Antioxidant deficiency in cystic fibrosis: when is the right time to take action?1–3

Evelyn I Back, Claudia Frindt, Donatus Nohr, Juergen Frank, Rita Ziebach, Martin Stern, Michael Ranke, and Hans K Biesalski

ABSTRACT
Background: Little is known about age- and disease-related changes in prooxidant and antioxidant systems in patients with cystic fibrosis (CF).
Objective: We investigated changes in antioxidant concentrations and oxidative stress in plasma, buccal mucosal cells (BMCs), and breath condensate in patients with CF in relation to age and disease progression.
Design: We recruited 22 patients with CF as well as 35 healthy control subjects and conducted a cross-sectional study by dividing the participants into 4 age groups (<6 y, 6–11 y, 12–17 y, ≥18 y). We collected fasting blood samples, BMCs, and breath condensate. Carotenoids, α-tocopherol, vitamin C, protein carbonyls, thiobarbituric acid–reactive substances, and F2-isoprostanes were assessed.

Results: In patients with CF, plasma vitamin C concentrations, plasma and BMC α-tocopherol concentrations, and forced expiratory volume in 1 s (percentage predicted) decreased significantly with age. Plasma β-carotene, β-cryptoxanthin, and total lycopene were significantly lower in patients than in control subjects in all age groups. Furthermore, α-tocopherol and vitamin C plasma concentrations as well as α-tocopherol concentrations in BMCs were significantly lower in CF patients ≥18 y old, whereas all indicators of oxidative stress assessed were significantly higher than those same indicators in control subjects.

Conclusions: Adult patients with CF in particular showed distinct vitamin deficits and elevated indicators of oxidative stress in plasma, BMCs, and breath condensate along with a progression of clinical status. We suggest that early in life dietary habits should be improved and that innovative supplementation strategies should be applied to optimize the antioxidant status of patients with CF.


KEY WORDS Antioxidants, α-tocopherol, vitamin E, vitamin C, ascorbic acid, β-carotene, cystic fibrosis, oxidative stress, F2-isoprostanes, thiobarbituric acid–reactive substances

INTRODUCTION
In a healthy organism, free radicals and reactive oxygen species are controlled by various enzymes and antioxidants in the body, and a balance exists between prooxidant and antioxidant processes (1). In patients with cystic fibrosis (CF), however, this balance is disturbed (2) because the availability of important dietary antioxidants such as vitamin E and carotenoids is diminished as a result of maldigestion, malabsorption, and increased turnover (3, 4). Furthermore, chronically stimulated immune cells as well as invading pathogens are able to produce free radicals, thus increasing the free radical load (5–7). Thanks to therapeutic advances, eg, improved antibiotic treatment, the life expectancy of patients with CF has increased, yet the abovementioned imbalance is thought to contribute to disease progression in CF. Against this background, it seems important to strengthen the antioxidant defense to ensure health and well-being for an “aging” CF patient population. The aim of our study was to assess whether potential changes in antioxidant systems and oxidative stress were attributable to disease progression in CF or merely an effect of age per se; ie, whether the same trends observed in patients with CF could be identified in a healthy reference population. Furthermore, we wanted to investigate whether there were differences in antioxidant status and in the amount of oxidative stress between patients and healthy control subjects of various age groups.

SUBJECTS AND METHODS
Study design
Twenty-two patients with CF from the CF outpatient clinic and 35 control subjects from the endocrinologic outpatient clinic as well as from the clinic staff of the University Children’s Hospital Tübingen were enrolled in our cross-sectional study. Patients and control subjects were divided into 4 age groups: <6 y, 6–11 y, 12–17 y, and ≥18 y. Inclusion criteria were fasting for at least 10 h before sample collection and not taking any vitamin supplements for 24 h (patients) or 7 days (control subjects) before sample collection. Exclusion criteria were pulmonary exacerbations in the 4 wk before study participation (patients); smokers, pregnancy, or breastfeeding; severe liver disease (≥2-fold increase of transaminases for ≥6 mo plus ultrasonographic signs of morphologic liver changes); and acute or...
chronic diseases of the respiratory and digestive system (control subjects). The study was approved by the ethical committee of the Medical Faculty of the University of Tübingen. Informed consent was obtained from the subject or parents or guardians.

Subject characteristics

Personal and clinical data as well as fasting blood samples were collected by trained medical staff members during a routine consultation. Body mass index (BMI) z scores were calculated with use of German reference databases (8, 9). Lung function measurements were carried out by a trained nurse with use of a MasterScreen Body plethysmograph (Viasys Healthcare GmbH, Hoechberg, Germany). Maximum vital capacity and forced expiratory volume in 1 s (FEV₁) were recorded and compared with predicted values. Dietary intake data (3-d weighed food record was analyzed with EBISPRO for WINDOWS; J. Erhardt, University of Hohenheim), fat absorption data, and information about the use of supplements were obtained from the patients’ clinical files. Fat absorption was assessed as follows: dietary fat intake was calculated separately for days 1 and 2 of the 3-d weighed food record. On days 2 and 3, 2 separate 24-h stool samples were collected, and their fat content was analyzed. Percentage fat absorption was then calculated as fat intake of day 1 divided by fat excretion of day 2 and fat intake of day 2 divided by fat excretion of day 3. Pancreatic insufficiency was defined as total daily stool fat excretion > 7 g (before enzyme replacement therapy) in combination with pathologically elevated fecal elastase values.

Collection of blood samples

Blood for all analyses was drawn into EDTA-coated S-Monovettes (Sarstedt AG & Co, Nümbrecht, Germany) after the participants had fasted for at least 10 h. The tube was shaken well and then immediately put on ice in the dark. The tubes were then centrifuged at 3000 × g for 10 min at 4 ºC. Plasma was separated from blood cells, stabilized as required, and then frozen at −80 ºC.

Collection of buccal mucosa samples

Buccal mucosal cells (BMCs) were collected by a noninvasive method described by Gilbert et al (10). Subjects were instructed how to collect the BMCs before sample collection. The study staff members or the parents collected the samples from younger children. Briefly, subjects were asked to rinse their mouth thoroughly with drinking water. Then the subjects brushed the inside of one cheek 20 times (1 time = 1 downstroke) with use of a soft surgical toothbrush (select TE 227 special care; Medico-Service Beyer GmbH, Herdorf, Germany), which was soaked beforehand in sterile isotonic NaCl (0.9%) or glucose (5.4%) solution. Only the topmost cell layers were brushed off in a completely pain-free manner. After brushing, the participants rinsed the mouth with 20 mL isotonic salt or glucose solution. The procedure was repeated for the second cheek, and the toothbrush was washed with the remaining 10 mL solution. The 3 fractions of the cell suspension were collected and transferred to a 50-mL tube precoated with 200 μL 2% butylhydroxytoluene (B-1378; Sigma-Aldrich, Steinheim, Germany) in ethanol. The tube was immediately placed on ice in the dark. The samples were then washed twice with phosphate-buffered saline (pH 7.2). Centrifugations between washes were carried out at 1000 × g for 5 min at 4 ºC. The resulting cell pellets were divided into aliquots and placed in reaction tubes, and the tubes were flooded with argon gas before closing and storing them at −80 ºC until analysis.

Collection of breath condensate

Breath condensate was collected noninvasively with use of ECOScreen (Erich Jaeger GmbH, Hoechberg, Germany). The participants were asked to breathe for 5 min regularly and not deeper than usual through the attached mouthpiece. After the collection period, the breath condensate (0.5–1 mL) was divided into aliquots, placed in 2 tubes, and immediately put on dry ice in the dark. The aliquots were then transferred to a freezer and stored at −80 ºC for a maximum of 4 wk until analysis.

Vitamin C in plasma

Vitamin C in plasma was analyzed with use of the method by Ihara et al (11, 12). The method was adapted at our institute to be used with a Cobas Mira S (Roche, Grenzach-Wyhlen, Germany). Briefly, a 200-μL plasma aliquot was transferred to a tube containing 20 μL freshly prepared 40% m-phosphoric acid directly after separating the plasma from blood cells. The sample was mixed by vortex for 10 s and incubated for 10 min at room temperature in the dark. The mixture was then centrifuged at 13 000 × g for 1 min at 4 ºC. All samples were frozen at −80 ºC until analysis. For analysis, samples were thawed and centrifuged at 13 000 × g for 1 min at 4 ºC. The supernatant was transferred to a fresh tube. The supernatant (25 μL) was then mixed with 10 μL potassium phosphate buffer (0.1 mol/L, pH 6.5) and 200 μL 4-hydroxy-2,2,6,6-tetramethylpiperidinyloxy free radical (2 mg TEMPO/10 mL phosphate buffer, catalog no. H-8258; Sigma-Aldrich Chemie GmbH, Steinheim, Germany). After incubation, 85 μL o-diphenylamine (5 mg/10 mL phosphate buffer, catalog no. D-2385; Sigma-Aldrich Chemie GmbH) and 10 μL potassium phosphate buffer (0.1 mol/L, pH 6.5) were added. The formation of the colored product was measured at 340 nm and was compared with the concentration of a known standard (56.6 μmol vitamin C/L).

Fat-soluble vitamins in plasma and buccal mucosa

Plasma aliquots were taken directly after separating the plasma from blood cells and frozen until analysis at −80 ºC. For extraction of fat-soluble vitamins, 20-μL plasma aliquots were mixed with 100 μL extraction solvent [50% ethanol pro analysis (pa), 50% 1-butanol pa, containing 5 mg butylhydroxytoluene/mL and 8 μmol Tocol/L (Matreya Inc, State College, PA) as an internal standard] to extract the vitamins and to denature plasma proteins. The sample was then mixed by vortex for 30 s and centrifuged at 21 000 × g for 10 min at 4 ºC. The supernatant was transferred to a polypropylene vial (catalog no. LAPH 11191217; VWR International GmbH, Bruchsal, Germany), which was closed with a cap (catalog no. 548-3014; VWR International GmbH).

For the extraction of fat-soluble vitamins from BMCs, the cell pellets were thawed at room temperature in the dark. The cells were cracked by immersing the tubes overall 3 times alternately in lukewarm water and liquid nitrogen for 2 min. Ethanol pa (200 μL) was added to each sample. The samples were then mixed by vortex, and 1000 μL hexane was added. The samples were then mixed by vortex for 30 s, incubated for 15 min on a covered shaker incubator at room temperature, and centrifuged at 3000 × g. The resulting cell pellets were divided into aliquots and placed in reaction tubes, and the tubes were flooded with argon gas before closing and storing them at −80 ºC until analysis.

Downloaded from https://academic.oup.com/ajcn/article-abstract/80/2/374/4690320 by guest on 07 March 2019
g for 2 min at 4 °C. The hexane phase was extracted and collected in a glass test tube. The extraction process was then repeated. In the second run, samples were only incubated for 5 min on the shaker incubator and centrifuged at 13,000 × g instead of 3000 × g. The hexane fractions were combined and dried for about 10 min under a gentle stream of nitrogen at room temperature in the dark. The residue was redissolved in 100 µL extraction solvent.

Fat-soluble vitamins were analyzed with use of the method of Erhardt et al (13) with slight modifications. The HPLC systems consisted of a ProStar pump 210 (Varian Deutschland GmbH, Darmstadt, Germany), a UV-VIS Dual λ Absorbance Detector Waters 2487 (Waters, Arcade, NY), a Scanning Fluorescence Detector Waters 474 (Waters), an autosampler, and a STAR chromatography workstation HPLC software (version 5.31; Varian Deutschland GmbH). The UV-VIS spectrophotometer was programmed as follows: 0–2.5 min: 325 nm; 2.5–13.5 min: 450 nm; 13.5–15 min: 325 nm. The fluorescence detector (Waters 747) was constantly measuring at 298 and 328 nm. A spherisorb column, 3 µm, 250 mm × 4 mm (Grom, Herrenberg, Germany), which was kept at 40 °C, was used for the separation. The isocratic mobile phase consisted of 82% acetonitrile, 15% dioxane, and 3% methanol [containing 100 mmol/L ammonium acetate (catalog no. 1116.0500; Merck, Darmstadt, Germany) and 0.1% triethylamine (catalog no T-0886; Sigma-Aldrich Chemie) as related to the amount of methanol used]. The flow rate was constant at 1.25 mL/min. For quantification, the internationally certified NIST standards (standard reference material 968c; National Institute of Standards and Technology, Gaithersburg, MD) were used. The area under the curve (AUC) of the respective vitamin was divided by the AUC of the internal standard, and the corrected AUC was used for the calculation of the vitamin concentration of the respective sample.

**DNA content of buccal mucosa samples**

As a measure for the amount of cells in each BMC aliquot, the DNA content of each cell pellet was determined after extraction of fat-soluble vitamins. For determination of the DNA content of BMCs, the method according to Natarajan et al (14) was used. The dried cell pellets were incubated for 24 h at 37 °C with 200 µL 1:5 (by vol) mixture of 0.16% acetaldehyde and 20% perchloric acid plus 320 µL 4% (wt:vol) solution of diphenylamine (catalog no. D-2385; Sigma-Aldrich Chemie GmbH) in acetic acid. After incubation, the mixture was mixed by vortex. Then 200 µL solution was transferred to a microtiter plate (PS Microplate 96-flat bottom well; Greiner bio-one, Frickenhausen, Germany) and analyzed at 590 nm with a plate reader (Bio Kinetics Microplate Reader EL 340; Bio Tek Instruments, Winooski, VT). An 8-point standard curve made from fish sperm DNA (Boehringer Ingelheim Bioproducts Partnership, Heidelberg, Germany) in ultrapure water and ranging from 0.38 to 5.769 µg DNA/mL was used for quantification.

**Total cholesterol in plasma**

Total cholesterol in plasma was determined with use of the ABX Diagnostics Cholesterol kit (REF.A11A00051; Axon Lab AG, Stuttgart, Germany) for Cobas Mira S (Roche) according to the manufacturer’s instructions.

**C-reactive protein**

C-reactive protein (CRP) was assessed with use of the ABX Diagnostics CRP 400 kit (REF.A11A00153; Axon Lab AG) for Cobas Mira S (Roche) according to the manufacturer’s instructions. CRP concentrations ≥5 mg/L were considered as a marker of acute infection, i.e., CRP positive.

**Thiobarbituric acid–reactive substances in plasma**

An improved method for the analysis of malondialdehyde was established at our institute by Jentzsch et al (15). Briefly, 50 µL plasma was mixed with 3 µL butylhydroxytoluene in ethanol pa (20 mg/mL) directly after centrifugation and was frozen at −80 °C. Samples were thawed for analysis, and 50 µL phosphoric acid (0.2 mol/L) and 6 µL thiobarbituric acid (16 g/L) were added. The mixture was incubated for 45 min at 90 °C. Then the samples were cooled on ice, and 125 µL 1-butanol pa and 10 µL saturated NaCl solution were added. This mixture was mixed by vortex for 60 s. After centrifugation for 1 min at 12,000 × g at room temperature, 62.5 µL supernatant was transferred to a microtiter plate (PS Microplate 96-flat bottom well; Greiner bio-one) and measured in a FL 600 Microplate Fluorescence Reader (Bio Tek Instruments) at an excitation wavelength of 530 nm and an emission wavelength of 590 nm. A standard curve with 7 different concentrations of malondialdehyde-[bis(dimethyl acetate] in phosphoric acid (0.2 mol/L), ranging from 0.05 to 2.0 µmol/L, was used for quantification.

**Protein carbonyls in plasma**

Carbonyl groups in plasma proteins were determined with use of an enzyme-linked immunosorbent assay as described by Buss et al (16) with slight modifications. Briefly, carbonyl groups were derivatized with dinitrophenylhydrazine. The derivatized samples were transferred to a 96-well plate (Nunc Immuno Plate Maxisorb; Nunc GmbH & Co KG, Wiesbaden, Germany) and incubated overnight. An anti-dinitrophenol antibody from rabbit (dilution 1:1000; D-9656; Sigma, Taufkirchen, Germany) was used for the separation. Then the mixture was mixed by vortex. An anti-dinitrophenol antibody from rabbit was used for quantification. The absorbance values were
measured with a plate reader (Bio Kinetics Microplate Reader EL 340; Bio Tek Instruments) at a wavelength of 405 nm.

**Evaluation of antioxidant status by way of plasma concentrations of antioxidant vitamins**

Definitions of cutoffs for various stages of vitamin depletion vary between laboratories and institutions. The stages we used for classification of patients and control subjects in Results were vitamin deficit (17) (plasma vitamin C < 11 μmol/L), suboptimal vitamin status (17) (plasma α-tocopherol < 16.2 μmol/L, plasma β-carotene < 0.03 μmol/L), and optimal plasma concentrations (18) (plasma vitamin C > 50 μmol/L, plasma α-tocopherol > 30 μmol/L, plasma β-carotene > 0.4 μmol/L). According to Biesalski et al (18, p 152), “Optimal plasma concentrations are ‘target parameters’ which are currently derived from prospective studies and case studies and from comparisons between countries with large study populations. . . . When these threshold levels are reached, the relative risk of diseases such as coronary heart disease and certain neoplastic disorders is low as long as they derive from a suboptimal antioxidant status.”

**Statistical analysis**

Plasma concentrations below the detection were assigned half the value of the detection limit (ie, β-cryptoxanthin: 0.015 μmol/L; α-carotene: 0.04 μmol/L; β-carotene: 0.04 μmol/L; total lycopene: 0.065 μmol/L) to be able to consider the respective sample in statistical analyses. Plasma α-carotene concentrations of patients with CF were, however, not statistically analyzed, because they were all below the detection limit and, thus, consisted completely of surrogate values. The described procedure was furthermore not applied to buccal mucosa samples, because the detection limit here refers to vitamin concentrations in the extract made from each BMC aliquot. Assigning the same value to those extracts would introduce a large error, because the BMC aliquots consisted of varying amounts of cells.

First of all, the continuous variables were tested for normal distribution by Shapiro-Wilk W test. In addition, the homogeneity of variances of subgroups was tested with Levene test. All variables except BMI z scores were not normally distributed, and for them nonparametric methods were used. The patient group <6 y old is not listed in the tables, because only one child with CF <6 y old met all criteria for inclusion. This child was, however, included in the graphs that show the age-related trends that were identified by linear regression. For the cholesterol data, a two-factor model on the ranks, and for BMI z scores, a two-factor analysis of variance were specified to identify differences between patients and control subjects as well as between age groups. Antioxidant status and oxidative stress indicators of patients and control subjects were compared separately for the different age groups with use of the nonparametric Mann-Whitney U test. Differences in dietary and supplemental vitamin intake among the 3 patient age groups were analyzed with use of the Kruskal-Wallis test. All differences were considered significant with P < 0.05. SPSS for WINDOWS software (version 9.0; SPSS Inc, Chicago) was used for all statistical tests. Data are reported as median and interquartile range (ie, quartile 1–quartile 3), except for BMI z scores, which, as a normally distributed variable, are reported as mean ± SD. When the number of samples for a specific analysis was lower than the maximum number of subjects available, the exact n is specified in the respective table.

**RESULTS**

**Characteristics of the study population**

Table 1 and Table 2 show the most important general and clinical characteristics of the study population according to the respective age group. Patients and control subjects were age matched, yet they were not sex matched in all groups, as can be seen in Table 1. We had, however, no evidence that sex had an influence on antioxidant status or indicators of oxidative stress and, therefore, did not further consider it in the interpretation of our results.

**Age- and disease-related trends and changes**

As FEV₁ decreased significantly with age (Figure 1), we used both variables to investigate the effects of disease progression on antioxidant status and oxidative stress in patients with CF. Figure 2 shows which age-related trends could be identified by linear regression in patients with CF. The concentration of vitamin C decreased significantly with age. Furthermore, the concentration of α-tocopherol in both plasma and BMCs decreased significantly with age, whereas we observed no significant
change of plasma α-tocopherol when corrected for total plasma cholesterol concentrations. Absolute concentrations of α-tocopherol in plasma were thus significantly reduced in older patients, whereas relative loading of lipoproteins with α-tocopherol remained rather constant. Figure 3 shows the results of linear regression analysis with FEV₁ instead of age. Probably because of the smaller data set (actual FEV₁ measurements were not available from all patients), only α-tocopherol in BMCs was significantly higher in patients with better FEV₁, whereas P for vitamin C was slightly above 0.05 and for the other variables distinctly above 0.05. We did not identify significant changes with age or FEV₁ for the other variables distinctly above 0.05. We did not statistically compare the results of our patients with results of the control group.

To differentiate between effects attributable to age per se as compared with age in the sense of disease progression as mentioned earlier, we also looked for age-related changes in our control group. Plasma total lycopene (n = 35; R² = 0.1656, P = 0.015), total lycopene corrected for cholesterol concentration (n = 35; R² = 0.1124, P = 0.049), and plasma α-carotene (n = 35; R² = 0.1151, P = 0.046) were the only variables that changed significantly with age in control subjects. Unlike the vitamin concentrations in plasma and BMCs of patients with CF, however, these vitamin concentrations significantly increased with age in the control group.

**Differences in antioxidant status between patients with cystic fibrosis and control subjects**

Table 3 displays plasma and tissue antioxidant concentrations of patients and control subjects. Plasma β-carotene, β-cryptoxanthin, and total lycopene concentrations (both absolute and corrected for cholesterol) were significantly lower in patients of all age groups than for control subjects. In addition, plasma vitamin C, plasma α-tocopherol, and BMC α-tocopherol were significantly lower in patients aged ≥18 y than in control subjects. All except one patient had BMC carotenoid concentrations below the detection limit. We, therefore, did not statistically compare the results of our patients with results of the control subjects as explained in Subjects and Methods.

**Differences in the markers of oxidative stress between patients with cystic fibrosis and control subjects**

Table 4 specifies which significant differences in the indicators of oxidative stress we observed between patients with CF and control subjects. In patients with CF aged 6–11 y, plasma protein carbonyl concentrations (marker of oxidative modification of proteins) were significantly higher than in the respective control group. In patients aged ≥18 y, all markers of oxidative stress assessed were significantly higher.
Vitamin intake of patients with cystic fibrosis

Table 5 shows the patients’ vitamin intake (in mg) as well as percentage coverage of requirements [German Nutrition Society (19): recommended intake for vitamin C and estimated adequate intake for vitamin E; Institute of Medicine (20): dietary reference intake for vitamin C, dietary β-carotene, and dietary plus supplemental vitamin E]. No significant correlation between dietary intake and respective plasma concentrations of any of the 3 vitamins could be identified (vitamin C: n = 14, R² = 0.0961, P > 0.05; β-carotene: n = 9, R² = 0.334, P > 0.05; vitamin E: n = 14, R² = 0.0005, P > 0.05).

There were no significant differences in antioxidant vitamin intake among the 3 age groups.

Vitamin deficits and optimal plasma concentrations in patients with cystic fibrosis and control subjects

Table 6 presents the percentage of patients and control subjects with vitamin deficits or suboptimal vitamin status and the percentage reaching optimal plasma concentrations. None of the patients with CF suffered from vitamin C deficit, yet the percentage reaching optimal plasma concentrations decreased in the older age groups. The percentage of patients with CF with suboptimal vitamin E status increased with age, whereas only a few control subjects in the 12–17 y age group had a suboptimal vitamin E status. Most patients with CF, but only 20% of control subjects, in all age groups had suboptimal β-carotene plasma concentrations.

DISCUSSION

Our study demonstrated that the concentration of antioxidants in plasma and tissue (BMCs) decreased significantly with age in patients with cystic fibrosis (CF) but not in healthy control subjects. Furthermore, antioxidant concentrations in plasma and tissue of patients with CF were reduced when compared with healthy control subjects. The differences in antioxidant status were most pronounced in patients aged ≥18 y. This patient group also had significantly higher markers of oxidative stress than did healthy control subjects. Overall, our results show that antioxidant depletion in patients with CF is a progressive process. Therefore, antioxidant status should be monitored carefully, and antioxidant supplementation should be considered before severe deficits develop to provide optimum antioxidant protection.

Antioxidant status

In general, our results correspond well with results of previous studies. Winklhofer-Roob et al (21) as well as Brown et al (22) documented a similar decrease of vitamin C plasma concentrations with age. However, an explanation for why vitamin C “vanishes” from the plasma of older patients despite dietary intakes similar to those of younger patients has not yet been...
offered. In general, mostly fat-soluble vitamin concentrations are thought to be a problem in CF, because patients with CF often suffer from fat maldigestion and malabsorption. We assume that in older patients with CF there is an increased turnover of vitamin C, resulting from an increased demand to reduce the tocopheryl radical. Clinical studies dealing with patients suffering from severe oxidative stress have, for example, documented low plasma concentrations of vitamin C that increased after oxidative stress had subsided (23, 24). Ascorbate utilization by cycling vitamin E is, however, not well demonstrated or accepted in vivo, although it is easily demonstrated in vitro.

Vitamin E and carotenoids, notably β-carotene and lycopene, exert antioxidant functions in lipid phases and, therefore, were determined in our study. In accordance with other literature data (4, 25, 26), the plasma carotenoid concentrations of our patients with CF were significantly reduced when compared with control subjects. The exact plasma values measured vary between different studies, probably because of seasonal and methodologic effects. The α-tocopherol plasma concentrations measured in our patients with CF fall within the range reported in the literature (4, 25, 26). As mentioned before, we could not identify any dietary pattern to explain why some of the patients had a less favorable antioxidant status than others. It is particularly striking that so many patients suffered from a biochemical vitamin E deficit, although most of them had vitamin E supplements prescribed. As we did not ask the patients whether they actually took the supplements every single day as prescribed, we are, unfortunately, unable to provide an exact compliance rate. It is, thus, conceivable that the suboptimal vitamin E status observed in a lot of our patients with CF was either due to unsatisfactory compliance, to insufficient bioavailability of vitamin E from the supplements used, or to incorrect vitamin dosage.

As for the antioxidant concentration in BMCs, we are, to our knowledge, the first ones to investigate antioxidant stores in patients with CF with this method. Buccal mucosa can be sampled noninvasively and is, thus, readily and repeatedly available for subsequent analysis. Furthermore, we observed in another study conducted at our institute that β-carotene concentrations in BMCs increased after supplementation, whereas signs of oxidative stress as measured by TBARS simultaneously decreased (13). Vitamin concentrations in BMCs, therefore, respond to dietary intake and can be used to monitor the effect of supplementation on vitamin availability in the tissue.

Oxidative stress

When looking at the potentially deleterious consequences of decreased antioxidant capacity, various researchers report increased concentrations of oxidative stress in their patient cohorts. McGrath et al (7), for example, measured significantly elevated TBARS:cholesterol concentrations in patients with CF. Montuschi et al (27) reported increased F2α-isoprostane concentrations in breath condensate in stable patients with CF, and
TABLE 3
Differences in antioxidant status between cystic fibrosis (CF) patients and control subjects

<table>
<thead>
<tr>
<th></th>
<th>6–11 y age group</th>
<th>12–17 y age group</th>
<th>≥18 y age group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CF patients</td>
<td>Control subjects</td>
<td>CF patients</td>
</tr>
<tr>
<td><strong>Plasma</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vitamin C (μmol/L)</td>
<td>68.4 (66.2–72.5)</td>
<td>65.4 (55.8–82.9)</td>
<td>53.3 (42.0–68.8)</td>
</tr>
<tr>
<td>β-Carotene (μmol/L)</td>
<td>0.158 (0.14–0.43)</td>
<td>0.612 (0.40–0.89)</td>
<td>0.158 (0.040–0.22)</td>
</tr>
<tr>
<td>β-Carotene:cholesterol (μmol/mmol)</td>
<td>0.0482 (0.036–0.14)</td>
<td>0.164 (0.10–0.18)</td>
<td>0.0377 (0.016–0.076)</td>
</tr>
<tr>
<td>β-Cryptoxanthin (μmol/L)</td>
<td>0.0381 (0.015–0.052)</td>
<td>0.164 (0.080–0.24)</td>
<td>0.0303 (0.015–0.045)</td>
</tr>
<tr>
<td>β-Cryptoxanthin:cholesterol (μmol/mmol)</td>
<td>0.117 (0.0085–0.012)</td>
<td>0.0386 (0.018–0.052)</td>
<td>0.00843 (0.0059–0.019)</td>
</tr>
<tr>
<td>Total lycopene (μmol/L)</td>
<td>0.0650 (0.065–0.065)</td>
<td>0.564 (0.35–0.69)</td>
<td>0.0650 (0.065–0.16)</td>
</tr>
<tr>
<td>Total lycopene:cholesterol (μmol/mmol)</td>
<td>0.0178 (0.016–0.037)</td>
<td>0.135 (0.084–0.16)</td>
<td>0.0253 (0.020–0.039)</td>
</tr>
<tr>
<td>α-Tocopherol (μmol/L)</td>
<td>21.8 (18.5–29.9)</td>
<td>24.3 (18.3–27.2)</td>
<td>15.3 (14.5–25.8)</td>
</tr>
<tr>
<td>α-Tocopherol:cholesterol (μmol/mmol)</td>
<td>5.6 (4.6–9.4)</td>
<td>5.4 (4.8–6.1)</td>
<td>5.9 (4.1–6.5)</td>
</tr>
<tr>
<td><strong>BMC</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>α-Tocopherol (pmol/μg DNA)</td>
<td>154.0 (93.0–199.5)</td>
<td>158.1 (76.2–207.6)</td>
<td>117.6 (62.5–138.5)</td>
</tr>
</tbody>
</table>

1 Values are median; interquartile range in parentheses. BMC, buccal mucosal cells.
2 n lower than maximum number of observations: for vitamin C, n = 6; for α-tocopherol in BMCs, n = 5.
3 n lower than maximum number of observations: for α-tocopherol in BMCs, n = 4.
4 n lower than maximum number of observations: for all fat-soluble vitamins, n = 6; for vitamin C, n = 5; for α-tocopherol in BMCs, n = 5.
5 n lower than maximum number of observations: for α-tocopherol in BMCs, n = 12.
Dominguez et al (28) observed elevated plasma protein carbonyl concentrations in patients with CF. The same findings could be confirmed in our study in which TBARS:cholesterol, \( F_2 \)-\( \alpha \)-isoprostane, and carbonylated protein concentrations were significantly increased but mainly in older (\( \geq 18 \) y old) patients with CF who presented the lowest antioxidant concentrations.

**Interactions between antioxidant status, oxidative stress, and clinical status**

It is well known that one of the most important variables for disease progression in CF, percentage predicted FEV\(_1\), decreases with age in patients with CF (22), as our study confirmed. The problem is that the design used in most of the studies, including our own, only shows the coincidence of certain effects, eg, decrease of FEV\(_1\) and depletion of antioxidant stores, not their causality or correlation.

The problem with most of the reported intervention trials in the literature is that the researchers did not assess the direct clinical relevance of the intervention (administration of vitamin E, \( \beta \)-carotene, or both) for the patients. Most researchers stated either that a biochemical vitamin E deficit was improved after supplementation (29, 30) or that the correction of low vitamin E or \( \beta \)-carotene plasma concentrations corresponded with improved in vitro test results (eg, increased resistance of LDL to copper-induced lipid peroxidation in vitro) (25, 31). In contrast, Wood et al (32) carried out an 8-wk, double-blind, randomized intervention trial, providing one patient group with low-dose (10 mg vitamin E, 500 \( \mu \)g vitamin A) and one group with high-dose (200 mg vitamin E, 300 mg vitamin C, 25 mg \( \beta \)-carotene, 90 \( \mu \)g selenium, 500 \( \mu \)g vitamin A) vitamin supplements. They observed significant changes of clinical indicators after treatment, eg, increased plasma antioxidant concentrations and a correlation between improved \( \beta \)-carotene status and lung function as well as between improved selenium status and lung function. However, the study had some flaws, because blood samples were not collected in the fasting state, and the intervention period was possibly too short to observe further clinical changes. Renner et al (33) are the only researchers who actually reported distinct clinical benefits from high-dose (1 mg \( \cdot \) kg\(^{-1}\) body weight \( \cdot \) d\(^{-1}\); maximum 50 mg/d) \( \beta \)-carotene supplements. Their patients required significantly less antibiotics during the phase of high-dose \( \beta \)-carotene supplementation.

Finally, one observation deserves further interest when looking at the increased indicators of oxidative stress in older patients with CF. Martin-Gallán et al (34) reported that markers of oxidative stress (protein carbonyls) were elevated in diabetic patients with and without microangiopathy when compared with healthy control subjects. Many patients with CF will indeed ultimately develop CF-related diabetes mellitus. This factor might, therefore, contribute to increased indicators of oxidative stress in the oldest patient group. However, the development of diabetes might be favored by increased indicators of oxidative stress.

### TABLE 4

Differences in oxidative stress between cystic fibrosis (CF) patients and control subjects

<table>
<thead>
<tr>
<th>Variable</th>
<th>CF patients</th>
<th>Control subjects</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma protein carbonyls (nmol/mg protein)</td>
<td>0.608 (0.572–0.811)(^{2,3})</td>
<td>0.531 (0.524–0.547)</td>
</tr>
<tr>
<td>( \geq 18 ) y age group</td>
<td>0.623 (0.536–0.686)(^{2,4})</td>
<td>0.536 (0.513–0.552)(^{3})</td>
</tr>
<tr>
<td>TBARS:cholesterol (nmol MDA/nmol cholesterol)</td>
<td>99.8 (90.1–135.9)(^{2,7})</td>
<td>67.8 (56.8–79.5)</td>
</tr>
<tr>
<td>( F_2 )-( \alpha )-isoprostane in breath condensate (pg/mL)</td>
<td>15.7 (12.2–43.1)(^{6})</td>
<td>11.3 (8.3–12.7)(^{3})</td>
</tr>
</tbody>
</table>

\(^{1}\) All values are median; interquartile range in parentheses. TBARS, thiobarbituric acid–reactive substances; MDA, malondialdehyde.

\(^{2,3,5,6,9}\) n lower than maximum number of observations; \(^{4}\) n = 4, \(^{7}\) n = 6, \(^{8}\) n = 12.

\(^{3,4,7,8}\) Significantly different from control subjects: \(^{4}\) \( P = 0.025\), \(^{7}\) \( P = 0.046\), \(^{8}\) \( P = 0.008\), \(^{4}\) \( P = 0.028\).

### TABLE 5

Vitamin intake and coverage of vitamin requirements of cystic fibrosis (CF) patients

<table>
<thead>
<tr>
<th>Vitamin</th>
<th>6–11 y age group</th>
<th>12–17 y age group(^{2})</th>
<th>( \geq 18 ) y age group(^{3,4})</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vitamin C</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intake (mg)</td>
<td>76 (26–142)</td>
<td>113 (37–233)</td>
<td>102 (71–349)</td>
</tr>
<tr>
<td>Percentage coverage of recommended intake (%)</td>
<td>95 (36–158)</td>
<td>113 (37–233)</td>
<td>102 (71–349)</td>
</tr>
<tr>
<td>Percentage coverage of DRI (%)</td>
<td>209 (99–380)</td>
<td>150 (50–343)</td>
<td>113 (94–537)</td>
</tr>
<tr>
<td>Total vitamin E</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intake (IU)(^{3})</td>
<td>148 (66–206)</td>
<td>116 (15–261)</td>
<td>140 (10–653)</td>
</tr>
<tr>
<td>Percentage coverage of estimated adequate intake (%)</td>
<td>898 (541–1322)</td>
<td>585 (66–1212)</td>
<td>629 (55–3600)</td>
</tr>
<tr>
<td>Percentage coverage of DRI (%)</td>
<td>925 (580–1250)</td>
<td>517 (65–1164)</td>
<td>629 (44–2920)</td>
</tr>
<tr>
<td>( \beta )-Carotene</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intake (mg)(^{5})</td>
<td>0.73 (0.48–5.93)</td>
<td>1.32 (0.70–4.25)</td>
<td>4.93 (1.6–19.8)</td>
</tr>
</tbody>
</table>

\(^{1}\) All values are median; interquartile range in parentheses. Vitamin requirements from reference 20, and vitamin intakes from reference 19. DRI, dietary reference intake. There were no significant differences between age groups.

\(^{2,3}\) n lower than maximum number of observations; \(^{5}\) n = 6, \(^{7}\) n = 3.

\(^{4}\) Values are median; minimum–maximum in parentheses.

\(^{5}\) 1 IU = 1 mg all-rac-\( \alpha \)-tocopherol acetate.

\(^{6}\) Recommended daily intake 2–4 mg (19).
In summary, plasma and tissue antioxidant status of patients with CF was lower than that of healthy control subjects and was particularly low in older patients. This lack of antioxidant capacity probably contributed to the elevated markers of oxidative stress detected in our adult patient population. A lot of data allude to the relevance of sufficient antioxidant capacity in CF to maintain better health and well-being in the long run. However, studies that demonstrate a clear clinical benefit would be required to prove the effectiveness of antioxidant supplements and to identify the correct dosage and combination of micronutrients. We have, thus, developed an innovative antioxidant supplement with improved bioavailability in the form of Jelly Babies, which will help to overcome the problem of malabsorption by way of its improved galenics and will surely increase patient compliance because it is more fun to eat. It will be tested for its clinical benefits in an upcoming randomized double-blind controlled intervention trial with patients with CF.

We thank all participating staff members as well as patients from the cystic fibrosis and endocrinologic outpatient clinic, in particular Dr. A Busch (study physician) and A Hofmann (collection and evaluation of dietary protocols), for their assistance in data collection; J Erhardt, A Flaccus, M Langer, B Wendt, and M Wolter for technical assistance; and the company Biotest for making available one of their EcoScreens. We are particularly indebted to Katrin Kromeyer-Hauschild for her assistance in calculating the BMI z scores.

EIB conceived and designed the study, collected the samples, contributed to lab work, managed the database, analyzed and interpreted the data, and wrote the manuscript; EIB was employed through a fund and received travel grants from Mukoviszidose e. V., Bonn, Germany, and the results of this paper are part of her doctoral dissertation. CF helped with all statistical techniques. 18 All values are n/total; percentage in parentheses.

TABLE 6
Vitamin deficits and optimal plasma concentrations in cystic fibrosis (CF) patients and control subjects by age group

<table>
<thead>
<tr>
<th>Vitamin</th>
<th>Deficit or suboptimal status</th>
<th>Optimal plasma concentrations</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>6–11 y</td>
<td>12–17 y</td>
</tr>
<tr>
<td>Vitamin C</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CF patients</td>
<td>0/6 (0)</td>
<td>0/7 (0)</td>
</tr>
<tr>
<td>Control subjects</td>
<td>0/9 (0)</td>
<td>0/5 (0)</td>
</tr>
<tr>
<td>Vitamin E</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CF patients</td>
<td>1/7 (14)</td>
<td>4/7 (57)</td>
</tr>
<tr>
<td>Control subjects</td>
<td>0/9 (0)</td>
<td>2/5 (40)</td>
</tr>
<tr>
<td>β-Carotene</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CF patients</td>
<td>5/7 (71)</td>
<td>7/7 (100)</td>
</tr>
<tr>
<td>Control subjects</td>
<td>1/9 (11)</td>
<td>1/5 (20)</td>
</tr>
</tbody>
</table>

15. Jentzsch AM, Bachmann H, Fürst P, Biesalski HK. Improved analysis of...