Association of cholesterol oxidation and abnormalities in fatty acid metabolism in cystic fibrosis¹⁻³

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ABSTRACT

Background: Disarrangement in fatty acids and oxidative stress are features of cystic fibrosis. Cholesterol is very sensitive to oxidative stress.

Objectives: The objectives were to examine whether cholesterol oxidation products are altered in cystic fibrosis and whether they are associated with fatty acids and with characteristics of the disease state.

Design: 7-Ketocholesterol and 7β-hydroxycholesterol (prototype molecules of free radical–mediated cholesterol oxidation) and the fatty acid profile were assessed by mass spectrometry in patients and in sex- and age-matched control subjects.

Results: In a comparison with control subjects, mean (±SD) cholesterol oxidation was higher (7-ketocholesterol: 11.31 ± 5.1 compared with 8.33 ± 5.5 ng/mL, P = 0.03; 7β-hydroxycholesterol: 14.5 ± 6.8 compared with 9.7 ± 4.1 ng/mL, P = 0.004), total saturated fatty acids were higher (31.90 ± 1.93% compared with 30.31 ± 0.98%, P < 0.001), monounsaturated fatty acids were higher (29.14 ± 3.85% compared with 25.88 ± 2.94%, P = 0.004), ω-6 (n−6) polyunsaturated fatty acids were lower (34.84 ± 4.77 compared with 39.68 ± 2.98%, P < 0.0001), and ω-3 (n−3) polyunsaturated fatty acids were comparable in patients with cystic fibrosis. Oxysterols were inversely associated with 24:0 and 18:2 ω-6 fatty acids but did not correlate with the increased oleic acid or with any of the ω-3 fatty acids.

Conclusions: Cystic fibrosis is characterized by relevant cholesterol oxidation that is associated with an abnormal fatty acid profile. The interplay between oxysterols and fatty acids potentially provides insight into the biological mechanisms that underlie this complex disease. Am J Clin Nutr 2009;90:477–84.

INTRODUCTION

Cystic fibrosis is a hereditary disease associated with mutations in the gene encoding for a conductance channel, the cystic fibrosis transmembrane regulator (CFTR) (1). It is not clear how alterations in CFTR expression lead to a complex disease with multiorgan involvement, including the lung, pancreas, and liver. Whereas possible explanations of disease mechanism must include the increased viscosity in ductal fluid, additional candidates for disease mechanism are emerging, including altered essential fatty acids profile (2) and increased oxidative stress in biological fluids and at the tissue level (3).

An abnormal polyunsaturated fatty acid pattern has been attributed to CFTR mutation-driven alterations in the absorption and/or metabolism of dietary lipids (4, 5). Reduced docosahexaenoic acid and increased arachidonic acid concentrations have been suggested to up-regulate the formation of both enzymatic- and free radical–derived inflammatory mediators, including leukotrienes and isoprostanes, which may contribute to the inflammatory state observed in cystic fibrosis.

The occurrence of oxidative stress, defined as the increased formation of free radicals escaping the antioxidant scavenging system, has been reported in cystic fibrosis patients as measured by various markers (6). Isoprostanes, specific markers originating from arachidonic acid peroxidation, have been reported in urine and breath condensate of patients with cystic fibrosis (7, 8). Consistent with the increased oxidative stress status, vitamin E, an antioxidant that inhibits lipid peroxidation, is frequently deficient in patients with cystic fibrosis (3).

Cholesterol may undergo free radical–mediated oxidation with generation of oxysterols, reaction products that may serve as lipid peroxidation markers. In addition, oxysterols have biological activities relevant in the disease mechanism, including regulation of enzymes involved in inflammation and fatty acid metabolism (9, 10).

To date, neither free radical–derived oxysterols nor the interplay between them and fatty acids has been reported in cystic fibrosis patients. Therefore, the present study was designed to examine whether free radical–derived oxysterols, vitamin E, and fatty acid concentrations are altered in cystic fibrosis in vivo and whether such abnormalities are associated with each other and with characteristics of the disease state.

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SUBJECTS AND METHODS

Study participants

Study participants were individuals with cystic fibrosis as defined by the consensus-statement requirements for the diagnosis of this disease (11) and were followed at the Cystic Fibrosis Center (Policlinico Umberto I University Hospital, Rome). All participants provided written informed consent before enrollment, which began in March 2006. The protocols were approved by the institutional review board at Sapienza University, Faculty of Medicine, and by Policlinico Umberto I University Hospital.

Definitions

Liver disease was defined according to criteria reported by Colombo et al (12). The diagnostic criteria of cystic fibrosis–related diabetes are those reported by Kerem et al (13). Pancreatic insufficiency was defined as the requirement for exogenous pancreatic enzymes for the treatment of steatorrhea. A total of 36 participants were enrolled (27 with pancreatic insufficiency and 9 with pancreatic sufficiency). The following medications were annotated on the patients’ data sheets: use of ω-3 fatty acids, vitamin E and multivitamin supplements, nonsteroid antiinflammatory drugs, antibiotics, and steroids.

Controls

A total of 24 healthy control subjects were recruited among the University personnel, after a review of their medical history. Exclusion criteria for control participants included a family history of cystic fibrosis or features consistent with the presence of cystic fibrosis (chronic lung disease, male infertility, chronic sinusitis, and chronic pancreatitis) or the use of drugs that affect fatty acids (systemic corticosteroids, isotretinoin, and ursodiol) and/or oxidative stress (antioxidants and hypolipemic drugs).

Outcomes

In the present study, the by-group difference in oxysterols was the primary outcome. By-group differences in other markers of oxidative stress, including fatty acids, were secondary outcomes.

Laboratory methods

FEV<sub>1</sub> (forced expiratory volume in 1 s) as a percentage of predicted values, body mass index (BMI, the weight in kg divided by the square of height in cm), and the National Institutes of Health prognostic score, as defined by Taussig et al (14), were obtained. All participants were encouraged to eat a high-fat (40% of calories, with 47% of total dietary lipids as extra virgin olive oil), high-calorie diet and had regular visits to a nutritionist, in accordance with the guidelines of the Cystic Fibrosis Foundation (15). Mutation was assessed by first-level analysis polymerase chain reaction and additional analysis by cycle sequencing.

Blood and lipid analysis

Peripheral venous blood was obtained from participants after they fasted overnight. The plasma or serum was then removed and stored at −80°C until assayed. For cholesterol oxidation markers, we measured the 2 oxysterols 7-ketocholesterol and 7β-hydroxycholesterol, which are generated via a free radical–mediated mechanism (10). α-Tocopherol, the main and most active vitamin E stereoisomer, was measured to assess vitamin E status. Herein, oxysterols refer to 7-ketocholesterol and 7β-hydroxycholesterol, and vitamin E refers to α-tocopherol. Oxysterols and vitamin E were analyzed by mass spectrometry with isotope-dilution methods as previously reported (16, 17). As 7-ketocholesterol and 7β-hydroxycholesterol undergo mutual interconversion in vivo (18) and because of the association between oxysterols and α-tocopherol with cholesterol (19), results are also presented as relative values corrected for cholesterol concentration.

FIGURE 1. An overview of the fatty acid profile analyzed in the study population. The trivial name is reported with the short-hand nomenclature, which designates the fatty acid carbon chain length and the number of double bonds. The notation “ω-x” is used to describe the position of the double bond nearest to the methyl group. Of the polyunsaturated fatty acids, linoleic acid and α-linolenic acid are also known as essential fatty acids because they are necessary and cannot be synthesized in the body.
Fatty acids were analyzed in plasma by gas chromatography with a flame ionization detector, according to Masood et al (20), and are expressed as the percentage of total fatty acids. An overview of the saturated, monounsaturated, and polyunsaturated fatty acids measured in the study population is depicted in Figure 1. Total cholesterol and triglycerides were measured by using commercial kits (Abbott Diagnostics, Abbott Park, IL).

**Statistical analysis**

The present study was designed to have 80% power to detect a true by-group difference of not less than 8 ± 11 ng/mL in oxysterols (the primary outcome), assuming a 2-sided α of 0.05 and a cystic fibrosis:control ratio of 1.5:1.

Data analysis was performed by using SPSS 10.0 statistical analysis software (1999; SPSS, Chicago, IL). The normality of distribution of continuous variables was assessed by using the Kolmogorov-Smirnov test (cutoff \( P = 0.01 \)). Descriptive statistics for continuous variables are reported as means ± SDs or medians (minimum to maximum) as appropriate. Blood chemistry measures, fatty acid values, and markers of oxidative stress were compared between patients and control subjects and, separately, with cystic fibrosis patients by disease manifestation, nutrition supplement, and medication use, by using the \( t \) test for independent variables or the Mann–Whitney \( U \) test. In addition, because the biological effects of fatty acids are dependent not only on their absolute concentrations but also on the ratio of \( \omega-6 \) to \( \omega-3 \) series, ratios were calculated and compared by participant status and, among cystic fibrosis patients, by disease manifestation and medication/supplement use. Categorical variables were described by using frequency distributions and are presented as frequency (%). The chi-square test was used to associate categorical variables between cases and controls and, separately, within cystic fibrosis patients by category of disease manifestation and nutrition supplement and medication use. Associations between chemistry measures and markers of oxidative stress were described by calculating Pearson’s or Spearman’s correlation coefficients. All tests were 2-sided and were considered significant at a \( P \) value < 0.05.

**RESULTS**

**Oxidative stress markers and lipids in plasma**

The demographic characteristics of the study population are shown in Table 1. No participant had a weight loss of >5% in the 3 mo preceding blood withdrawal. A large proportion of patients (75%) had pancreatic insufficiency. Approximately 50% of patients were taking vitamin E supplements, and 27% were taking docosahexaenoic acid.

Cholesterol oxidation markers and plasma lipids for cases and controls are shown in Table 2. As shown, oxysterols, the primary outcome variables, were significantly elevated, whereas vitamin E was significantly lower in cystic fibrosis patients than in healthy subjects. Differences in markers of oxidative stress between cases and controls were preserved even after correction for total cholesterol. Differences in the vitamin E:cholesterol ratio were not detected between cases and controls.

Among patients with cystic fibrosis, cholesterol oxidation markers were higher in those with pancreatic insufficiency, diabetes mellitus, and liver disease than in patients without these manifestations (Table 3). Subjects who reported taking \( \omega-3 \) fatty acid supplements had significantly increased oxysterol concentrations. Differences in oxysterols were not detected by vitamin E supplementation, respiratory tract infection status, or use of nonsteroid antiinflammatory drugs, steroids, antibiotics, or multivitamin supplements.

**Plasma fatty acid profiles**

The complete fatty acid profile of the study population is presented in Table 4. Total saturated fatty acids and mono-unsaturated fatty acids were higher, whereas polyunsaturated fatty acids and \( \omega-6 \) fatty acids were lower in cystic fibrosis patients than in the control subjects. Mean (±SD) \( \omega-6 \) polyunsaturated fatty acid concentrations were significantly lower in cystic fibrosis patients than in the control subjects.
A significant correlation was not detected between oxidative stress variables and monounsaturated fatty acids. No association was found between markers of oxidative stress and oleic acid, the major contributor to the increase in monounsaturated fatty acids in cystic fibrosis patients.

**TABLE 3**

Cholesterol oxidation in cystic fibrosis patients according to clinical characteristics

<table>
<thead>
<tr>
<th></th>
<th>7/3OH-chol (ng/mL)</th>
<th>7K-chol (ng/mL)</th>
<th>7/3OH-chol + 7K-chol (ng/mL)</th>
<th>7/3OH-chol/cholesterol</th>
<th>7K-chol/cholesterol</th>
<th>7/3OH-chol + 7K-chol/cholesterol</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pancreas</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sufficient (n=9)</td>
<td>14.03 ± 8.65</td>
<td>9.31 ± 4.00</td>
<td>23.35 ± 12.34</td>
<td>0.08 ± 0.04</td>
<td>0.05 ± 0.01</td>
<td>0.13 ± 0.05</td>
</tr>
<tr>
<td>Insufficient (n=27)</td>
<td>14.67 ± 6.36</td>
<td>11.91 ± 5.31</td>
<td>26.58 ± 11.10</td>
<td>0.13 ± 0.05</td>
<td>0.11 ± 0.05</td>
<td>0.24 ± 0.01</td>
</tr>
<tr>
<td>Diabetes</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No (n=26)</td>
<td>13.08 ± 6.33</td>
<td>9.94 ± 3.91</td>
<td>23.03 ± 9.61</td>
<td>0.11 ± 0.05</td>
<td>0.08 ± 0.05</td>
<td>0.20 ± 0.10</td>
</tr>
<tr>
<td>Yes (n=10)</td>
<td>18.12 ± 6.94</td>
<td>14.74 ± 6.27</td>
<td>32.86 ± 12.60</td>
<td>0.15 ± 0.05</td>
<td>0.13 ± 0.06</td>
<td>0.28 ± 0.11</td>
</tr>
<tr>
<td>Liver disease</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No (n=19)</td>
<td>15.62 ± 7.90</td>
<td>10.60 ± 4.73</td>
<td>26.23 ± 12.24</td>
<td>0.10 ± 0.05</td>
<td>0.07 ± 0.03</td>
<td>0.17 ± 0.08</td>
</tr>
<tr>
<td>Yes (n=17)</td>
<td>13.36 ± 5.43</td>
<td>12.07 ± 5.51</td>
<td>25.43 ± 10.56</td>
<td>0.14 ± 0.05</td>
<td>0.12 ± 0.06</td>
<td>0.26 ± 0.11</td>
</tr>
<tr>
<td>α-3 Fatty acid supplement use</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No (n=26)</td>
<td>14.23 ± 7.00</td>
<td>11.21 ± 5.19</td>
<td>25.45 ± 11.55</td>
<td>0.10 ± 0.04</td>
<td>0.09 ± 0.05</td>
<td>0.19 ± 0.09</td>
</tr>
<tr>
<td>Yes (n=10)</td>
<td>15.46 ± 6.55</td>
<td>11.61 ± 5.13</td>
<td>26.98 ± 11.10</td>
<td>0.15 ± 0.07</td>
<td>0.12 ± 0.06</td>
<td>0.28 ± 0.13</td>
</tr>
</tbody>
</table>

1 7/3OH, 7β-hydroxycholesterol; chol, cholesterol; 7K, 7-cholesterol.
2 Mean ± SD (all such values).
3 Values were derived by using an independent-samples or Mann-Whitney U test, depending on the normality of the variable’s distribution.
Regarding the associations between cholesterol oxidation markers and ω-6 series fatty acids, a negative association was found between oxysterols and linoleic acid (7-ketocholesterol compared with 18:2; $r = -0.42$, $P = 0.033$; 7-ketocholesterol/ cholesterol compared with 18:2; $r = -0.57$, $P = 0.004$; 7β-hydroxycholesterol/cholesterol compared with 18:2; $r = -0.42$, $P = 0.045$; oxysterols/cholesterol compared with 18:2; $r = -0.51$, $P = 0.012$). No correlation was observed between oxidative stress variables and arachidonic acid or between ω-3 series fatty acids and oxidative stress variables in patients with cystic fibrosis, both in the entire patient population and in the ω-3 fatty acid–supplemented patients.

**Influence of clinical status**

Pancreatic insufficiency and liver disease were associated with the largest alteration in fatty acid profiles. In the cystic fibrosis patients, 24:0 fatty acid concentrations were significantly lower in patients with pancreatic insufficiency and with liver disease (Table 5), and in patients who took ω-3 fatty acid or vitamin E supplements (Table 6), than in the other patients.

**TABLE 4**

Complete fatty acid profile in plasma

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>Cystic fibrosis patients (n = 36)</th>
<th>Healthy control subjects (n = 24)</th>
<th>$P^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saturated</td>
<td>% of total fatty acids</td>
<td>% of total fatty acids</td>
<td></td>
</tr>
<tr>
<td>14:0</td>
<td>0.90 ± 0.37</td>
<td>0.65 ± 0.20</td>
<td>&lt;0.02</td>
</tr>
<tr>
<td>16:0</td>
<td>22.54 ± 2.05</td>
<td>21.29 ± 0.96</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>18:0</td>
<td>7.91 ± 1.01</td>
<td>7.95 ± 0.69</td>
<td>NS</td>
</tr>
<tr>
<td>20:0</td>
<td>0.10 ± 0.03</td>
<td>0.13 ± 0.08</td>
<td>&lt;0.02</td>
</tr>
<tr>
<td>22:0</td>
<td>0.33 ± 0.08</td>
<td>0.27 ± 0.04</td>
<td>&lt;0.02</td>
</tr>
<tr>
<td>24:0</td>
<td>0.10 ± 0.03</td>
<td>0.15 ± 0.03</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Total</td>
<td>31.90 ± 1.93</td>
<td>30.31 ± 0.98</td>
<td>0.001</td>
</tr>
<tr>
<td>Monounsaturated</td>
<td>14:1 0.12 ± 0.03</td>
<td>0.08 ± 0.03</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>C16:1</td>
<td>1.91 ± 0.74</td>
<td>1.45 ± 0.03</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>18:1o-7</td>
<td>1.65 ± 0.37</td>
<td>1.61 ± 0.19</td>
<td>NS</td>
</tr>
<tr>
<td>18:1o-9</td>
<td>25.02 ± 3.44</td>
<td>22.33 ± 2.75</td>
<td>0.005</td>
</tr>
<tr>
<td>20:1</td>
<td>0.21 ± 0.05</td>
<td>0.19 ± 0.06</td>
<td>NS</td>
</tr>
<tr>
<td>24:1</td>
<td>0.21 ± 0.05</td>
<td>0.22 ± 0.03</td>
<td>NS</td>
</tr>
<tr>
<td>Total</td>
<td>29.14 ± 3.85</td>
<td>25.88 ± 2.94</td>
<td>0.004</td>
</tr>
<tr>
<td>PUFA ω-6</td>
<td>18:2o-6 25.02 ± 4.00</td>
<td>29.29 ± 2.62</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>18:3o-6</td>
<td>0.49 ± 0.17</td>
<td>0.39 ± 0.09</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>20:2o-6</td>
<td>0.22 ± 0.04</td>
<td>0.22 ± 0.02</td>
<td>NS</td>
</tr>
<tr>
<td>20:3o-6</td>
<td>1.86 ± 0.37</td>
<td>1.76 ± 0.27</td>
<td>NS</td>
</tr>
<tr>
<td>20:4o-6</td>
<td>6.79 ± 1.50</td>
<td>7.56 ± 0.96</td>
<td>0.045</td>
</tr>
<tr>
<td>22:4o-6</td>
<td>0.25 ± 0.08</td>
<td>0.26 ± 0.04</td>
<td>NS</td>
</tr>
<tr>
<td>22:5o-6</td>
<td>0.18 ± 0.10</td>
<td>0.18 ± 0.03</td>
<td>NS</td>
</tr>
<tr>
<td>Total</td>
<td>34.84 ± 4.77</td>
<td>39.68 ± 2.98</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>PUFA ω-3</td>
<td>18:3o-3 0.27 ± 0.07</td>
<td>0.27 ± 0.09</td>
<td>NS</td>
</tr>
<tr>
<td>20:5o-3</td>
<td>0.47 ± 0.28</td>
<td>0.35 ± 0.12</td>
<td>NS</td>
</tr>
<tr>
<td>22:5o-3</td>
<td>0.34 ± 0.09</td>
<td>0.35 ± 0.08</td>
<td>NS</td>
</tr>
<tr>
<td>22:6o-3</td>
<td>1.57 ± 0.99</td>
<td>1.54 ± 0.33</td>
<td>NS</td>
</tr>
<tr>
<td>Total</td>
<td>2.66 ± 1.21</td>
<td>2.53 ± 0.49</td>
<td>NS</td>
</tr>
</tbody>
</table>

1 All values are means ± SDs. PUFA, polyunsaturated fatty acid.
2 Values were derived by using an independent-samples or Mann-Whitney $U$ test, depending on the normality of the variable’s distribution.

In addition, patients who took vitamin E supplements had lower linoleic acid concentrations (18:2ω-6; 23.4 ± 4.2% compared with 26.7 ± 3.0%; $P = 0.042$), lower total ω-6 polyunsaturated fatty acids (33.0 ± 4.6% compared with 36.7 ± 4.3%; $P = 0.049$), and lower total polyunsaturated fatty acids (ω-3 + ω-6) (35.6 ± 4.4% compared with 39.5 ± 4.2%; $P = 0.032$).

Compared with cystic fibrosis patients without liver disease, cystic fibrosis patients with liver disease had significantly higher monounsaturated fatty acid concentrations accompanied by a reciprocal reduction of linoleic acid and polyunsaturated fatty acids concentrations (Table 5).

**DISCUSSION**

Measures of free radical–derived oxysterols have not been reported in cystic fibrosis. In the present study, we used a specific mass spectrometry–based assay to measure plasma 7β-hydroxycholesterol and 7-ketocholesterol in patients with cystic fibrosis.
fibrosis. These oxysterols are validated markers of lipid peroxidation in vivo (16), which have been shown to be elevated in several clinical conditions characterized by high systemic oxidative stress status and suggested as potential disease markers in atherosclerosis, liver disease, and adolescence metabolic syndrome (10, 21, 22).

Our data showed elevated free radical–derived oxysterol concentrations and a distorted fatty acid profile in the plasma of cystic fibrosis patients. The pathophysiological relevance of these findings is further supported by the identification of increased free radical–derived oxysterol concentrations in patients with a more severe disease, as manifested by pancreatic insufficiency, liver disease, and/or cystic fibrosis–related diabetes.

Elevated plasma concentrations of 7β-hydroxycholesterol and 7-ketocholesterol in cystic fibrosis occurs in the presence of relative hypocholesterolemia, highlighting the major involvement of cholesterol in the free radical–mediated oxidative challenge to circulating lipids in cystic fibrosis. This is further supported by a previous study that showed normal concentrations of 7α-hydroxycholesterol and reduced 7β-hydroxycholesterol in the plasma of cystic fibrosis patients, which indicates that the 2 enzymatic pathways of bile acid synthesis—CYP7α and CYP27—are not up-regulated in cystic fibrosis (23).

The finding of oxysterol accumulation is consistent with previous studies of lipid peroxidation (6, 24), including those based on isoprostanes (7, 8), which are considered accurate markers of oxidative stress (25). In analogy to other lipid peroxidation products, oxysterols exert many biological activities relevant to the pathophysiological process operating in cystic fibrosis, such as apoptosis, cytotoxicity, impairment of endothelial function, and regulation of enzymes involved in inflammation and in fatty acid metabolism, including nitric oxide synthase, cyclooxygenase 2, phospholipase A₂, and fatty acid desaturases (9, 10, 26).

We have proposed the simultaneous measurement of oxysterols and vitamin E as an essential tool in correctly studying the oxidant/antioxidant balance at the plasma and tissue levels (16). Consistent with previous reports, our study found a 20% lower plasma vitamin E concentrations in the patients with cystic fibrosis than in the control subjects.

These findings support the hypothesis that reduced antioxidant protection of blood lipids underlies the observed increase in lipid peroxidation. However, it is well known that absolute vitamin E concentrations correlate with plasma lipids (27) and, as a standard procedure, lipid-corrected values are used to express α-tocopherol status (19). Indeed, vitamin E concentrations adjusted for cholesterol were not lower in the patients with cystic fibrosis than in the control subjects. In addition, there were no differences in vitamin E or oxysterol concentrations in patients who were taking vitamin E supplements. These data should be interpreted in the context of the current Cystic Fibrosis Foundation Consensus Conference on nutrition, which recommends vitamin E supplementation to normalize blood vitamin E concentrations (15).

Consistent with previous studies, we detected an altered fatty acid profile in cystic fibrosis patients characterized by an increase in saturated and monounsaturated fatty acids (28, 29) and a decrease in ω-6 polyunsaturated fatty acids. The results of the present study indicate that alterations in free radical–mediated cholesterol oxidation and fatty acids are closely related. Whereas no correlation was found between oxysterols and any of the fatty acids in the healthy control subjects, significant associations emerged from the correlation analysis between oxysterols and fatty acids in cystic fibrosis patients.

 Plasma cholesterol and vitamin E were positively correlated with 24:0 and were significantly negatively correlated with oxysterols, possibly identifying 24:0 as an indicator of adequate nutriture. This notion was further supported by the positive correlation between 24:0 and BMI in cystic fibrosis, coupled with the observation that 24:0 concentrations were lower in patients with pancreatic insufficiency.

Oleic acid concentrations were higher whereas linoleic acid concentrations were lower in cystic fibrosis patients than in control subjects—a finding consistently reported in the literature (2, 5, 29, 30). Arachidonic acid concentrations were lower in cystic fibrosis patients, which is consistent with some studies (29, 31) but contradicts others (5, 32). In contrast with reports of low docosahexaenoic acid concentrations in cystic fibrosis,
alterations in ω-3 fatty acids were not observed in the present study, neither in total amount nor as single components, both in the whole population and in subgroups.

The altered pattern of fatty acids observed in our study indicated that the changes in oleic acid and arachidonic acid are not harmful because no correlation was observed between these 2 fatty acids and oxysterols. In contrast, we observed that linoleic acid was inversely associated with oxidative stress and was even lower in the presence of pancreatic insufficiency or liver disease.

It is now accepted that reduced concentrations of the essential fatty acid linoleic acid reflect not only fat malabsorption (30) but are also associated with the CFTR gene mutation (33). Oxidative stress–associated changes in cystic fibrosis may contribute to altering fatty acid trafficking and metabolism.

In contrast with other reports, the present study detected a normal plasma ω-3 class pattern, a discrepancy perhaps explained by dietary habits. Whereas previous studies were from countries consuming typically Western diets (34), the present study enrolled individuals consuming the Mediterranean diet. Patients in the present study consumed 47% of total dietary lipids as extra virgin olive oil, which is rich in various anti-oxidants. The antioxidants in extra virgin olive oil could conceivably confer protection to the highly oxidizable ω-3 fatty acids from oxidative degradation in the intestine or in blood and tissues. Manipulation of the diet with regard to lipid composition has been performed in cystic fibrosis patients. The widespread notion of beneficial effects of ω-3 fatty acids in diverse chronic conditions, including atherosclerosis, suggested the use of ω-3 supplements in cystic fibrosis. In addition, oral administration of docosahexaenoic acid reversed the arachidonic acid to docosahexaenoic acid ratio in cftr−/− mice, which adds biochemical justification to the use of ω-3 supplements in cystic fibrosis. On the other hand, studies of ω-3 supplement use in cystic fibrosis patients, aimed at down-regulating arachidonic acid concentrations and ameliorating inflammation, were disappointing as highlighted in a recent Cochrane meta-analysis on fish-oil supplementation (35). It is interesting to note that, in the present study, patients who took docosahexaenoic acid supplements did not have increased plasma ω-3 fatty acids concentrations and showed more severe oxidative stress, an observation not previously reported.

In light of the association between nutritional status and life expectancy in cystic fibrosis patients, the manipulation of dietary lipids and the management of oxidative stress in these patients must be further explored. A Mediterranean diet is a potential candidate for future studies (34). The increase in dietary oleic acid, obtainable with a virgin olive oil–fortified diet typical in Mediterranean areas, has been shown to be beneficial both in vitro and in vivo experimental settings. Oleic acid renders liposomes and LDL cholesterol less susceptible to oxidation (36, 37). Furthermore, individuals following a Mediterranean-style diet rich in olive-derived oleate reduced their proinflammatory potential and increased resistance to LDL oxidation, compared with subjects following a Western diet (38). These findings were recently supported by a clinical trial on diet shift that focused on a high-monounsaturated-fat diet obtained by adding virgin olive oil (39).

In conclusion, the present study indicates that despite normal vitamin E–corrected values, cystic fibrosis patients have increased concentrations of oxysterols closely related to the altered fatty acid profile. This emerging association highlights the usefulness of combined measurement of oxysterols and fatty acids as a means of understanding disease mechanisms in cystic fibrosis and of monitoring therapeutic strategies, including those based on antioxidants, and dietary manipulation.

The authors’ responsibilities were as follows—LI: planned the study protocol, reviewed the literature, and wrote the manuscript; RM and GS: conducted the biochemical analyses; SZ: collected the blood samples and clinical data; FG: prepared the deuterated standards; MB: conducted the statistical analysis; SQ: was in charge of the patients; and RM, GS, SZ, FG, MB, and SQ: participated in reviewing the final version of the manuscript. All authors read and approved the final manuscript. The manuscript is not under consideration elsewhere. All authors declared that they had no conflicts of interest to disclose.

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