Supplementation with carotenoids corrects increased lipid peroxidation in children with cystic fibrosis¹–³

Guy Lepage, Josée Champagne, Nancy Ronco, André Lamarre, Iris Osberg, Ronald J Sokol, and Claude C Roy

ABSTRACT Evidence of lipid peroxidation previously documented in cystic fibrosis (CF) implies an imbalance between free radical generation and antioxidant defense mechanisms. The aim of the present study was to examine the relation between plasma concentrations of malondialdehyde, a marker of lipid peroxidation, and the exogenous antioxidant line of defense. Malondialdehyde concentrations (90.2 ± 4.7 nmol/L) in 25 children with CF aged 9.6 ± 0.8 y were higher (P < 0.001) than concentrations (69.1 ± 2.6 nmol/L) in 17 children used as control subjects and were not correlated with any marker of disease severity. In contrast with their all-rac-α-tocopherol status, which was normal as a result of routine supplementation with a 200-mg dose of all-rac-α-tocopherol acetate/d, β-carotene was very low. A 2-mo open trial in which 12 children with CF aged 11.5 ± 0.8 y were given 4.42 mg (8.23 μmol) β-carotene three times per day led to normalization of the malondialdehyde concentration in all but 1 patient, in conjunction with an increase of plasma β-carotene from 0.08 ± 0.03 to 3.99 ± 0.92 μmol/L. Their plasma concentrations were inversely correlated (r = −0.42, P < 0.05) with malondialdehyde when the values measured pre- and posttreatment were pooled. We conclude that β-carotene deficiency contributes to lipid peroxidation in CF and that supplementation may eventually prove to be a useful adjunct for the management of the disease. Am J Clin Nutr 1996;64:87–93.

KEY WORDS Lipid peroxidation, free radicals, oxidant stress, cystic fibrosis, β-carotene, carotenoids, all-rac-α-tocopherol, malondialdehyde, antioxidants

INTRODUCTION Increased oxidative damage occurs in many human diseases (1) and is likely to play a role in the pathophysiology of lung injury in patients with cystic fibrosis (CF) in view of the severity of the inflammation that accompanies chronic infection (2). Evidence of lipid peroxidation in CF has been documented by several authors (3–10) and is likely attributable to the imbalance between an increased generation of free radicals and a decreased availability of antioxidant defense mechanisms. Overproduction of reduced oxygen molecules could be due in part to increased activation of neutrophils (11) and of the arachidonic acid pathway (12). Moreover, ibuprofen significantly slows the progression of lung disease in CF patients (13). Analysis of bronchoalveolar lavage fluid has revealed a 1000-fold increase in the number of neutrophils recovered from the lungs compared with control subjects (14). It is well established that neutrophils, when activated, are a major source of free radicals (15). In addition to neutrophils, two secretory products of Pseudomonas aeruginosa, the most commonly found pathogenic bacterium in CF, are capable of damaging local tissues through the generation of hydroxyl radicals (16). Both endogenous and exogenous antioxidants are deficient in patients with CF. There is evidence of low concentrations of glutathione (8) and of glutathione peroxidase (17). Furthermore, the activities of superoxide dismutase and catalase in erythrocytes of CF patients and their parents has been shown to be increased, thereby suggesting increased intracellular oxidative stress (18). Low vitamin E concentrations in plasma have led to the routine administration of all-rac-α-tocopherol to compensate for its poor absorbability (19). Furthermore, low concentrations of carotenoids in CF patients have led to the suggestion that supplementation with α-tocopherol could be considered (2, 7, 20, 21) in view of its potent antioxidant properties (22).

The purpose of the present study was to examine the relation between lipid peroxidation and exogenous antioxidant vitamin concentrations and to test the possibility that carotenoids could reduce the extent of lipid peroxidation.

SUBJECTS AND METHODS

Subjects Twenty-five children with CF (11 boys and 14 girls) with a mean age of 9.6 y (range: 2.7–20.3 y) regularly followed in the CF clinic of l’Hôpital Ste-Justine were recruited for the first part of the study. Seventeen children with a mean age of 10.4

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y (range: 4.1–19.2 y) who had blood work done for conditions other than gastrointestinal, hepatic, or nutritional problems were used as control subjects. The patients were maintained on their usual medication and were treated with supplemented pancreatic enzymes. They were all-receiving 4000 IU vitamin A/d and 200 mg all-rac-α-tocopheryl acetate/d. Seventy-two-hour fecal fat collections were analyzed by the method of van de Kamer (23). A fasting blood sample was obtained for analysis of γ-glutamyl transferase, β-carotene, vitamin E, malondialdehyde, and fatty acids. The results of these determinations were then examined in relation to their nutritional and essential fatty acid (EFA) status, fecal fat excretion, and pulmonary function.

For the second part of the study, 12 children with CF (8 boys and 4 girls) with a mean age of 11.5 y (range: 7.0–15.9 y) were enrolled in a 2-mo open study of carotenoid supplementation. The study was undertaken during the months of July and August. A β-carotene preparation purchased from Swiss Herbal Remedies Ltd (Markham, Ontario) contained 25 000 IU β-carotene/caplet. It was taken after each meal with appropriate pancreatic enzyme supplementation. The HPLC profile of the carotenoids revealed that 49% was β-carotene and that each caplet contained 4.42 mg (8.23 μmol) β-carotene. Fasting blood was obtained for determination of β-carotene, all-rac-α-tocopherol, retinol, retinyl palmitate, retinol binding protein (RBP), the fatty acid profile, and malondialdehyde. As in the first part of the study, the results of these determinations were examined in relation to nutritional and EFA status, fecal fat excretion, and pulmonary function.

The study protocol was approved by the Ethics Committee of l’Hôpital Ste-Justine and consent was obtained from the parents.

Plasma samples

Plasma was collected from fasting subjects with EDTA as an anticoagulant. To avoid isomerization of carotenoids, aluminium foil was used to protect all blood samples from light as soon as they were drawn. The plasma specimens were stored in vials at −80 °C until analyzed.

Chemicals

Standards of retinol, retinol acetate, all-rac-α-tocopherol, tocopheryl acetate, β-carotene, retinyl palmitate, fatty acids, and 1,1,3,3-tetraoctoxyp propane were purchased from Sigma Chemical Co (St Louis). HPLC-grade water, methanol, methylene chloride, and acetone from BDH Inc (Ville St-Laurent, Canada). All solvents used for gas-liquid chromatographic analysis and vitamin extraction were gas-distilled in our laboratory.

Vitamin determinations

At the time of the assay, samples of plasma were thawed in the dark and aliquots were processed for analysis under subdued light; 500-μL samples of the different specimens were mixed in tubes for 0.5 min in a vortex mixer with 500 μL of the internal standards (12 μg each of retinyl acetate and tocopheryl acetate in C2H5OH with 125 μg ascorbic acid). After the addition of n-hexane (twice, 2.5 mL each), tubes were shaken for 10 min, sonicated for 3 min, and centrifuged for 5 min at 1000 × g and room temperature. The n-hexane layer was transferred into a tube and the pooled organic extracts of each sample were evaporated to dryness under a gentle stream of nitrogen at 20 °C. Tubes were rapidly removed from the water bath and the residue was reconstituted with 150 μL acetonitrile:methylene chloride:methanol (70:20:10, by vol), to which 25 μg ascorbic acid in 50 μL ethanol was added. The tubes were then vortexed for 30 s and sonicated for 3 min. Twenty microliters of this solution was injected into the HPLC system.

The chromatographic analysis was performed on a Hewlett-Packard (Palo Alto, CA) spherical 5-μm C18 octadecylsilsane Hypersil column (20 cm × 2.1 mm internal diameter). A guard column of the same package preceded the main column. This reversed-phase octadecylsilsane column was used for the simultaneous determination of the liposoluble vitamins by using isocratic elution with methanol:acetonitrile (60:40, by vol). The flow rate was 400 μL/min. The light absorption of the compounds was measured with a photodiode-array detector at wavelengths 282 nm for all-rac-α-tocopheryl acetate; 290 nm for all-rac-α-tocopherol; 322 nm for retinol, retinyl palmitate, and retinyl acetate; and 446 nm for β-carotene. The amounts were calculated by using all-rac-α-tocopheryl acetate and retinyl acetate as internal standards. The areas under the curve of the chromatographic peaks were used in the calculations. All manipulations were carried out under subdued light to avoid photoisomerization of the compounds. All analyses were run on a Hewlett-Packard 1090 HPLC. The photodiode-array detector acquires chromatographic signals and spectra over the wavelength range of 190–600 nm.

Malondialdehyde, fatty acid, and RBP determinations

Proteins from plasma samples (500 μL) were precipitated as described previously (24) and the supernate was filtered through a 0.2-μm cellulose acetate syringe filter. After complexing malondialdehyde to thiobarbituric acid (TBA), an aliquot was injected into the HPLC apparatus for separation and measurement at 532 nm of the malondialdehyde–TBA complex (24). The analyses were performed on the Hewlett-Packard 1090 HPLC. Both the precolumn and the column were stainless steel Hypersil octadecylsilsane C18 with a particle size of 5 μm. The precolumn measured 4 X 20 mm, whereas the dimensions of the column were 100 × 4.6 mm. The mobile phase consisted of methanol and water in a gradient mode (23).

Plasma fatty acids were analyzed after direct transesterification as published previously (25) with a Hewlett-Packard 5880 gas chromatograph as described earlier (26). The resulting fatty acid methyl esters were injected into a 60-m fused-silica capillary column coated with SP-2331. RBP was determined by rate nephelometry (Beckman Auto-ICS; Beckman, Montreal, Canada) with Beckman reagents and standards.

Statistical analysis

All results are expressed as means ± SEs. Statistical analyses were performed by using Student’s t test, analysis of variance, and linear-regression analysis (27). A P value of 0.05 was used as the level of significance.

RESULTS

The clinical characteristics of the 25 children with CF recruited for the first part of the study (survey) as well as for the
second part (clinical trial) are shown in Table 1. Fasting plasma concentrations of all-rac-α-tocopherol and β-carotene in patients who were supplemented with 200 mg all-rac-α-tocopheryl acetate and of control subjects are shown in Figure 1. Although concentrations of all-rac-α-tocopherol did not differ between CF patients (20.7 ± 1.5 μmol/L) and control subjects (19.0 ± 0.8 μmol/L), fasting plasma β-carotene concentrations were much lower in CF patients (85.7 ± 13.3 nmol/L) than in control subjects (372.5 ± 35.7 nmol/L, P < 0.0001). When expressed as molar ratios to total plasma fatty acids, all-rac-α-tocopherol (mmol/mol) and β-carotene (μmol/mol) values in CF patients were 2.16 ± 0.15 and 9.51 ± 1.65, respectively. The corresponding values in control subjects were 1.97 ± 0.08 (mmol/mol) and 36.8 ± 4.0 (μmol/mol), respectively.

Malondialdehyde concentrations in CF patients were higher than in control subjects (P < 0.001) and fatty acid profiles differed markedly (Table 2). The percentage of polyunsaturated fatty acids (PUFAs) (P < 0.0001) as well as the ratio of 20:3n-9 to 20:4n-6 (P < 0.0001) revealed that CF patients as a group had evidence of EFA deficiency (EFAD).

The clinical characteristics of the 12 children with CF recruited for the open trial with β-carotene are summarized in Table 1. In response to 4.42 mg (8.23 μmol) β-carotene three times per day over 2 mo, plasma β-carotene concentrations increased from 0.08 ± 0.03 to 3.99 ± 0.92 μmol/L (P < 0.005; Figure 2), concentrations above those observed in healthy pediatric control subjects. A wide range in response was observed. Malondialdehyde concentrations decreased from 95.4 ± 13.4 to 56.6 ± 4.8 nmol/L (P < 0.02), similar to control concentrations in all but one patient. This teenager was seen for an exacerbation of his pulmonary disease and required antibiotics once the β-carotene supplementation started. It is interesting that his β-carotene concentration is the lowest point plotted in the left panel of Figure 2 even though he had been supplemented. As shown in Figure 3, plasma malondialdehyde concentrations were inversely related to plasma β-carotene concentrations when the values obtained before and after treatment were plotted together (r = −0.42, P < 0.05).

In view of the possibility that β-carotene supplementation could have contributed to hypervitaminosis A, plasma retinol, retinol palmitate, and RBP were measured. There was no significant change in retinol or RBP Table 3. Values for RBP were generally low; one-third of the patients had values below the range in normal control subjects (1.4–4.4 μmol/L). There was excellent correlation between retinol and RBP plasma concentrations before (r = 0.96, P < 0.001) as well as after β-carotene supplementation (r = 0.91, P < 0.001). Moreover, the ratio of retinol to RBP significantly increased toward normal with β-carotene supplementation. However, retinyl palmitate represented 4.55 ± 0.70% of total plasma retinol in CF patients, whereas it was undetectable in control subjects. Furthermore, this fraction of total plasma retinol increased to 7.03 ± 0.86% with β-carotene supplementation (P < 0.008); however, β-carotene was not associated with a change in all-rac-α-tocopherol.

The fatty acid profiles obtained before and after β-carotene supplementation revealed little change in total fatty acids or in

**TABLE 1**
Characteristics of patients with CF

<table>
<thead>
<tr>
<th></th>
<th>Normal range</th>
<th>Survey (n = 25)</th>
<th>Clinical trial (n = 12)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y)</td>
<td>—</td>
<td>9.6 ± 0.8</td>
<td>11.5 ± 0.8</td>
</tr>
<tr>
<td>Weight (% expected)</td>
<td>85–115</td>
<td>96.2 ± 4.8</td>
<td>87.9 ± 5.7</td>
</tr>
<tr>
<td>Height (% expected)</td>
<td>85–115</td>
<td>98.7 ± 3.7</td>
<td>88.8 ± 4.2</td>
</tr>
<tr>
<td>Weight-for-height (% expected)</td>
<td>85–115</td>
<td>97.7 ± 3.5</td>
<td>99.1 ± 4.3</td>
</tr>
<tr>
<td>Fecal fat (g/d)</td>
<td>&lt; 5</td>
<td>24.8 ± 3.0</td>
<td>13.9 ± 3.7</td>
</tr>
<tr>
<td>γ-Glutamyltransferase (IU/L)</td>
<td>&lt; 30</td>
<td>15.8 ± 3.6</td>
<td>11.0 ± 1.4</td>
</tr>
<tr>
<td>FVC (%)</td>
<td>80–120</td>
<td>96.7 ± 3.4</td>
<td>104.7 ± 5.5</td>
</tr>
<tr>
<td>FEV1 (%)</td>
<td>85–100</td>
<td>91.0 ± 4.4</td>
<td>97.4 ± 4.8</td>
</tr>
</tbody>
</table>

* * * ± SEM. FVC, predicted forced vital capacity; FEV1, predicted forced expiratory volume at 1 s.

Figure 2, concentrations above those observed in healthy pediatric control subjects. A wide range in response was observed. Malondialdehyde concentrations decreased from 95.4 ± 13.4 to 56.6 ± 4.8 nmol/L (P < 0.02), similar to control concentrations in all but one patient. This teenager was seen for an exacerbation of his pulmonary disease and required antibiotics once the β-carotene supplementation started. It is interesting that his β-carotene concentration is the lowest point plotted in the left panel of Figure 2 even though he had been supplemented. As shown in Figure 3, plasma malondialdehyde concentrations were inversely related to plasma β-carotene concentrations when the values obtained before and after treatment were plotted together (r = −0.42, P < 0.05).

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The fatty acid profiles obtained before and after β-carotene supplementation revealed little change in total fatty acids or in

**TABLE 2**
Plasma malondialdehyde and fatty acid profiles

<table>
<thead>
<tr>
<th></th>
<th>Normal range</th>
<th>Control subjects (n = 16)</th>
<th>CF patients (n = 25)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Malondialdehyde (nmol/L)</td>
<td>&lt; 80</td>
<td>69.1 ± 2.6</td>
<td>90.2 ± 4.7</td>
</tr>
<tr>
<td>Total fatty acids (mmol/L)</td>
<td>8–14</td>
<td>9.80 ± 0.15</td>
<td>9.72 ± 0.50</td>
</tr>
<tr>
<td>PUFA (% of total)</td>
<td>40–46</td>
<td>42.97 ± 0.77</td>
<td>32.13 ± 0.76</td>
</tr>
<tr>
<td>20:3n-9/20:4n-6</td>
<td>0.01–0.04</td>
<td>0.017 ± 0.001</td>
<td>0.053 ± 0.006</td>
</tr>
</tbody>
</table>

* * * ± SEM. PUFA, polyunsaturated fatty acids; CF, cystic fibrosis.

2, 3Significantly different from control subjects: 2P = 0.001, 3P = 0.0001.
FIGURE 2. Effect of 4.42 mg (122 μmol) β-carotene three times per day during a 2-mo open trial in 12 children with cystic fibrosis. Plasma concentrations in 11 of 12 children before supplementation were well below the range (x ± 2 SD, 0.61 ± 0.40 μmol/L) of normal values shown by the shaded box. In response to β-carotene, malondialdehyde concentrations, which were initially higher, fell within the range (0.53 ± 0.13 nmol/L) of normal values shown by the shaded box in all patients except one.

the percentage of PUFAs (Table 4). However, the ratio of 20:3n-9 to 20:4n-6 decreased slightly (NS).

DISCUSSION

This study confirms previous work reporting ongoing lipid peroxidation and low β-carotene concentrations in CF patients (2-9, 15, 16, 20, 21, 28). Elevated plasma concentrations of malondialdehyde, despite normal concentrations of all-rac-α-tocopherol, suggest that there is a relation between lipid peroxidation and the β-carotene status of children with CF. In fact, plasma malondialdehyde concentrations were inversely related to plasma β-carotene concentrations when all the values before and after treatment were pooled. This finding was further substantiated in the second part of the study by the observation that malondialdehyde concentrations decreased in response to β-carotene to concentrations observed in control children.

FIGURE 3. Relation between plasma β-carotene and plasma malondialdehyde concentrations before and after β-carotene supplementation (r = -0.42, P < 0.05).

TABLE 3
Effects of β-carotene supplementation on plasma β-carotene, retinol binding protein (RBP), retinol, and tocopherol

<table>
<thead>
<tr>
<th></th>
<th>Normal range</th>
<th>Unsupplemented</th>
<th>Supplemented</th>
</tr>
</thead>
<tbody>
<tr>
<td>β-carotene (μmol/L)</td>
<td>2.0-2.8</td>
<td>0.08 ± 0.03$^2$</td>
<td>3.99 ± 0.92$^2$</td>
</tr>
<tr>
<td>RBP (μmol/L)</td>
<td>1.4-3.8</td>
<td>1.52 ± 0.15</td>
<td>1.42 ± 0.14</td>
</tr>
<tr>
<td>Retinol (μmol/L)</td>
<td>0.7-2.8</td>
<td>1.07 ± 0.07</td>
<td>1.10 ± 0.08</td>
</tr>
<tr>
<td>Retinol:RBP</td>
<td>0.8-0.9</td>
<td>0.72 ± 0.02</td>
<td>0.79 ± 0.03$^4$</td>
</tr>
<tr>
<td>Retinyl palmitate</td>
<td>(nmol/L)</td>
<td>&lt;87</td>
<td>50.9 ± 8.9</td>
</tr>
<tr>
<td>(%)$^6$</td>
<td></td>
<td>3</td>
<td>4.55 ± 0.70</td>
</tr>
<tr>
<td>dl-α-Tocopherol (μmol/L)</td>
<td>12-28</td>
<td>17.2 ± 1.4</td>
<td>18.3 ± 1.7</td>
</tr>
</tbody>
</table>

$^1$ n = 12.
$^2$ x ± SEM.
$^3$-5, 7 Significantly different from unsupplemented: $^1$ P = 0.001, $^4$ P = 0.007, $^7$ P = 0.004. $^7$ P = 0.008.
$^6$ Percent of total retinol circulating as retinyl palmitate.

All patients who were part of this study received a regular supplement of vitamin E (Aquasol E; Rhône-Poulenc Inc., Rorer, Montreal, Canada). Because β-carotene is a lipidsoluble provitamin, low concentrations documented previously in CF patients have been explained by malabsorption (29). However, two recent reports suggested that there was a better correlation between β-carotene concentrations and the severity of ongoing inflammation with its attendant generation of free radicals than with the degree of fat malabsorption (2, 30). Additionally, carotenoid concentrations correlated negatively with serum immunoglobulin G concentrations, an indirect measure of inflammation (2). The differences in plasma carotenoid concentrations between children with CF and healthy children may be due to rapid turnover of carotenoids, perhaps through quenching of toxic oxygen species generated by chronic inflammation in CF (2).

Malondialdehyde is an important biomarker of lipid peroxidation as an end product of the oxidation and decomposition of PUFAs containing three or more double bonds. It is also an endogenous byproduct of prostaglandin and leukotriene biosynthesis (31). Of the many assays available to measure malondialdehyde, the TBA–reactive substances test is the most popular (32). TBA binds to the aldehyde, malondialdehyde, which accounts for 15% of lipid peroxidation products (33). The TBA test for malondialdehyde lacks specificity and many refinements have been made to the technique first described by Sinnhuber and Yu (34). The HPLC technique used in this study is highly specific and reproducible because it measures only the malondialdehyde-TBA adduct (24). Malondialdehyde concentrations were significantly higher in CF patients than in control subjects (P < 0.001). Furthermore, there was a strong inverse

TABLE 4
Effect of β-carotene supplementation on plasma fatty acid profiles

<table>
<thead>
<tr>
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</tr>
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<tr>
<td>Total fatty acids (mmol/L)</td>
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</tr>
<tr>
<td>PUFAs (%)</td>
<td>40-46</td>
<td>37.13 ± 1.53</td>
<td>36.04 ± 1.65</td>
</tr>
<tr>
<td>20:3n-9/20:4n-6</td>
<td>0.01-0.04</td>
<td>0.037 ± 0.005</td>
<td>0.035 ± 0.006</td>
</tr>
</tbody>
</table>

$^1$ x ± SEM; n = 12. PUFA, Polyunsaturated fatty acids.
correlation between malondialdehyde and β-carotene in the 12 CF patients before and after supplementation with the antioxidant vitamin \((P < 0.05)\).

EFAD is a well-known manifestation of CF (35-42). An earlier study by our group found that it was biochemically present in 47% of 163 patients (42). Two major hypotheses have been raised to explain this deficiency, namely fat malabsorption and a mutation-derived metabolic defect of EFAs. We reported previously that steatorrhoea in the EFAD group was more than twofold that in the group with a normal EFA status (42). Nevertheless, the possibility of a metabolic defect of EFAs has received some support in view of the failure of most dietary interventions to correct the deficit (35, 40, 43, 44) and indirect evidence of an increased diversion of EFA through the eicosanoid pathway (38). It is also possible that EFAD could be secondary to EFA losses through peroxidation. PUFAs, whether free or incorporated into membrane lipids, are readily attacked by free radicals and are therefore lost. However, the present data do not support this mechanism because there was no correlation between malondialdehyde and the traditional marker of EFAD, the ratio of 20:3n-9 to 20:4n-6 (45). Furthermore, the EFA status did not change during 2 mo of supplementation. However, the patients who had the best response in terms of their malondialdehyde concentration were those with a more significant degree of steatorrhoea. Whether a study of longer duration would have improved the EFA status remains to be seen.

β-carotene is a metabolic precursor of vitamin A. Using \(^{13}C\)β-carotene in a human study, Parker et al (46) reported recently that 64%, 21%, and 14% of the absorbed \(^{13}C\) entered the plasma as retinyl esters, retinol, and unaltered β-carotene, respectively (46). Of note is the observation that retinol esters expressed as retinyl palmitate were high and increased by >50% with β-carotene supplementation. Measurement of serum retinyl esters has been recommended as a good marker of hepatic accumulation of vitamin A because a greater prevalence of abnormal liver-function tests was observed in patients with high plasma retinyl ester concentrations (47). However, even if retinyl esters normally represent <3% of total vitamin A (48), the percentage of circulating retinol as retinyl esters was very small compared with the percentage recorded in hypervitaminosis (49).

There are potentially two major sources of oxygen-free radicals in CF: those produced by an underlying primary defect in intracellular oxygen metabolism (50), and those produced by neutrophils in the lung as a consequence of the pulmonary infection associated with the disease (28). Combined antioxidant deficiencies are more common in CF patients with liver disease than in those without (51). Both intracellular and extracellular antioxidant compartments come into contact with free radicals produced by activated neutrophils in the lungs of CF patients (10). Adequate plasma concentrations of all-rac-α-tocopherol do not seem to be sufficient to inhibit lipid peroxidation. For a given concentration of plasma or erythrocyte all-rac-α-tocopherol, erythrocytes of patients with CF were shown to be more susceptible to peroxide-induced hemolysis than were those of control subjects (52).

Carotenoids, β-carotene in particular, have received much attention in the past few years as major exogenous antioxidants. β-carotene prevents peroxidation of PUFAs by singlet oxygen or by radical initiators (53). In carotene-depleted women, production of hexanal, pentanal, and pentane by copper-oxidized plasma low-density lipoproteins was greater than the production of these compounds when they were repleted with β-carotene (54). Erythrocyte superoxide dismutase activity was depressed in carotene-depleted women and it recovered with repletion, suggesting that β-carotene may have acted as a direct scavenger of superoxide radicals. TBA-reactive substances in their plasma were elevated and decreased with repletion (54). Allard et al (55) reported that breath-pentane output was higher in smokers than in nonsmokers and was reduced significantly only in smokers receiving β-carotene. Furthermore, supplementation seems to improve some indexes of immune response in humans with a previously stressed immune response (56), albeit not in healthy individuals (57). β-carotene has also been shown to inhibit free radical-induced peroxidation in membranes (58) and to have additive effects when given in conjunction with all-rac-α-tocopherol (59). Others have produced evidence that both act synergistically (60). A combination of β-carotene and all-rac-α-tocopherol results in an inhibition of lipid peroxidation significantly greater than the sum of individual contributions in liver microsomal membranes submitted to free radical attack (61). In CF, β-carotene has been shown to increase the resistance of low-density lipoproteins to oxidation and to decrease plasma malondialdehyde concentrations in CF patients (62). Should our preliminary observations be confirmed by a double-blind trial, CF would constitute one of the first in vivo models in which the essentiality of β-carotene as an antioxidant has been shown.

In conclusion, this study showed that in CF there is an imbalance between free radical generation and antioxidant defense mechanisms despite an adequate supply of all-rac-α-tocopheryl acetate. The clinical implications of these findings cannot be underestimated because free radical–mediated damage could play a role in the progressive deterioration of lung function. The observation that malondialdehyde concentrations were inversely correlated with those of β-carotene but reverted to normal in response to supplementation suggests that this antioxidant provitamin is essential in preventing lipid peroxidation. A controlled trial should confirm these findings before β-carotene supplements are advocated for all CF patients.

REFERENCES