Influence of Probucol on Enhanced LDL Oxidation After Fish Oil Treatment of Hypertriglyceridemic Patients

Suzanne Lussier-Cacan, Suzanne Dubreuil-Quidoz, Ghislaine Roederer, Nicole Leboeuf, Lucie Boulet, Ghislaine C. de Langavant, Jean Davignon, Marek Naruszewicz

The susceptibility of low-density lipoprotein (LDL) to oxidation was studied in hypertriglyceridemic men (5 with type III and 5 with type IV) at baseline on a low-saturated-fat, low-cholesterol diet, after 6 weeks of dietary supplementation with fish oil (Promega, 12 g/d), and after 6 weeks of fish oil combined with probucol (500 mg BID). The relative content of n-3 polyunsaturated fatty acids in plasma and LDL was increased during the two treatment periods, and a low a-tocopherol to n-3 polyunsaturated fatty acids ratio was observed. Plasma thiobarbituric acid–reactive substances (TBARS) levels were unchanged after 6 weeks of fish oil, but the ratio of lipid peroxides to the reduced triglyceride (TG) levels (MDA: TG) was significantly higher (P<.01). Addition of probucol lowered both absolute levels of TBARS (P<.01) and the MDA to TG ratio (P<.001). The susceptibility of LDL to Cu²⁺-catalyzed oxidation was evaluated over a 5-hour time course by determining TBARS formation, free amino group levels, and changes in LDL electrophoretic mobility. TBARS levels that were higher in native LDL (1.019«/< 1.050 g/mL) after 6 weeks of fish oil than at baseline (P<.01) were reduced 52.3±11.3% by the addition of probucol (P<.001). With fish oil alone, TBARS production after exposure of LDL to Cu²⁺ for 5 hours was increased 17.0±5.8% compared with corresponding baseline values (P<.001), whereas a 64.1±14.3% reduction from the previous period was observed with fish oil+probucol (P<.001). Determination of LDL reactive amino groups further documented the structural changes occurring with peroxide formation, which were opposed by probucol. Our observations emphasize a potential risk associated with intake of large doses of fish oil in the treatment of hyperlipidemia and further demonstrate the ability of probucol as an antioxidant.

(ARTERIOSCLEROSIS & THROMBOSIS. 1993;13:1790-1797.)

KEY WORDS • fish oil • probucol • hypertriglyceridemia • lipid peroxides • LDL oxidation • antioxidants

We have previously documented the efficacy of dietary supplementation with fish oil in the treatment of type III and type IV hypertriglyceridemia. Both groups responded to 6 g/d of n-3 fatty acids with an approximate 50% decrease in very-low-density lipoprotein (VLDL) triglyceride (TG), similar to that observed in other studies conducted in various population samples. In some individuals, especially type IV hypertriglyceridemic patients, a significant increase in LDL cholesterol was noted. For several patients, however, the diet–fish oil therapeutic combination appeared to be a satisfactory alternative to more classic drug therapy. The current study was undertaken to assess lipid peroxidation and the susceptibility of LDL to in vitro oxidation during dietary supplementation with fish oil in hyperlipidemia. Probucol, a hypolipidemic agent with potent antioxidant properties, was given in combination with fish oil, and the effect of probucol on the aforementioned parameters was evaluated as well as the effect of the combined therapy on lipid and lipoprotein levels.

Methods

Subjects

Ten men aged 30 to 58 years, 5 with dysbetalipoproteinemia (type III) and 5 with endogenous hypertriglycer-
TABLE 1. Biologic Features of Patients at Baseline*  

<table>
<thead>
<tr>
<th>Patients</th>
<th>Age, y</th>
<th>Type</th>
<th>BMI</th>
<th>Total Chol</th>
<th>VLDL Chol</th>
<th>IDL Chol</th>
<th>LDL Chol</th>
<th>HDL Chol</th>
<th>HDL₂ Chol</th>
<th>HDL₃ Chol</th>
<th>Total TG</th>
<th>VLDL TG</th>
<th>α-Tocopherol</th>
<th>β-Carotene</th>
</tr>
</thead>
<tbody>
<tr>
<td>V.P.</td>
<td>42</td>
<td>III</td>
<td>23.8</td>
<td>6.22</td>
<td>2.43</td>
<td>0.70</td>
<td>2.38</td>
<td>0.71</td>
<td>0.17</td>
<td>0.55</td>
<td>3.01</td>
<td>2.51</td>
<td>60.22</td>
<td>0.07</td>
</tr>
<tr>
<td>D.P.†</td>
<td>30</td>
<td>III</td>
<td>25.6</td>
<td>5.91</td>
<td>2.50</td>
<td>0.85</td>
<td>1.68</td>
<td>0.89</td>
<td>0.20</td>
<td>0.69</td>
<td>2.78</td>
<td>2.30</td>
<td>60.00</td>
<td>0.23</td>
</tr>
<tr>
<td>M.R.†</td>
<td>49</td>
<td>III</td>
<td>26.7</td>
<td>9.24</td>
<td>5.93</td>
<td>1.02</td>
<td>1.67</td>
<td>0.62</td>
<td>0.14</td>
<td>0.48</td>
<td>5.15</td>
<td>4.53</td>
<td>76.06</td>
<td>0.34</td>
</tr>
<tr>
<td>H.L.</td>
<td>35</td>
<td>III</td>
<td>30.8</td>
<td>14.08</td>
<td>11.41</td>
<td>0.98</td>
<td>1.03</td>
<td>0.64</td>
<td>0.14</td>
<td>0.50</td>
<td>10.78</td>
<td>10.13</td>
<td>77.71</td>
<td>1.09</td>
</tr>
<tr>
<td>C.B.</td>
<td>46</td>
<td>III</td>
<td>29.0</td>
<td>10.57</td>
<td>6.87</td>
<td>1.24</td>
<td>2.88</td>
<td>0.82</td>
<td>0.21</td>
<td>0.61</td>
<td>4.95</td>
<td>4.22</td>
<td>59.79</td>
<td>0.76</td>
</tr>
<tr>
<td>L.C.</td>
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<td>IV</td>
<td>28.8</td>
<td>6.01</td>
<td>1.31</td>
<td>0.19</td>
<td>3.68</td>
<td>0.84</td>
<td>0.14</td>
<td>0.70</td>
<td>3.26</td>
<td>2.92</td>
<td>48.56</td>
<td>0.30</td>
</tr>
<tr>
<td>B.R.</td>
<td>43</td>
<td>IV</td>
<td>25.7</td>
<td>6.94</td>
<td>4.66</td>
<td>0.15</td>
<td>1.59</td>
<td>0.54</td>
<td>0.08</td>
<td>0.46</td>
<td>10.00</td>
<td>9.54</td>
<td>97.12</td>
<td>0.21</td>
</tr>
<tr>
<td>B.J.</td>
<td>58</td>
<td>IV</td>
<td>25.1</td>
<td>5.64</td>
<td>1.64</td>
<td>0.16</td>
<td>3.06</td>
<td>0.78</td>
<td>0.14</td>
<td>0.64</td>
<td>2.85</td>
<td>2.43</td>
<td>47.31</td>
<td>0.55</td>
</tr>
<tr>
<td>P.R.†</td>
<td>50</td>
<td>IV</td>
<td>28.4</td>
<td>5.41</td>
<td>1.63</td>
<td>0.18</td>
<td>2.85</td>
<td>0.75</td>
<td>0.11</td>
<td>0.64</td>
<td>3.89</td>
<td>3.49</td>
<td>47.74</td>
<td>0.53</td>
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<tr>
<td>L.Y.</td>
<td>44</td>
<td>IV</td>
<td>27.1</td>
<td>5.80</td>
<td>4.38</td>
<td>0.25</td>
<td>0.67</td>
<td>0.51</td>
<td>0.14</td>
<td>0.37</td>
<td>13.29</td>
<td>12.59</td>
<td>60.23</td>
<td>0.39</td>
</tr>
</tbody>
</table>

BMI indicates body mass index, ie, weight in kilograms divided by height in meters squared; Chol, cholesterol; VLDL, very-low-density lipoprotein; IDL, intermediate-density lipoprotein; LDL, low-density lipoprotein; HDL, high-density lipoprotein; TG, triglycerides. LDL chol is defined as cholesterol in the d> 1.019 g/mL fraction less HDL chol. Lipid values are in millimoles per liter; α-tocopherol and β-carotene are in nanomoles per milliliter.

*Baseline is the mean of the last two samples of period I (diet alone).
†Cigarette smokers.

Plasma Lipid and Lipoprotein Determinations

Plasma lipoproteins were separated under standard conditions by a combination of ultracentrifugation (at d=1.006 and d=1.019 g/mL) and heparin-manganese precipitation of the apo B-containing lipoproteins according to the Lipid Research Clinics protocol. Inter-

**Procedure**

The study protocol (Fig 1) was approved by the institutional ethics committee and informed consent was obtained from each participant. All had an electrocardiogram performed within the preceding 4 months, and control coagulation tests were done before and during the last period of the study. After a weight-

**TABLE 1. Biologic Features of Patients at Baseline**

- **Patients**
- **Age, y**
- **Type**
- **BMI**
- **Total Chol**
- **VLDL Chol**
- **IDL Chol**
- **LDL Chol**
- **HDL Chol**
- **HDL₂ Chol**
- **HDL₃ Chol**
- **Total TG**
- **VLDL TG**
- **α-Tocopherol**
- **β-Carotene**

- **V.P.** 42 III 23.8 6.22 2.43 0.70 2.38 0.71 0.17 0.55 3.01 2.51 60.22 0.07
- **D.P.†** 30 III 25.6 5.91 2.50 0.85 1.68 0.89 0.20 0.69 2.78 2.30 60.00 0.23
- **M.R.†** 49 III 26.7 9.24 5.93 1.02 1.67 0.62 0.14 0.48 5.15 4.53 76.06 0.34
- **H.L.** 35 III 30.8 14.08 11.41 0.98 1.03 0.64 0.14 0.50 10.78 10.13 77.71 1.09
- **C.B.** 46 III 29.0 10.57 6.87 1.24 2.88 0.82 0.21 0.61 4.95 4.22 59.79 0.76
- **L.C.** 45 IV 28.8 6.01 1.31 0.19 3.68 0.84 0.14 0.70 3.26 2.92 48.56 0.30
- **B.R.** 43 IV 25.7 6.94 4.66 0.15 1.59 0.54 0.08 0.46 10.00 9.54 97.12 0.21
- **B.J.** 58 IV 25.1 5.64 1.64 0.16 3.06 0.78 0.14 0.64 2.85 2.43 47.31 0.55
- **P.R.†** 50 IV 28.4 5.41 1.63 0.18 2.85 0.75 0.11 0.64 3.89 3.49 47.74 0.53
- **L.Y.** 44 IV 27.1 5.80 4.38 0.25 0.67 0.51 0.14 0.37 13.29 12.59 60.23 0.39

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*Baseline is the mean of the last two samples of period I (diet alone).
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**Plasma Lipid and Lipoprotein Determinations**

Plasma lipoproteins were separated under standard conditions by a combination of ultracentrifugation (at d=1.006 and d=1.019 g/mL) and heparin-manganese precipitation of the apo B-containing lipoproteins according to the Lipid Research Clinics protocol. Inter-
mediate-density lipoprotein (IDL) cholesterol and IDL-TG concentrations were determined as the difference between measurements made on the d = 1.006 and d = 1.019 g/mL ultracentrifugation infranatants. In this study, LDL cholesterol is equal to the value obtained on the d = 1.006 and d = 1.019 fraction less IDL and HDL cholesterol. High-density lipoprotein (HDL) and HDL cholesterol were obtained after precipitation of HDL with dextran sulfate. For the oxidation study, LDL was isolated by sequential ultracentrifugation of normal human plasma between densities 1.019 and 1.050 g/mL. Density solutions were made with potassium bromide and contained 1 mmol/L EDTA. The isolated LDL fraction was dialyzed against phosphate-buffered saline (PBS), pH 7.4, and 0.3 mmol/L EDTA and stored at 4°C. Plasma and lipoprotein cholesterol and TGs were obtained after precipitation of HDL2 with polyethylene glycol, after transmethylation of total extracted lipids, by gas-liquid chromatography at Wisconsin Analytical Research Services, Madison, Wis. Total protein content of the LDL preparation was determined using bovine serum albumin as the standard.

**Lipid Oxidation Study**

Total plasma or LDL lipid peroxides were determined by a spectrophotometric assay as thiobarbituric acid–reactive substances (TBARS), and results are expressed as nanomoles of malondialdehyde (MDA) equivalents per milliliter plasma, or for LDL, as nanomoles per milligram protein. Freshly diluted tetramethoxyxylene was used as the standard. LDL (250 µg protein per milliliter PBS) free from EDTA was oxidized with 10 µmol/L CuCl2 over a 5-hour time course. The reactivity to 2,4,6-trinitrobenzenesulfonic acid (TNBS) of LDL free amino groups before and after oxidation was measured as described. Changes in electrophoretic behavior of LDL, before and during oxidation, were determined by agarose gel electrophoresis using the Beckman Paragon System (Beckman Instruments, Palo Alto, Calif).

**Other Analyses**

α-Tocopherol and carotenoid levels were determined in plasma and lipoprotein fractions by high-performance liquid chromatography at Wisconsin Analytical Research Services, Madison, Wis. Total protein content of the LDL preparation was determined using bovine serum albumin as the standard.

**Statistical Analyses**

Pairwise comparisons of results obtained at the end of period I (dietary period or baseline) and after 6 weeks of fish oil supplementation (period II) and those between period II and period III (fish oil + probucol) were effected for each group separately or, when appropriate, for pooled type III and type IV patients, and the differences were evaluated by Student’s t test.

**Table 2. Plasma Lipid and Lipoprotein Levels in Type III and Type IV Subjects Before and During Fish Oil Study**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Period I (Diet)</th>
<th>Period II (Diet+Fish Oil)</th>
<th>Period III (Diet+Fish Oil+Probucol)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Type III (n=5)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total chol</td>
<td>9.20±3.37</td>
<td>7.49±3.26</td>
<td>6.46±2.21*</td>
</tr>
<tr>
<td>VLDL chol</td>
<td>5.83±3.70</td>
<td>3.06±2.56†</td>
<td>3.03±1.95</td>
</tr>
<tr>
<td>HDL chol</td>
<td>0.96±0.20</td>
<td>0.96±0.20</td>
<td>1.09±0.34</td>
</tr>
<tr>
<td>LDL chol</td>
<td>1.93±0.72</td>
<td>2.34±0.22</td>
<td>1.71±0.48*</td>
</tr>
<tr>
<td>HDL chol</td>
<td>0.74±0.12</td>
<td>0.86±0.09</td>
<td>0.62±0.15†</td>
</tr>
<tr>
<td>HDL2 chol</td>
<td>0.17±0.03</td>
<td>0.18±0.06</td>
<td>0.10±0.05</td>
</tr>
<tr>
<td>HDL3 chol</td>
<td>0.57±0.09</td>
<td>0.68±0.10*</td>
<td>0.53±0.10*</td>
</tr>
<tr>
<td>Total TG</td>
<td>5.33±3.23</td>
<td>2.92±1.59†</td>
<td>3.02±1.36</td>
</tr>
<tr>
<td>VLDL-TG</td>
<td>2.37±1.33</td>
<td>2.28±1.41†</td>
<td>2.30±1.19</td>
</tr>
<tr>
<td><strong>Type IV (n=5)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total chol</td>
<td>5.96±0.59</td>
<td>5.59±0.48</td>
<td>4.51±0.79*</td>
</tr>
<tr>
<td>VLDL chol</td>
<td>2.72±1.65</td>
<td>1.29±0.73†</td>
<td>1.37±0.93</td>
</tr>
<tr>
<td>HDL chol</td>
<td>0.19±0.04</td>
<td>0.29±0.15*</td>
<td>0.31±0.28</td>
</tr>
<tr>
<td>LDL chol</td>
<td>2.37±1.22</td>
<td>3.28±1.17</td>
<td>2.37±1.32*</td>
</tr>
<tr>
<td>HDL chol</td>
<td>0.68±0.15</td>
<td>0.73±0.15</td>
<td>0.57±0.15*</td>
</tr>
<tr>
<td>HDL2 chol</td>
<td>0.12±0.03</td>
<td>0.13±0.04</td>
<td>0.07±0.03*</td>
</tr>
<tr>
<td>HDL3 chol</td>
<td>0.56±0.14</td>
<td>0.60±0.13</td>
<td>0.50±0.14*</td>
</tr>
<tr>
<td>Total TG</td>
<td>6.66±4.71</td>
<td>2.81±1.46†</td>
<td>3.81±3.68</td>
</tr>
<tr>
<td>VLDL-TG</td>
<td>6.19±4.59</td>
<td>2.36±1.33</td>
<td>3.29±3.44</td>
</tr>
</tbody>
</table>

Chol indicates cholesterol; VLDL, very-low-density lipoprotein; IDL, intermediate-density lipoprotein; LDL, low-density lipoprotein; HDL, high-density lipoprotein; and TG, triglyceride. Values are in millimoles per liter (mean±SD). Comparisons between last samples of periods I and II and of periods II and III: *P<.05, †P<.01, ‡P<.001.

**Results**

Dietary and drug compliance was generally good during this study, as assessed by diet monitoring, evaluation of plasma fatty acid profiles, and determination of plasma probucol levels. Probucol concentrations were 24.3±11.7 µg/mL (range, 12.85 to 43.6) after 2 weeks and 33.2±20.7 µg/mL (range, 18.1 to 62.6) at the end of the 6-week period. Patients with the highest plasma levels of probucol at 2 weeks also had the highest levels after 6 weeks of treatment. Two subjects reported some transient gastric discomfort with the fish oil concentrate, which was related to stressful periods in both cases. Body weight increased in 9 of the 10 participants, resulting in a significant mean increase of 1.4±1.6 kg (1.73±1.84%; P<.05) after 12 weeks. Interestingly, this increase is equivalent to the additional energy that was supplied during the study by the fish oil supplement (110 to 120 calories/d).

Lipid changes appear on Table 2 for the two treatment periods. Plasma VLDL cholesterol and total and VLDL-TG levels were reduced by fish oil in both groups of patients. Addition of probucol did not affect triglyceride levels except in one type IV subject whose total and VLDL-TG increased twofold during that period. This paradoxical increase occurred despite compliance.
TABLE 3. Plasma Thiobarbituric Acid-Reactive Substances During Fish Oil Study

<table>
<thead>
<tr>
<th>Variable</th>
<th>Period I (Diet)</th>
<th>Period II (Diet+Fish Oil)</th>
<th>Period III (Diet+Fish Oil+Probucol)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MDA, eq</td>
<td>4.51±0.72</td>
<td>4.49±0.61</td>
<td>3.55±1.06*</td>
</tr>
<tr>
<td>(nmol/mL)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MDA: TG ratio, μmol/mmol</td>
<td>1.11±0.53</td>
<td>2.00±0.69*</td>
<td>1.47±0.68†</td>
</tr>
</tbody>
</table>

MDA indicates malondialdehyde, and TG, triglyceride. Values are mean±SD; data are pooled for 5 type III and 5 type IV subjects.

Comparisons between last samples of periods I and II and of periods II and III: *P<.01, †P<.001.

to fish oil treatment as witnessed by the sustained higher proportions of plasma n-3 fatty acids during that period (not shown). Excluding this patient from the type IV group completely removed the nonsignificant TG increases shown in Table 2. The plasma TG decrease observed during the fish oil period was associated with a trend toward higher LDL cholesterol levels. These were significantly reduced by the addition of probucol. HDL cholesterol was lower under fish oil+probucol both in type III and type IV. The decrease also affected HDL1 and HDL2.

Levels of lipid peroxides (TBARS) were determined in total plasma and mean values are presented in Table 3. There were no significant changes with fish oil alone (period II), but absolute levels were significantly lower when probucol was added (period III). However, when results were expressed as the ratio of MDA equivalents to TG levels, a significant increase was found for pooled type III and type IV patients after fish oil treatment, followed by a significant decrease with combined fish oil and probucol (Table 3). This corresponded to significant changes (P<.001 from baseline) in the percent content of total plasma n-3 PUFAs. In type III, EPA (20:5) values were 1.33±0.64%, 8.87±4.42%, and 7.10±1.80% at baseline (period I), after 6 weeks of fish oil (period II), and at the end of period III (fish oil+probucol), respectively, and 0.95±0.44%, 9.24±3.95%, and 8.31±4.11% in type IV. After 4.5 hours of oxidation with CuCl2, the TBARS content of native and oxidized LDL showed structural changes consistent with the degree of peroxide formation: 5 hours of exposure to 10 μmol/L CuCl2 caused a reduction in LDL amino group reactivity of 28.8±2.9% at the end of the control period, of 42.0±6.5% after fish oil (difference from control at P<.01), and of 14.0±1.3% at the end of the fish oil+probucol period (difference from control and from fish oil alone at P<.001). The extent of LDL oxidation was assessed by agarose gel electrophoresis in a few samples and showed that the electrophoretic mobility of oxidized LDL from probucol-treated subjects was considerably decreased compared with that observed during the diet and fish oil only periods (not shown).

Plasma levels of natural antioxidants are presented for each group separately in Table 5. There were no changes in β-carotene, but total α-tocopherol levels were significantly lower in type III patients during the fish oil+probucol period (period III) than with fish oil.
TABLE 4. Changes in Low-Density Lipoprotein Fatty Acids

<table>
<thead>
<tr>
<th>Fatty Acid, % of Total</th>
<th>Period I (Diet)</th>
<th>Period II (Diet + Fish Oil)</th>
<th>Period III (Diet + Fish Oil + Probucol)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PA (C16:0)</td>
<td>22.12±2.33</td>
<td>20.17±3.63</td>
<td>23.74±4.70</td>
</tr>
<tr>
<td>SA (C18:0)</td>
<td>8.53±1.69</td>
<td>7.89±3.13</td>
<td>8.35±3.39</td>
</tr>
<tr>
<td>OA (C18:1, n-9)</td>
<td>20.00±2.99</td>
<td>17.17±2.47</td>
<td>18.17±2.62</td>
</tr>
<tr>
<td>LA (C18:2, n-6)</td>
<td>24.23±4.85</td>
<td>21.57±4.79</td>
<td>21.17±4.80</td>
</tr>
<tr>
<td>AA (C20:4, n-6)</td>
<td>10.17±2.92</td>
<td>7.72±1.39†</td>
<td>5.69±1.70</td>
</tr>
<tr>
<td>EPA (C20:5-n-3)</td>
<td>1.17±1.04</td>
<td>8.33±1.92*</td>
<td>6.60±3.86</td>
</tr>
<tr>
<td>DPA (C22:5, n-3)</td>
<td>0.49±0.19</td>
<td>1.20±0.53*</td>
<td>0.60±0.34†</td>
</tr>
<tr>
<td>DHA (C22:6, n-3)</td>
<td>1.56±0.79</td>
<td>4.76±0.74*</td>
<td>3.32±2.04†</td>
</tr>
</tbody>
</table>

PA indicates palmitic acid; SA, stearic acid; OA, oleic acid; LA, linoleic acid; AA, arachidonic acid; EPA, eicosapentaenoic acid; DPA, docosapentaenoic acid; and DHA, docosahexaenoic acid. Values are mean±SD; data are pooled for 3 type III and 3 type IV subjects.

Comparisons between last samples of periods I and II and of periods II and III: *P<.01, †P<.001.

Discussion

In the present study, the novel combination of fish oil and probucol in the treatment of hypertriglyceridemia produced several interesting results. First, except for one of the 10 participants in this study, the plasma TG-lowering effect of fish oil was maintained with the addition of probucol and in some cases was even greater. In addition, probucol was efficient in countering the increase in plasma LDL cholesterol resulting from fish oil consumption. The well-documented HDL cholesterol–lowering effect of probucol, also observed in this study, is of potential concern, since our patients had low baseline HDL cholesterol levels. However, there is indirect evidence that even severe lowering of HDL by probucol, alone or in combination with a fibrate, has no adverse effect on coronary artery disease.21-23 Furthermore, treatment with probucol in humans has been shown to increase plasma cholesteryl ester transfer activity24 and protein as well as apo E concentrations25 and to increase the selective uptake of HDL cholesterol esters in Hep G2 cells.26 These observations are consistent with a favorable effect of probucol on reverse cholesterol transport, either through facilitated delivery of cholesterol to the liver via apo E-rich VLDL remnant uptake25 or via HDL particles.26 If probucol is to be considered as an antioxidant adjunct in the treatment of hypertriglyceridemia, a lower dose than that used here is likely to be effective. Indeed, it was shown recently that 250 mg/d (1 tablet) of probucol was sufficient in hypercholesterolemic subjects who were on fish oil alone (period II). Levels of α-tocopherol measured in the d>1.006 g/mL plasma fraction were significantly higher during fish oil treatment (Table 5), consistent with the changing lipoprotein profile that characterized this study. Vitamin E levels were significantly higher, relative to plasma TG concentrations, in both groups during the fish oil only period (Table 5). However, the ratio of tocopherol to the sum of the highly oxidizable PUFAs was significantly and considerably lower throughout the periods of dietary fish oil supplementation.

TABLE 5. Plasma Levels of Natural Antioxidants

<table>
<thead>
<tr>
<th>Compound</th>
<th>Type III (n=5)</th>
<th>Type IV (n=5)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Period I (Diet)</td>
<td>Period II (Diet + Fish Oil)</td>
</tr>
<tr>
<td>β-Carotene</td>
<td>0.62±0.65</td>
<td>0.54±0.49</td>
</tr>
<tr>
<td>α-Tocopherol</td>
<td>73.01±19.63</td>
<td>83.64±27.63</td>
</tr>
<tr>
<td>α-Tocopherol in d&gt;1.006 g/mL</td>
<td>22.09±3.29</td>
<td>34.90±7.83*</td>
</tr>
<tr>
<td>α-Tocopherol/total TG</td>
<td>15.99±5.94</td>
<td>30.90±16.00*</td>
</tr>
<tr>
<td>α-Tocopherol/ C20:5+C22:5+C22:6 n-3 FAs</td>
<td>29.97±15.46</td>
<td>5.92±1.95†</td>
</tr>
<tr>
<td>β-Carotene</td>
<td>0.39±0.14</td>
<td>0.43±0.18</td>
</tr>
<tr>
<td>α-Tocopherol</td>
<td>59.15±21.28</td>
<td>54.56±10.18</td>
</tr>
<tr>
<td>α-Tocopherol in d&gt;1.006 g/mL</td>
<td>15.01±8.33</td>
<td>27.90±8.70*</td>
</tr>
<tr>
<td>α-Tocopherol/total TG</td>
<td>11.32±4.49</td>
<td>23.37±10.24†</td>
</tr>
<tr>
<td>α-Tocopherol/ C20:5+C22:5+C22:6 n-3 FAs</td>
<td>27.49±16.88</td>
<td>3.87±1.03†</td>
</tr>
</tbody>
</table>

TG indicates triglyceride and FAs, fatty acids. Values are in nanomoles per milliliter (mean±SD).

Comparisons between last samples of periods I and II and of periods II and III: *P<.05, †P<.001.
to protect LDL against in vitro oxidation while effecting a lower reduction in HDL cholesterol than that observed with the standard dose of 1 g/d.27 The interference with LDL oxidation by a low dose of probucol was also documented in a recent placebo-controlled trial in male volunteers, in whom no statistically significant decrease in HDL cholesterol levels was observed after 4 months of 250 mg/d of probucol.28

Absolute plasma TBARS levels were unchanged after 6 weeks of diet supplementation with fish oil. However, VLDL-TG was significantly reduced by fish oil consumption, and evaluation of MDA values relative to TG levels showed a significantly higher ratio with fish oil compared with baseline. The sustained levels of TBARS after fish oil therapy, despite lower plasma TG, suggest that the increased plasma content of EPA and DHA, two highly unsaturated fatty acids, provides excess substrate for oxidation. Probucol protected against this oxidation, as evidenced by both the reduced levels of TBARS and the lower TBARS to TG ratio during the fish oil + probucol period (period III).

The quantity of TBARS was significantly higher in native LDL, and LDL showed increased susceptibility to Cu²⁺ oxidation during fish oil therapy compared with baseline (diet alone). These differences were observed on a probable background of some preexisting susceptibility to oxidation. Indeed, it was assumed that the therapeutic diet our hypertriglyceridemic subjects were on at baseline, although not specifically rich in PUFAs, had a polyunsaturated to saturated fatty acid ratio higher than that of a typical “North American” diet (=0.7 to 0.8 versus =0.3 to 0.4). Further, “small,” dense LDL particles, which are characteristic of hypertriglyceridemia, have exhibited greater susceptibility to oxidation than “large” LDL of normal density.29 The enhanced oxidative susceptibility of small, dense LDL has also been confirmed in a study that evaluated LDL particles of different densities obtained from healthy volunteers30 and in hypertriglyceridemic subjects undergoing clofibrate therapy.31 Addition of probucol to fish oil not only prevented the fish oil-induced susceptibility to peroxidative modification but was also responsible for even lower levels of TBARS in LDL than those observed during the diet only period. This occurred despite the high EPA and DHA contents of LDL that persisted during fish oil + probucol treatment. Previous studies have shown that treatment of hypercholesterolemia with probucol, either as monotherapy or in combination with cholestyramine, imparts protection to LDL against oxidation. Our study shows that this protection also occurs in hypertriglyceridemia, even in the presence of higher than usual levels of oxidizable material.

The α-tocopherol contents of LDL samples from normal healthy individuals have not been found to be predictive of resistance to cell-mediated oxidative damage, suggesting that other intervening factors, including the fatty acid composition of LDL, are important determinants of LDL oxidative susceptibility. Nevertheless, tocopherol enrichment of LDL by high-dose vitamin E supplementation was shown to increase the resistance of LDL to cell-mediated and copper-catalyzed oxidation. In our study, the quantity of vitamin E added to the oil concentrate (1 IU/g oil) was clearly insufficient to compensate for the increased unsaturated lipid content of LDL and to prevent some degree of in vivo oxidation and increased susceptibility to in vitro oxidation with Cu²⁺. Higher plasma levels of lipid peroxides were also reported in fish oil studies conducted in normolipidemic men36,37 and women.38 Supplementation with vitamin E attenuated or prevented the fish oil–induced increase in plasma and LDL TBARS.36,37 In our study, plasma levels of α-tocopherol were or tended to be lower during the combined fish oil + probucol period (Table 5). This may be consistent with a recent observation that incubation with probucol, which inhibited the oxidative modification of LDL, both in a cell-free and in a cellular system, failed to preserve α-tocopherol and β-carotene during oxidation.29 In that study, incubation of LDL with ascorbate, in contrast, largely preserved the natural antioxidants in LDL. The possibility of reduced compliance to fish oil treatment in our patients when probucol was added cannot, however, be excluded. Still, this is unlikely to have caused the apparent decrease in tocopherol levels, since the percent content of n-3 fatty acids in total plasma was unchanged during that period (see “Results”).

There were three smokers among our subjects, but no differences could be detected between them and the nonsmokers with regard to any of the measured variables likely to be affected by smoking, either during the control period or in their response to treatment. This is in contrast to another study36 in which LDL from smokers has been shown to contain higher TBARS levels before treatment and during fish oil ingestion (10 g fish oil concentrate) than LDL from nonsmokers. This difference can possibly be accounted for by less frequent smoking by our subjects than by those in the previous study.

Although we and others36–38 have shown that fish oil intake is associated with increased lipid peroxides as reflected in higher levels of TBARS in native LDL and of the TBARS to TG ratio in plasma, our results can only caution against the potential risk of large amounts of n-3 fatty acids contributing to in vivo modification of LDL, to increased susceptibility of cell membranes to oxidation, and to atherogenesis. Indeed, various blood and cell components are involved in reactions that maintain or disrupt the integrity of the artery. Their combined presence in a biologic system differs considerably from the in vitro conditions used in our studies. Furthermore, it may be that substitution in cell membranes of EPA (20:5, n-3) for arachidonic acid (20:4, n-6), which affects several physiological functions, has one additional consequence. Indeed, a recent report40 that n-3 fatty acid–enriched, activated monocytes contributed low amounts of superoxide material and showed reduced capacity to oxidatively modify LDL suggested that EPA in membrane phospholipids may not be a good substrate for in vivo peroxide formation.

Nevertheless, this study showed that treatment of hypertriglyceridemia with fish oil was associated with an increased risk for lipid peroxidation, due to either inadequate vitamin E content in the oil concentrate or high susceptibility of the treated individuals. Thus, intake of large amounts of n-3 fatty acids should be carefully monitored in hyperlipidemic subjects, who may be at higher risk because of long-circulating and potentially atherogenic lipoproteins (cholesterol-enriched VLDL, β-VLDL, and cholesterol-rich or
small, dense LDL), of decreased HDL levels, and of depleted stores of natural antioxidants. Supplementation of fish oil therapy with large doses of vitamin E may help in preventing some of the fish oil–induced susceptibility of LDL to peroxidative modification. A vitamin E supplement of 400 mg/d added to a regimen of 10 g fish oil/d was shown to reduce LDL susceptibility to peroxidative modification in nonsmoking volunteers. It is uncertain, however, whether similar vitamin E supplementation would be adequate to protect LDL from oxidation in hyperlipidemic subjects taking fish oil. Supplementation with vitamin C might also be considered, as more and more observations point to the essential role of ascorbic acid for α-tocopherol regeneration.

Results from a recent experiment that used a new water-soluble analogue of probucol suggested that the effectiveness of probucol in vivo may be related both to its presence in LDL, acting as a nonspecific antioxidant, and to an additional ability to inhibit cell-mediated oxidation of LDL by virtue of its uptake into cells. Addition of a low dose of probucol to a therapeutic regimen may thus be warranted, especially when intervening circumstances known to be favorable to oxidation are identified.

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Influence of probucol on enhanced LDL oxidation after fish oil treatment of hypertriglyceridemic patients.
S Lussier-Cacan, S Dubreuil-Quidoz, G Roederer, N Leboeuf, L Boulet, G C de Langavant, J Davignon and M Naruszewicz

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