnostic software; Medgraphics, St Paul) with established normal values (29). Forced vital capacity (FVC) and forced expiratory volume in 1 s (FEV$_1$) were recorded and compared with predicted values. Height was measured with a Dyfed stadiometer (Holtain Ltd, Croswell, Crymych, United Kingdom). Weight was recorded by using digital scales (model number 824/890; GEC/Avery, Somerset, United Kingdom). Full blood counts were performed by using a Coulter GEN-S analyzer (Beckman-Coulter, Fullerton, CA). 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 (30).

Vitamin and mineral analysis

Blood samples were collected in EDTA-coated tubes and centrifuged at 3000 × *g* at 4 °C for 10 min. Plasma was collected and frozen at −70 °C within 0.5 h of blood collection. Vitamins A and E and β-carotene were separated on a reverse-phase HPLC column and measured with a programmable-wavelength ultraviolet-visible detector (31). The samples were thawed, mixed with ethanol to precipitate proteins, and mixed by vortex, after which hexane was added. After being mixed by vortex again, the samples were centrifuged, and after the hexane phase was removed, the samples were injected onto an HPLC column (5 μm, 300 mm × 3.5 mm internal diameter, laboratory-packed Whatman ODS 3; Whatman International, Kent, United Kingdom) with 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 with an electrochemical detector (32). The samples were mixed with trichloracetic acid to precipitate proteins, mixed by vortex, and centrifuged, and the supernatant fluid was injected onto an HPLC column (5 μm, 150 mm × 3.5 mm internal diameter, laboratory-packed Whatman ODS 3; Whatman International) with 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 a silver/silver chloride reference electrode. Plasma concentrations of zinc, selenium, and copper were analyzed by inductively coupled plasma mass spectrometry. The 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 addition in a pooled-plasma base.

Isoprostane assays

Plasma preparation

Blood samples were collected in EDTA-coated tubes containing reduced glutathione (Sigma Chemical Co, St Louis) as an antioxidant. The samples were centrifuged at $3000 \times g$ at $4\,°C$ for 10 min. The plasma fraction was removed and stored at $-70\,°C$ in tubes that were precoated with butylated hydroxytoluene (Sigma Chemical Co) for isoprostane analysis. A known amount of tritium-labeled prostaglandin ($PGF_{2\alpha}$; Amersham, Arlington Heights, IL) was added to an aliquot of plasma to allow determination of the recovery rate after the purification procedure. Ethanol was added, and the sample was chilled at $4\,°C$ and then centrifuged at $1500 \times g$ for 10 min to remove the precipitated proteins. The supernatant fluid was decanted, an equal volume of 15% KOH was added, and the resultant solution was incubated at $40\,°C$ for 1 h to cleave any esterified isoprostane. The sample was diluted with water, and then the pH was lowered with hydrochloric acid to < 3.0. The sample was passed through a Sep-Pak C-18 reverse-phase cartridge (Waters, Milford, MA), which had been activated by rinsing it first with methanol and then with a 1-mmol HCl/L solution. After passing the sample through the cartridge, the cartridge was rinsed again with a 1-mmol HCl/L solution and then with heptane, and then 8-*iso*-$PGF_{2\alpha}$ was eluted with ethyl acetate:heptane (1:1). A Sep-Pak silica cartridge (Waters) was then activated by rinsing it first with methanol and then with ethyl acetate. After passing the eluate from the C-18 cartridge through the silica cartridge, 8-*iso*-$PGF_{2\alpha}$ was finally eluted with ethyl acetate:methanol (1:1). This solvent was evaporated using N_2, and the sample was reconstituted with assay buffer. A quantity of purified sample was added to aqueous biodegradable counting scintillant (Amersham) and counted using a liquid scintillation counter to determine recovery rates (12).

Enzyme immunoassay

Purified plasma was analyzed with an 8-isoprostane enzyme immunoassay kit (Cayman Chemical, Ann Arbor, MI) (12). Absorbance values were measured with a plate reader (Multiskan Ascent; Thermo Labsystems, Helsinki) that used a wavelength of 405 nm, and the raw data were corrected for recovery. The assay was validated by adding a series of known amounts of pure 8-*iso*-$PGF_{2\alpha}$ standard to base volumes of purified plasma. The concentration of these samples was determined by using enzyme immunoassay. A high correlation (0.99) was obtained between the known amounts of pure 8-isoprostane added and the concentration determined by enzyme immunoassay. The antiserum used in this assay has 100% cross-reactivity with 8-*iso*-$PGF_{2\alpha}$, 0.2% cross-reactivity each with $PGF_{2\alpha}$, $PGF_{3\alpha}$, PGE_1, and PGE_2, and 0.1% cross-reactivity with 6-*keto*-$PGF_{1\alpha}$. The detection limit of the assay is 4 ng/L. This kit has been used to measure 8-*iso*-$PGF_{2\alpha}$ concentrations in human plasma, BAL, and other fluids (12, 33, 34).

Plasma fatty acid analysis

Whole blood was collected into EDTA tubes and centrifuged at $3000 \times g$ at $4\,°C$ for 10 min. The plasma was separated and stored at $-70\,°C$ until analyzed. Total fatty acids were measured by using the method of Lepage and Roy (35). Two milliliters of a methanol-toluene mixture (4:1, by vol) containing 0.02 g 21:0/L and 0.12 g butylated hydroxytoluene/L was added to 200 μL plasma. Fatty acids were methylated by adding 200 μL acetyl chloride dropwise while mixing by vortex and heating to $100\,°C$ for 1 h. After cooling, the reaction was stopped by adding 5 mL 6% K_2CO_3. The sample was centrifuged at 3000 rpm at $4\,°C$ for 10 min to facilitate separation of the layers. The upper toluene layer was used for analysis of the fatty acid methyl esters by gas chromatography with a fused carbon-silica column coated with cyanopropylphenyl (30 m × 0.25 mm, DB-225; J & W Scientific, Folsom, CA). The port temperatures of both the injector and the detector were set at $250\,°C$. The oven temperature was initially set at $170\,°C$ for 2 min and was then increased $10\,°C$/min until a temperature of $190\,°C$ was reached and held for 1 min, at which point the temperature was increased $3\,°C$/min until a temperature of $220\,°C$ was reached and maintained; the total run time was 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 for GC analysis; Hewlett-Packard, Palo Alto, CA).

Glutathione peroxidase enzyme assay

Whole blood was collected into EDTA-coated tubes and centrifuged at $8500 \times g$ at $4\,°C$ for 10 min. Plasma was discarded, and the cells were washed with 10 volumes of cold buffer (50 mmol tris-HCl/L, pH 7.5, containing 5 mmol EDTA/L and 1 mmol dithiothreitol/L). Samples were centrifuged again at $8500 \times g$ at $4\,°C$ for 10 min, and the supernatant fluid was discarded. The cells were then lysed by adding exactly 4 volumes of ice-cold, deionized water. After centrifuging again at $8500 \times g$ at $4\,°C$ for 10 min, the supernatant fluid was collected and stored at $-70\,°C$ until analyzed. Erythrocyte GSHPx activities were measured by using a GPx-340 spectrophotometric assay kit (Bioxytech; OXIS International, Portland, OR) to obtain values in U/mL. The hemoglobin concentration of the samples was measured by using Sigma kit number 525 for total hemoglobin (Sigma Chemical Co) to allow erythrocyte GSHPx activity to be expressed as U/g hemoglobin.

Superoxide dismutase enzyme assay

Whole blood was collected into EDTA-coated tubes and centrifuged at $3000 \times g$ at $4\,°C$ for 10 min. The erythrocyte pellet was separated and stored at $-70\,°C$ until analyzed, at which point it was thawed and resuspended in 4 volumes of 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, which was mixed by vortex for 30 s. The samples were centrifuged at $3000 \times g$ at $4\,°C$ for 10 min. The upper phase was collected and stored at $-70\,°C$ until analyzed. Erythrocyte Zn/Cu superoxide dismutase (EC 1.15.1.1) activities were measured by using an SOD-525 spectrophotometric assay kit (Bioxytech; OXIS International) to obtain values in U/mL. The hemoglobin concentration of the samples was measured by using Sigma kit number 525 for total hemoglobin (Sigma Chemical Co) to allow erythrocyte Zn/Cu superoxide dismutase activity to be expressed as U/mg hemoglobin.

TABLE 1
Subject characteristics at baseline[1]

	Group A (n = 8 M, 16 F)	Group B (n = 13 M, 9 F)
Age (y)	10.6 ± 0.7[2]	12.6 ± 0.8
Height (cm)	143.8 ± 4.8	152.2 ± 4.8
z Score for height	−0.12 ± 0.34	−0.48 ± 0.31
Weight (kg)	41.0 ± 3.7	42.7 ± 3.6
z Score for weight	0.06 (−1.05, 1.17)[3]	−0.23 (−1.05, 0.14)
BMI (kg/m^2)	18.8 ± 0.9	18.0 ± 0.6
Shwachman score[4]	90 (80–95)	85 (73–86)
Genotype		
Homozygous ΔF508	14	13
Heterozygous ΔF508	8	7
Other	2	2
Pancreatic sufficient		
Yes	5	3
No	19	19
No. of hospital admissions in past 2 y	0 (0–2)	0 (0–2)
No. of courses of intravenous antibiotics in past 2 y	0 (0–2)	0 (0–2)
No. of courses of nebulized antibiotics in past 2 y	3 (0–20)	4 (1–24)
No. of missed school days in past 12 mo	7 (0–20)	4 (0–12)
No. of months since last admission	24 (5–100)	11 (0–36)
No. of days on antibiotics during study	14 (0–17)	18 (0–56)
No. of days of hospitalization during study	0 (0–0)	0 (0–0)
No. of days of missed school during study	0 (0–3)	0 (0–3)
Self-reported compliance (%)	100 (97–100)	99 (86–100)

[1] There were no significant differences between the groups.
[2] $\overline{x}$ ± SEM of normally distributed data.
[3] Median; interquartile range (quartile 1–quartile 3) in parentheses; nonparametric data.
[4] Range: 0–100.

Dietary intake

Dietary intake was assessed by using 3-d food records (36). Analysis of food records was conducted by using the Foodworks 210 Nutrient Calculation Software, which is based on the 1995 Nuttab database (37). The mean nutrient intakes for each group were determined from these 3-d food records.

Statistical analysis

Results were analyzed by using MINITAB 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 Student's t test for normally distributed data, the Mann-Whitney U test for nonparametric unpaired data, and the Wilcoxon test for nonparametric paired data. Differences were considered significant at $P < 0.05$. Relations between variables were studied by linear regression: Pearson's correlation coefficient for normal data and Spearman's rank correlation coefficient for nonparametric data (38).

RESULTS

Of the 46 CF patients who completed the study, 24 were randomly assigned to group A (low-dose supplement) and 22 were randomly assigned to group B (high-dose supplement). There was no difference in clinical status between the patients in group A and those in group B at baseline (**Table 1**). There were no significant differences between the groups in average age, height, weight, or Shwachman score. Similarly, there were no significant differences between the groups in the number of hospitalizations in the past 2 y, in the number of courses of intravenous or nebulized antibiotics

in the past 2 y, in the number of missed school days in the past 12 mo, or in the number of months since the last hospital admission. At baseline, all subjects had a vitamin E concentration > 8 μmol/L (lower limit of normal range; 27).

As shown in Table 1, the clinical management required during the study was similar in both groups. There were no significant differences between the groups in the number of days on antibiotics, in the number of days of hospitalization, or in the number of days of missed school during the study. Analysis of patient diaries suggested that compliance with the supplements was also similar between the groups. The medications used to treat the patients in each group during the study are shown in **Table 2**.

After 8 wk of supplementation, antioxidant defenses improved in group B but not in group A (**Table 3**). Significant increases in

TABLE 2
Percentages of subjects who used various medications during the supplementation trial[1]

	Group A (n = 24)	Group B (n = 22)
Pancreatic enzymes (%)	21 [5]	14 [3]
Antibiotics (%)	67 [16]	68 [15]
Bronchodilators (%)	29 [7]	27 [6]
Inhaled corticosteroids (%)	8 [2]	18 [4]
Oral steroids (%)	0 [0]	5 [1]
α-Dornase and mucolytic agents (%)	21 [5]	45 [10]
Laxatives (%)	0 [0]	5 [1]
Insulin (%)	0 [0]	5 [1]

[1] n in brackets.

TABLE 3
Biochemical markers of oxidative stress: plasma concentrations of 8-*iso*-prostaglandin F$_{2\alpha}$ (8-*iso*-PGF$_{2\alpha}$), vitamin E, vitamin C, β-carotene, vitamin A, zinc, selenium, and copper and erythrocyte glutathione peroxidase (GSHPx) and superoxide dismutase (SOD) activities at baseline and changes (Δ) after 8 wk of supplementation in groups A and B[1]

	Group A ($n = 24$)		Group B ($n = 22$)		P
	Week 4 (baseline)	Change	Week 4 (baseline)	Change	(Δgroup B vs Δgroup A)
8-*iso*-PGF$_{2\alpha}$ (ng/L)[2]	167 ± 9	1 ± 9	177 ± 10	2 ± 9	NS
Vitamin E (μmol/L)[3]	18.8 ± 1.5	−1.9 ± 0.9	16.5 ± 1.1	10.6 ± 1.5	<0.001
Vitamin C (μmol/L)[2]	45 ± 5	25 ± 7.7	47 ± 6	33 ± 8.9	NS
β-Carotene (μmol/L)[3]	0.1 ± 0.04	0.0 ± 0.02	0.0 ± 0.02	0.1 ± 0.04	0.007
Vitamin A (μmol/L)[3]	1.5 ± 0.1	0.4 ± 0.5	1.5 ± 0.1	−0.2 ± 0.1	NS
Zinc (μmol/L)[2]	10.7 ± 0.3	−1.0 ± 0.4	10.5 ± 0.3	−0.8 ± 0.4	NS
Selenium (μmol/L)[2]	1.04 ± 0.05	−0.09 ± 0.04	0.98 ± 0.03	0.51 ± 0.10	<0.001
Copper (μmol/L)[2]	15.3 ± 0.8	0.2 ± 0.3	15.3 ± 0.6	0.0 ± 0.4	NS
GSHPx (U/g Hb)[2]	18.2 ± 1.4	−0.3 ± 0.6	15.4 ± 0.7	1.3 ± 0.3	0.016
SOD (U/mg Hb)[3]	2.15 ± 0.06	0.07 ± 0.06	2.17 ± 0.05	−0.03 ± 0.05	NS

[1] $\overline{x}$ ± SEM. Hb, hemoglobin.
[2] Normally distributed data (two-sample *t* test).
[3] Nonparametric data (Mann-Whitney *U* test).

plasma concentrations of vitamin E, β-carotene, and selenium and in GSHPx activity were observed in group B. Vitamin C concentrations increased significantly from baseline in group B ($P < 0.001$). However, a similar increase was also seen in the subjects in group A ($P = 0.003$), who did not receive vitamin C supplements.

Despite significant improvements in antioxidant defenses in group B after supplementation, there was no significant difference between the groups in the mean change in plasma 8-*iso*-PGF$_{2\alpha}$ concentrations. At baseline there was no significant difference between the groups in clinical status, including percentage of FEV$_1$, percentage of FVC, and quality of well-being (**Table 4**). After 8 wk of supplementation, there were no significant differences between the groups in the changes in percentage of FEV$_1$, in percentage of FVC, and in quality of well-being. At baseline, both groups had similar hematologic results, and after supplementation there was no significant difference between the groups in the change in cell counts.

There was no significant difference between the groups in dietary micronutrient intakes at baseline except that zinc intake at baseline was significantly higher in group B than in group A (**Table 5**). There were also no significant differences between the groups in the change in nutrient intake during the supplementation period. There was no significant difference between the groups in plasma fatty acid composition at baseline (**Table 6**). Furthermore,

after supplementation, there was no significant difference between the groups in the change in fatty acid concentrations.

Within group B, the change in β-carotene concentrations correlated with the change in percentage of FVC ($r = 0.586$, $P = 0.005$) (**Figure 1**). This correlation remained significant when the one outlier with a high change in β-carotene concentration was removed ($r = 0.525$, $P = 0.017$). Similarly, the change in selenium concentrations correlated with the change in percentage of FEV$_1$ ($r = 0.440$, $P = 0.046$) (Figure 1). However, the correlations between the change in vitamin E concentrations and both the change in percentage of FEV$_1$ ($r = 0.363$, $P = 0.106$) and the change in percentage of FVC ($r = 0.148$, $P = 0.523$) were not significant. Similarly, the correlations between the change in vitamin C concentrations and both the change in percentage of FEV$_1$ ($r = 0.125$, $P = 0.588$) and the change in percentage of FVC ($r = 0.113$, $P = 0.627$) were not significant. Nor were the correlations between the change in GSHPx activity and either the change in percentage of FEV$_1$ ($r = −0.341$, $P = 0.130$) or the change in percentage of FVC ($r = 0.185$, $P = 0.422$) significant. Within group B, the change in total plasma fatty acid concentrations correlated with the change in percentage of FEV$_1$ ($r = 0.583$, $P = 0.006$) (**Figure 2**) and with the change in 8-*iso*-PGF$_{2\alpha}$ concentrations ($r = 0.538$, $P = 0.010$) (**Figure 3**).

TABLE 4
Key clinical and hematologic markers at baseline and changes after 8 wk of supplementation in groups A and B[1]

	Group A ($n = 24$)		Group B ($n = 22$)	
	Week 4 (baseline)	Change	Week 4 (baseline)	Change
Percentage of FEV$_1$ (%)[2]	76.0 ± 4.3	1.3 ± 1.8	67.5 ± 5.1	−3.0 ± 2.7
Percentage of FVC (%)[2]	87.9 ± 3.8	4.8 ± 2.0	85.3 ± 5.4	0.6 ± 3.0
QOWB[3]	0.734 ± 0.013	0.058 ± 0.024	0.736 ± 0.012	−0.005 ± 0.012
White blood cell count (×10^9/L)[3]	8.8 ± 0.7	−0.4 ± 0.5	10.1 ± 0.8	1.0 ± 0.7
Neutrophils (×10^9/L)[3]	5.0 ± 0.7	0.1 ± 0.7	6.6 ± 0.8	1.3 ± 0.8
Lymphocytes (×10^9/L)[2]	2.9 ± 0.2	−0.4 ± 0.2	2.7 ± 0.2	−0.1 ± 0.4
Monocytes (×10^9/L)[2]	0.5 ± 0.1	0.0 ± 0.1	0.6 ± 0.1	0.1 ± 0.1
Eosinophils (×10^9/L)[2]	0.2 ± 0.0	0.0 ± 0.1	0.3 ± 0.1	0.0 ± 0.1
Platelets (×10^9/L)[2]	333 ± 20	15 ± 10	356 ± 18	−6 ± 17

[1] $\overline{x}$ ± SEM. There were no significant differences between the changes in group A and those in group B. FEV$_1$, forced expiratory volume in 1 s; FVC, forced vital capacity; QOWB, quality of well-being.
[2] Normally distributed data (two-sample *t* test).
[3] Nonparametric data (Mann-Whitney *U* test).

TABLE 5
Nutrient intakes at baseline and changes after 8 wk of supplementation in groups A and B[1]

Nutrient	Group A (n = 24)		Group B (n = 22)	
	Week 4 (baseline)	Change	Week 4 (baseline)	Change
Energy (kJ)[2]	267 ± 24	−13 ± 11	295 ± 22	20 ± 12
Protein (g)[3]	2.1 ± 0.2	−0.1 ± 0.1	2.7 ± 0.2	−0.3 ± 0.1
Fat (g)[3]	2.5 ± 0.2	−0.1 ± 0.1	3.0 ± 0.3	−0.2 ± 0.2
Saturated (%)[2]	50.9 ± 1.0	−0.2 ± 0.04	49.8 ± 1.3	2.6 ± 0.03
Monounsaturated (%)[2]	37.8 ± 0.6	−0.5 ± 0.03	37.5 ± 0.8	−1.1 ± 0.02
Polyunsaturated (%)[2]	11.3 ± 0.8	0.7 ± 0.03	12.7 ± 0.9	−1.2 ± 0.02
Carbohydrate (g)[3]	8.4 ± 0.8	−0.4 ± 0.4	8.4 ± 0.6	−0.5 ± 0.5
Vitamin A equivalents (μg)[2]	23.3 ± 3.5	−0.3 ± 2.6	29.9 ± 5.9	−4.4 ± 4.1
Vitamin C (mg)[2]	5.5 ± 1.4	−1.2 ± 1.3	5.2 ± 1.3	−1.8 ± 1.3
Iron (mg)[2]	0.29 ± 0.03	−0.02 ± 0.02	0.34 ± 0.04	−0.05 ± 0.03
Zinc (mg)[3]	0.25 ± 0.02	0.0 ± 0.02	0.32 ± 0.03[4]	−0.02 ± 0.02

[1] $\bar{x}$ ± SEM. All values are per kilogram body weight. There were no significant differences between the changes in group A and those in group B.
[2] Nonparametric data (Mann-Whitney U test).
[3] Normally distributed data (two-sample t test).
[4] Significantly different from group A, $P < 0.05$.

DISCUSSION

To our knowledge, this is the first double-blind, randomized control trial in CF patients in which the effect of antioxidant supplementation on isoprostane concentrations was examined. Although antioxidant defenses improved with high doses of antioxidants, there was no corresponding decrease in oxidative stress (as measured by 8-iso-PGF$_{2\alpha}$ concentrations). Plasma fatty acid concentrations were found to have the strongest influence on plasma 8-iso-PGF$_{2\alpha}$ concentrations, suggesting that a high fat intake contributes to oxidative stress. However, a correlation

TABLE 6
Plasma fatty acid composition at baseline and changes after 8 wk of supplementation in groups A and B[1]

Fatty acid	Group A (n = 24)		Group B (n = 22)	
	Week 4 (baseline)	Change	Week 4 (baseline)	Change
	mg/L		mg/L	
14:0[2]	28 ± 4	−3 ± 4	29 ± 3	8 ± 6
16:0[3]	445 ± 29	−25 ± 23	454 ± 26	23 ± 29
16:1n−7[3]	54 ± 7	−5 ± 4	54 ± 7	4 ± 4
18:0[3]	159 ± 9	−12 ± 7	164 ± 9	4 ± 10
18:1n−9[2]	425 ± 25	−23 ± 26	439 ± 24	40 ± 34
18:1n−7[2]	42 ± 3	−1 ± 2	42 ± 3	5 ± 3
18:2n−6[3]	447 ± 28	−22 ± 18	414 ± 24	−15 ± 14
18:3n−6[3]	9 ± 1	−1 ± 1	10 ± 1	0 ± 1
18:3n−3[2]	9 ± 1	−1 ± 1	10 ± 1	0 ± 1
20:3n−6[2]	34 ± 2	−3 ± 1	34 ± 1	0 ± 2
20:4n−6[2]	106 ± 6	−3 ± 3	109 ± 6	−1 ± 3
20:5n−3[2]	17 ± 1	−2 ± 1	20 ± 1	−1 ± 1
22:0[3]	13 ± 1	−1 ± 1	13 ± 1	−1 ± 1
22:6n−3[3]	13 ± 1	0 ± 1	12 ± 1	0 ± 1
PUFAs[3]	636 ± 35	−32 ± 21	608 ± 29	−17 ± 19
MUFAs[3]	521 ± 32	−28 ± 32	536 ± 31	48 ± 39
SFAs[3]	645 ± 40	−40 ± 32	660 ± 36	35 ± 43
Total[3]	1801 ± 90	−100 ± 68	1804 ± 80	66 ± 94

[1] $\bar{x}$ ± SEM. PUFA, polyunsaturated fatty acid; MUFA, monounsaturated fatty acid; SFA, saturated fatty acid. There were no significant differences between the changes in group A and those in group B.
[2] Nonparametric data (Mann-Whitney U test).
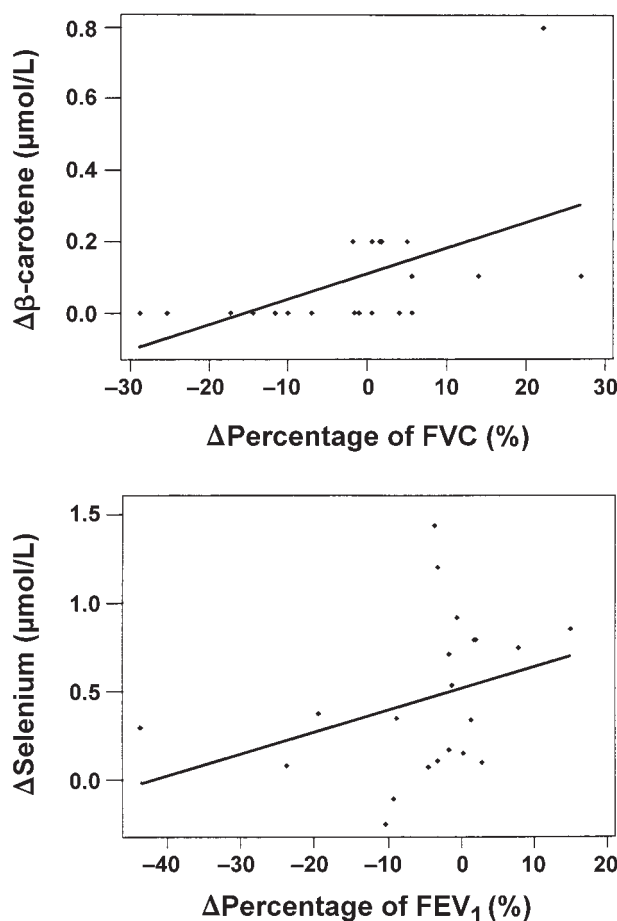[3] Normally distributed data (two-sample t test).

FIGURE 1. Changes (Δ) in β-carotene concentration plotted against Δpercentage of forced vital capacity (FVC; $r = 0.586$, $P = 0.005$) and Δselenium plotted against Δpercentage of forced expiratory volume in 1 s (FEV$_1$; $r = 0.440$, $P = 0.046$) in group B between baseline (week 4) and week 12. Data are nonparametric and were analyzed by using Spearman's rank correlation.
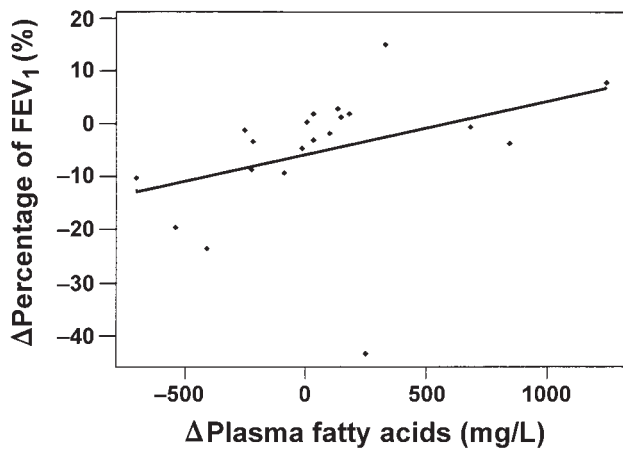
FIGURE 2. Changes (Δ) in percentage of forced expiratory volume in 1 s (FEV$_1$) plotted against Δplasma fatty acid concentration in group B between baseline (week 4) and week 12 ($r = 0.583$, $P = 0.006$). Data are nonparametric and were analyzed by using Spearman's rank correlation.

between increased plasma fatty acid concentrations and improved lung function suggests that high fat diets have clinical benefit. Relations also exist between increased antioxidant (plasma β-carotene and selenium) concentrations and improved lung function. Thus, both antioxidant supplementation and high-fat diets may have clinical relevance for CF patients.

The correlation between the change in 8-*iso*-PGF$_{2α}$ concentrations and the change in plasma fatty acid concentrations in group B agrees with the strong correlation previously observed between 8-*iso*-PGF$_{2α}$ concentrations and plasma fatty acid concentrations ($r = 0.768$, $P = 0.001$) (21). This suggests that the high-fat diet recommended to meet the increased energy demands of CF patients may predispose them to oxidative stress by increasing their plasma fatty acid concentrations. Diets rich in polyunsaturated fatty acids increase susceptibility to oxidative stress (39–41), and susceptibility increases with the number of double bonds (42).
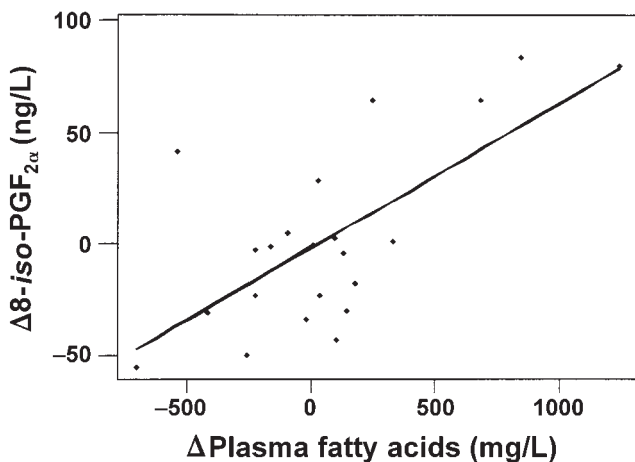

FIGURE 3. Changes (Δ) in 8-*iso*-prostaglandin F$_{2α}$ (8-*iso*-PGF$_{2α}$) concentration plotted against Δplasma fatty acid concentration in group B between baseline (week 4) and week 12 ($r = 0.689$, $P < 0.001$). Data are normal and were analyzed by using Pearson's correlation coefficient.
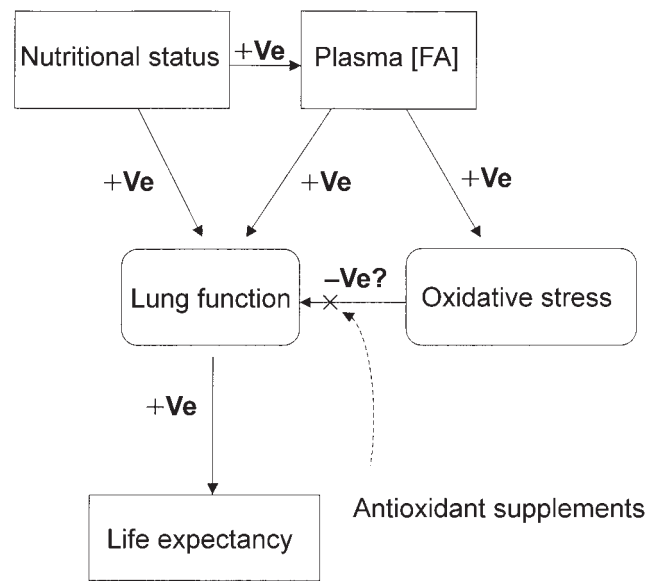

FIGURE 4. Model describing the relation between oxidative stress, nutritional status, antioxidants, and clinical status in cystic fibrosis. +Ve, positive effect; −Ve, negative effect; [FA], fatty acid concentration.

Indeed, a higher content of polyunsaturated fatty acids increases the oxidative susceptibility of LDL in CF (43). Evidence also suggests that high-fat diets increase oxidative stress irrespective of the degree of saturation of the fat (44–47). Although the underlying mechanism is uncertain, this effect may be a direct effect of increased free radical generation during fat metabolism or an indirect effect of increased metabolic rate resulting from increased energy intake (44). It is unlikely that elevated 8-*iso*-PGF$_{2α}$ concentrations in association with increased fatty acid concentrations are simply a result of the increased availability of substrate. From the plasma arachidonic acid (20:4n−6) and 8-*iso*-PGF$_{2α}$ values shown in Tables 6 and 3, respectively, it is apparent that arachidonic acid is available in abundance before and after supplementation (at concentrations that are 6 orders of magnitude higher than those of 8-*iso*-PGF$_{2α}$) and is thus not the limiting factor in isoprostane production. The contribution of dietary 8-*iso*-PGF$_{2α}$ to plasma 8-*iso*-PGF$_{2α}$ concentrations has been found to be small (28) but cannot be ruled out completely as a confounding factor.

Recent efforts have focused on promoting high-fat diets for CF patients because improved nutritional status and high fat intake are linked to improved survival (48). Furthermore, a clear association has been shown between poor nutritional status and deteriorating lung function (49). Our data also shows a correlation between the change in plasma fatty acid concentrations and the change in percentage of FEV$_1$. Thus, as increased fatty acid concentrations are linked to both elevated oxidative stress and improved lung function in the present study, we conclude that the clinical benefits gained from a high-fat diet outweigh any possible negative effects of oxidation, resulting in a net improvement in lung function (**Figure 4**).

These findings prompt us to question the importance of oxidative stress in CF. CF is a multifactorial disease, and many variables, such as infection, poor mucociliary clearance, and severity of the gene defect, determine the extent of lung damage. This

short-term supplementation trial does not provide any direct evidence that oxidative stress contributes to lung deterioration. Our previous study of pulmonary exacerbations in CF patients (21) supports the view that oxidative stress does not significantly affect lung function, because 8-*iso*-PGF$_{2\alpha}$ concentrations increased after antibiotic treatment at the same time that percentage of FEV$_1$ was improving. Direct evidence linking oxidative stress to clinical status is very limited (7, 50, 51), especially considering the numerous studies that have examined this issue. Although it is accepted that oxidative stress occurs in CF, further research is necessary to determine its contribution to lung deterioration before further resources are directed at minimizing oxidative stress.

Some data from our study that may be indirect evidence of a relation between oxidative stress and clinical status are the correlations between the change in β-carotene concentrations and the change in percentage of FVC and between the change in selenium concentrations and the change in percentage of FEV$_1$. These results agree with data that we (11) and others (12) have previously reported that showed correlations between lung function and β-carotene in CF. Other antioxidants that are linked to improved lung function in CF patients include vitamin E (50), vitamin A (52), and vitamin C (50). Although our data suggest a potential role for β-carotene and selenium in improving respiratory status, the mechanism by which this occurs cannot be elucidated from this study. It is possible that they influence lung function because of their role as antioxidants. However, the positive effect on lung function may be due to other factors, such as the enhancement of immune cell function, which has been observed as β-carotene concentrations increase (52, 53). The fact that increased concentrations of vitamin E (a well known lipid-soluble antioxidant) were not related to lung function suggests that β-carotene and selenium may act by mechanisms other than those of antioxidants.

Although our data suggest that plasma fatty acid concentrations have the strongest influence on 8-*iso*-PGF$_{2\alpha}$ concentrations, the antioxidant supplements may have had a greater effect than that of plasma fatty acids on 8-*iso*-PGF$_{2\alpha}$ concentrations if a greater increase in mean plasma β-carotene concentrations had been achieved. β-Carotene appears to play a unique role in antioxidant defense. Previous findings with other markers of oxidation (4, 10, 26, 54–56) showed elevated oxidative stress in CF despite normal vitamin E concentrations. However, when β-carotene status improved, oxidative stress was normalized (4, 26, 55). In these studies, although β-carotene uptake varied greatly between the patients and the studies, increases in β-carotene concentrations ranged from 6- to 50-fold. It is unclear why plasma β-carotene concentrations in our trial were so low after supplementation with a similar dose. The all-*trans* β-carotene isomer used in our study was previously shown to have the best bioavailability (57) and the best ability to effect malondialdehyde concentrations (57) and LDL oxidation (58). A study of β-carotene supplementation in healthy adults indicated that essentially no β-carotene is absorbed in the absence of dietary fat (59). Although the subjects in our trial consumed the supplement with their usual breakfast and pancreatic enzymes, their diet was not controlled; thus, fat intake varied. Future studies could be improved by having subjects consume capsules daily with the meal that contains the most fat (59) or with a food of specified fat content (55) or by having subjects take smaller, more frequent doses with their meals (4). The possibility that β-carotene absorption was influenced by the concurrent presence of high concentrations of other antioxidants or that β-carotene was used preferentially cannot be ruled out.

Diet analysis showed no difference between the 2 groups in their dietary intake. Unfortunately, intake data on vitamin E and selenium are not included in Foodworks software. However, there were no differences between the groups in other nutrient intakes, suggesting that the observed improvements in antioxidant defenses were due to the supplementation. Interestingly, plasma vitamin C concentrations increased from baseline values in both groups. Because the subjects in group A were not supplemented with vitamin C and because the subjects' dietary vitamin C intake did not change during the course of the study, we are unable to explain this increase. However, the increase is important because it may have masked the potential effects of the high-dose antioxidant supplement in group A.

A report suggested that peripheral blood markers may be less sensitive than are direct lung measurements (60). Thus, plasma antioxidant concentrations may be a poor reflection of local antioxidant defenses in the lungs, where most of the oxidative damage occurs. This highlights the importance in future studies of directly measuring antioxidant defenses in the lung lining fluid.

A limitation of conducting randomized control trials with antioxidants in a CF population is that a placebo group is inappropriate, as is a true washout period. These would require cessation of all fat-soluble vitamin supplements (which are ongoing treatment for many pancreatic insufficient patients), which is unethical and has possible adverse clinical outcomes. In the present study, this was addressed by using a low-dose supplement during the washout period for both groups and during the following 8 wk for group A (the control group). Thus, we cannot rule out the possibility that oxidative stress was stabilized by the low-dose supplement that was taken by all patients before the baseline sample collection, resulting in the inability of the high-dose supplement to further decrease 8-*iso*-PGF$_{2\alpha}$ concentrations.

In conclusion, improved plasma β-carotene, selenium, and fatty acid status are linked to improved lung function, despite the fact that increased fatty acid concentrations are also linked to increased oxidative stress. There is no direct evidence from our data that oxidative damage contributes to lung deterioration in CF patients. However, the relations that we observed between changes in β-carotene and selenium concentrations and changes in lung function suggest that improving antioxidant status may improve clinical status. Although these data may appear contradictory on the surface, it is possible that the correlations between changes in β-carotene and selenium concentrations and changes in lung function may result from actions of these nutrients that are unrelated to their antioxidant properties; these actions need to be further explored. Furthermore, an understanding of the relative contribution of oxidative stress to lung damage is needed. If oxidative stress is found to significantly affect the clinical status of CF patients, means of reducing oxidative damage while maintaining a high-fat diet need to be elucidated. Manipulation of dietary fat and antioxidant supplementation (in particular, β-carotene supplementation) may be part of the solution.

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