Effects of Lovastatin Therapy on Susceptibility of LDL to Oxidation During α-Tocopherol Supplementation

Ari Palomäki, Kimmo Malminiemi, Outi Malminiemi, Tiina Solakivi

Abstract—A randomized, double-masked, crossover clinical trial was carried out to evaluate whether lovastatin therapy (60 mg daily) affects the initiation of oxidation of low density lipoprotein (LDL) in cardiac patients on α-tocopherol supplementation therapy (450 IU daily). Twenty-eight men with verified coronary heart disease and hypercholesterolemia received α-tocopherol with lovastatin or with dummy tablets in random order. The two 6-week, active-treatment periods were preceded by a washout period of at least 8 weeks. The oxidizability of LDL was determined by 2 methods ex vivo. The depletion times for LDL ubiquinol and LDL α-tocopherol were determined in timed samples taken during oxidation induced by 2,2-azobis(2,4-dimethylvaleronitrile). Copper-mediated oxidation of LDL isolated by rapid density-gradient ultracentrifugation was used to measure the lag time to the propagation phase of conjugated-diene formation. α-Tocopherol supplementation led to a 1.9-fold concentration of reduced α-tocopherol in LDL (P<0.0001) and to a 2.0-fold longer depletion time (P<0.0001) of α-tocopherol compared with determinations after the washout period. A 43% prolongation (P<0.0001) was seen in the lag time of conjugated-diene formation. Lovastatin decreased the depletion time of reduced α-tocopherol in metal ion–dependent oxidation by 44% and shortened the lag time of conjugated-diene formation in metal ion–dependent oxidation by 7%. In conclusion, α-tocopherol supplementation significantly increased the antioxidative capacity of LDL when measured ex vivo, which was partially abolished by concomitant lovastatin therapy. (Arterioscler Thromb Vasc Biol. 1999;19:1541-1548.)

Key Words: α-tocopherol ■ clinical trials ■ lipid oxidation ■ lovastatin ■ ubiquinol

High serum cholesterol concentrations and oxidation of LDL seem to play key roles in the pathogenesis of an atherosclerotic lesion. An effective means to treat hypercholesterolemic patients is through the use of statins, or 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA ) reductase inhibitors. They effectively decrease serum LDL cholesterol, increase HDL cholesterol levels slightly, and improve the prognosis of coronary heart disease (CHD) patients.1,2 The prominent antioxidant in LDL is α-tocopherol, because in every LDL particle there are 6 to 8 molecules of α-tocopherol.1–5 α-Tocopherol acts as a powerful antioxidant after the very early stage of LDL oxidation and especially during intense oxidative stress.5–8 However, under mild oxidation, α-tocopherol may act as a prooxidant, thus shifting the peroxidative insult from the surface to the inner part of the LDL particle.5–10 This event could be prevented by ubiquinol.11

α-Tocopherol supplementation may have a significant role in the prevention of CHD. Retrospective data indicate an inverse relation between plasma concentrations of vitamin E and the risk of angina pectoris.12 According to 1 prospective study, the serum α-tocopherol concentration may affect cardiac risk (ie, nonfatal myocardial infarction or cardiovascular death) in patients with coronary disease.13 Similar results have been obtained in both men and women,14,15 with a high intake of vitamin E being correlated with a lowered risk of CHD. Although vitamin E supplementation at pharmacological doses may reduce the rate of nonfatal myocardial infarction, its effect on cardiac or all-cause mortality is unclear.16

We found earlier in hypercholesterolemic CHD patients not under any antioxidant treatment that the oxidation times of both LDL ubiquinol and LDL α-tocopherol were shortened significantly during lovastatin therapy.17 This finding was associated with a shortened lag time to conjugated-diene formation, suggesting a diminished resistance of LDL particles to the early phase of oxidative stress. This oxidative resistance, at least partially, may be restored with ubiquinone supplementation.18 α-Tocopherol supplementation prolongs the lag time to conjugated-diene formation, when LDL oxidation occurs at high concentrations of oxidants.19,20 However, even during α-tocopherol treatment, a prominent change in diet can affect the susceptibility of LDL to oxidation.21

In the present trial, we studied whether effective lovastatin therapy modifies the oxidizability of LDL, measured as the lag time to diene formation, in CHD patients who were on α-tocopherol supplementation. We also studied whether lov-
Lovastatin and α-Tocopherol Modify LDL Oxidation

astatin in this context affects the depletion times of LDL α-tocopherol or ubiquinol during metal ion–independent LDL oxidation.

Methods

Study Design

In this randomized, double-blind, crossover trial, the effects of lovastatin treatment were compared with those of placebo in patients receiving α-tocopherol. A 12-week-long period of α-tocopherol supplementation was preceded by a washout period of at least 8 weeks. During α-tocopherol treatment (300 mg/d, or 450 IU daily), concomitant lovastatin (LT period) or placebo resembling lovastatin (PT period) was taken. In other words, 14 patients started with the LT (lovastatin + α-tocopherol) period and continued, after 6 weeks, with PT (placebo + α-tocopherol), whereas the other half of the patients had the PT period first. The clinical part of the study was carried out in the cardiology outpatient clinic at Kanta–Häme Central Hospital, Hämeenlinna, Finland. The study was approved by the Ethics Committee of the hospital and the National Agency for Medicines. Written, informed consent was received in advance from every patient. Monitoring was carried out according to Good Clinical Trial Practice,22 and the recommendations of the Declaration of Helsinki were followed during the study protocol.

Inclusion criteria were checked during the prestudy visit. Except for the study medication, the entire trial was performed without any antioxidants, vitamins, or treatments affecting lipid metabolism while all other medications were kept constant. The study products were Esol (100 mg RRR-α-tocopherol, Leiras) and Lovacol (20 mg lovastatin, Orion, under license from MSD). The patients took 1 pill of α-tocopherol 3 times daily during both 6-week periods. One lovastatin tablet was taken daily during the first week, 2 tablets per day during the second week, and 3 tablets daily during weeks 3 through 6. This dosage protocol and the final dailyLovastatin dose of 40 mg had proved to be well tolerated and effective with a similar patient group.18 The placebos were taken in identical fashion. Compliance was checked by tablet counting, and diet was assessed on each visit. Clinical evaluation of the safety of therapy was completed by analyzing serum enzymes indicating the function of the muscles, liver, and kidneys.

Subjects

The study was carried out with 28 well-informed and cooperative men at an average age of 56±8 years (mean±SD; range, 40 to 69 years) and a body mass index of 25.4±1.4 kg/m². All of them had definite CHD and primary hyperlipidemia. The diagnosis of CHD was confirmed either by coronary angiography (17 patients) or by verified previous heart infarction (11 patients) at least 6 months before the study. The inclusion criteria for fasting serum lipids were as follows: LDL cholesterol >4.0 mmol/L or total to HDL cholesterol ratio >5.5. Exclusion criteria were concomitant steroid therapy; diabetes; alcoholism or misuse of narcotics; overt hypertriglyceridemia (>5 mmol/L); liver, renal, or endocrine disease; malignant tumor; or chronic terminal disease. All of the patients were on a cholesterol-lowering diet. There were 2 current smokers (>2 cigarettes per day). Twenty-seven of the patients were on β-blocking–agent therapy, 4 used angiotensin-converting enzyme inhibitors, 11 were taking long-acting nitrates, and 7 used calcium channel–blocking agents.

Blood Samples

Venous blood samples were drawn at 8 AM on all study visits. The patients were advised to fast; not to take any medication, coffee, or other beverages; and not to smoke for 12 hours before blood sampling. Alcohol was prohibited for 36 hours before sampling. Blood samples were taken in the sitting position after a rest of at least 15 minutes. Plasma was separated by centrifugation immediately after cooling the samples on ice in the dark for 5 minutes. After separation, the samples were kept frozen at −80°C until analyzed.

Metal Ion–Dependent Oxidation of LDL

The copper-induced LDL oxidation method used in this trial has been described earlier.19 In brief, LDL was isolated by single-step ultracentrifugation for 30 minutes at 338 000 g (Beckman TL-100) with a TLV-100 rotor. A 1-mL sample of desalted and gel–filtered LDL solution was standardized to 0.05 mg protein per mL with PBS. The sample was oxidized at 37°C as described previously.20 The final concentration of CuSO₄ in the mixture was 1.67 μmol/L. UV absorbance at 234 nm was monitored every minute for 300 minutes with the use of a Perkin Elmer Lambda Bio 10 spectrometer. Lag time to the start of the propagation phase of diene formation was defined as the intersection of the tangents of the initial phase (first 5 minutes) and maximal propagation.

Metal Ion–Independent Oxidation of LDL

The method of LDL oxidation induced by 2,2-azobis(2,4-dimethylvaleronitrile) (AMVN) has been described thoroughly in our previous article.17 In brief, LDL was precipitated from EDTA-plasma with heparin (Noparin, Novo Nordisk) and trisodium citrate (pH 5.0) in acid-washed Kimax glass tubes.24 The lipid fraction of LDL was dissolved in chloroform/methanol (1:1, vol/vol). Peroxyl radicals were produced by AMVN (Polysciences Inc), with a final AMVN concentration of 2.1 mmol/L. Reduced α-tocopherol and ubiquinol were determined by high-performance liquid chromatography and redox-sensitive electrode.25 Samples of 100 μL were taken every 3 minutes from a test tube that had been placed in a temperature-controlled incubator (37°C). Oxidation was stopped by deep-freezing the samples in LN₂, wherein they were stored until HPLC analyses. The results were standardized against LDL phosphate concentrations. LDL phosphate (Pi) concentration was determined using a colorimetric method and an inorganic ammonium molybdate reaction.

Determination of Lipids and Apolipoproteins

Total cholesterol, HDL cholesterol, and triglycerides were analyzed immediately after separation of the serum samples. LDL cholesterol concentration was calculated with the Friedewald formula. The other biochemical determinations of plasma samples were made after the entire clinical phase of the study had been completed. Apo A-I and B were measured by a nephelometric method (Behring) using highly specific antisera.

Numerical Analyses

The rates of individual consumption of ubiquinol and reduced α-tocopherol during AMVN-induced oxidation were calculated by linear regression analysis. The effects of the lovastatin and placebo interventions during α-tocopherol treatment were compared by 2-way ANOVA with equal replications (RANOVA) and with the factors treatment and treatment order. Data for the 3 periods were included in the overall analyses: initial washout (WO), lovastatin + tocopherol (LT), and placebo + tocopherol (PT). The treatment periods were further compared pairwise by t test–based contrast analysis (user contrast, BMDP Solo).26 A value of P≤0.05 was regarded as statistically significant. Mean±SEM are presented unless stated otherwise.

Results

During the trial, no severe adverse effects were reported or observed. Patient compliance was good, as measured by adherence to the protocol and the consumed–tablet counts, which were, on average, 96% for both lovastatin (or placebo resemblingLovastatin) and α-tocopherol. No clinically significant changes were observed in weight, diet, living habits, serum enzymes, hematological parameters, or clinical status (Table 1).

Lipids and Lipoproteins

LT treatment had significant effects on all lipid and lipoprotein parameters measured. The only parameters on which...
The elevations were more modest: 10.2% and 3.4%, respectively.

When treatments including α-tocopherol alone (ie, the PT phase) seemed to have an influence were HDL cholesterol and apo A-I. When LT was compared with the initial WO period, HDL cholesterol increased by 15.9% and apo A-I by 5.9%, whereas with PT, the elevations were more modest: 10.2% and 3.4%, respectively.

When treatments including α-tocopherol therapy were compared, lovastatin highly significantly decreased both serum total and LDL cholesterol, by 32.4% and 41.1%, respectively. Furthermore, a slight but significant increase in serum HDL cholesterol (5.2%) was measured during LT, as well as a decrease in triglycerides by 28.3%. Apo B decreased significantly by 31.1%, but apo A-I remained at the same level as during PT. The LDL to HDL ratio decreased by 44.1%, and the LDL cholesterol to apo B ratio dropped by 31.1%, but apo A-I remained at the same level as during PT. The LDL to HDL ratio decreased by 31.1%, but apo A-I remained at the same level as during PT.

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### Table 1. Fasting Serum Lipid Levels Before the Study and After the 6-Week Treatment Periods

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Before Treatments and After WO</th>
<th>LT</th>
<th>PT</th>
<th>P Between Treatments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cholesterol, mmol/L</td>
<td>6.71 ± 0.17</td>
<td>4.56 ± 0.16</td>
<td>6.75 ± 0.24</td>
<td>&lt;0.0001 NS &lt;0.0001</td>
</tr>
<tr>
<td>LDL cholesterol, mmol/L</td>
<td>4.70 ± 0.12</td>
<td>2.75 ± 0.13</td>
<td>4.67 ± 0.21</td>
<td>&lt;0.0001 NS &lt;0.0001</td>
</tr>
<tr>
<td>HDL cholesterol, mmol/L</td>
<td>0.88 ± 0.05</td>
<td>1.02 ± 0.04</td>
<td>0.97 ± 0.04</td>
<td>&lt;0.0001 0.0003 0.021</td>
</tr>
<tr>
<td>LDL to HDL ratio</td>
<td>5.88 ± 0.56</td>
<td>2.85 ± 0.23</td>
<td>5.10 ± 0.41</td>
<td>&lt;0.0001 NS &lt;0.0001</td>
</tr>
<tr>
<td>Triglycerides, mmol/L</td>
<td>2.49 ± 0.23</td>
<td>1.75 ± 0.14</td>
<td>2.44 ± 0.23</td>
<td>&lt;0.0001 NS 0.0001</td>
</tr>
<tr>
<td>Apo A-I, g/L</td>
<td>1.19 ± 0.04</td>
<td>1.26 ± 0.04</td>
<td>1.23 ± 0.04</td>
<td>0.0004 0.04 0.97</td>
</tr>
<tr>
<td>Apo B, g/L</td>
<td>1.35 ± 0.05</td>
<td>0.93 ± 0.04</td>
<td>1.35 ± 0.05</td>
<td>&lt;0.0001 NS &lt;0.0001</td>
</tr>
<tr>
<td>LDL cholesterol to apo B ratio, mmol/g</td>
<td>3.56 ± 0.10</td>
<td>3.03 ± 0.11</td>
<td>3.49 ± 0.11</td>
<td>&lt;0.0001 NS &lt;0.0001</td>
</tr>
<tr>
<td>LDL phosphorus, mmol/L plasma</td>
<td>1.05 ± 0.06</td>
<td>0.70 ± 0.05</td>
<td>1.03 ± 0.06</td>
<td>&lt;0.0001 NS &lt;0.0001</td>
</tr>
<tr>
<td>Alanine aminotransferase, U/L</td>
<td>33.4 ± 3.5</td>
<td>32.8 ± 2.7</td>
<td>30.6 ± 2.8</td>
<td>NS NS NS</td>
</tr>
</tbody>
</table>

Mean ± SEM (n = 28) and the P values from t test–based user contrast analyses (BMDP Solo) are presented. Contrast analysis was carried out after ANOVA with repeated measurements (RANOVA) and with the factors treatment and treatment order. NS when P > 0.05.

### Table 2. Kinetic Parameters of LDL Antioxidants Before the Study and After the 6-Week Treatment Periods

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Before Treatments and After WO</th>
<th>LT</th>
<th>PT</th>
<th>P Between Treatments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reduced LDL tocopherol</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Observed value before oxidation, μmol/L plasma</td>
<td>15.0 ± 1.4</td>
<td>18.6 ± 1.7</td>
<td>27.1 ± 2.6</td>
<td>NS &lt;0.0001 &lt;0.0001</td>
</tr>
<tr>
<td>Observed value before oxidation, mmol/mol Pi</td>
<td>14.5 ± 1.2</td>
<td>26.6 ± 2.0</td>
<td>27.3 ± 1.9</td>
<td>&lt;0.0001 &lt;0.0001 NS</td>
</tr>
<tr>
<td>Rate of consumption, mmol/(mol Pi/min)</td>
<td>1.41 ± 0.15</td>
<td>1.72 ± 0.16</td>
<td>1.29 ± 0.14</td>
<td>0.0283 NS 0.0096</td>
</tr>
<tr>
<td>Calculated depletion time, minutes</td>
<td>11.3 ± 0.92</td>
<td>12.7 ± 0.76</td>
<td>22.8 ± 2.51</td>
<td>NS &lt;0.0001 &lt;0.0001</td>
</tr>
<tr>
<td>LDL ubiquinol</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Observed value before oxidation, mmol/L plasma</td>
<td>665 ± 60</td>
<td>365 ± 47</td>
<td>657 ± 65</td>
<td>&lt;0.0001 NS &lt;0.0001</td>
</tr>
<tr>
<td>Observed value before oxidation, mmol/mol Pi</td>
<td>0.643 ± 0.051</td>
<td>0.532 ± 0.063</td>
<td>0.654 ± 0.054</td>
<td>NS NS 0.041</td>
</tr>
<tr>
<td>Rate of consumption, μmol/(mol Pi/min)</td>
<td>49.3 ± 0.4</td>
<td>67.7 ± 1.2</td>
<td>66.9 ± 1.0</td>
<td>0.046 NS NS</td>
</tr>
<tr>
<td>Calculated depletion time, minutes</td>
<td>12.6 ± 0.62</td>
<td>9.8 ± 0.68</td>
<td>10.8 ± 0.78</td>
<td>0.0007 0.024 NS</td>
</tr>
</tbody>
</table>

Mean ± SEM (n = 28) and the P values from t test–based user contrast analysis (after RANOVA) are presented. NS when P > 0.05.

### LDL Antioxidant Consumption During Metal Ion–Independent Oxidation

The effects of different therapy on the kinetic parameters of LDL antioxidants during oxidation induced by AMVN are compiled in Table 2. Tocopherol supplementation led to a significant increase in the reduced α-tocopherol content in LDL, by 81.4%. The consumption rate of α-tocopherol was decreased nonsignificantly by 15.6%, and the prolongation of depletion time was 100.9% (P < 0.0001). LT did not change LDL α-tocopherol levels when compared with the values after PT. However, the rate of α-tocopherol consumption was hastened and the depletion time shortened significantly, by
Conjugated-Diene Formation in LDL During Metal Ion–Dependent Oxidation

During copper-mediated oxidation, α-tocopherol therapy increased the lag time by 43.3% and decreased the maximal oxidation rate by 15.8% (Table 3). The maximal protein-normalized diene formation had a significant but small tendency to decrease when compared with WO.

During LT, the lag time decreased by 7.1% when compared with PT. The small decrease, 5.2%, in the oxidation rate during LT did not reach statistical significance (P = 0.096) when compared with PT. Lovastatin did not significantly alter the maximal, protein-normalized conjugated-diene level. A representative example of conjugated-diene formation during copper-induced oxidation of LDL is presented in Figure 3.

Ubiquinol concentration in LDL was related to the changes in LDL lag time. When the study group was evenly divided into 2 groups according to ubiquinol level during PT, the lag time diminished during LT, from 87.9 to 78.6 minutes in the lower-ubiquinol subgroup and from 93.6 to 90.0 minutes in the higher-ubiquinol subgroup. These changes did not differ significantly (P = 0.17) from each other. When the same subdivision was made according to the LDL ubiquinol level during LT, the changes in lag times were significantly different (P = 0.03): from 95.0 to 82.5 minutes in the lower-ubiquinol subgroup and from 86.5 to 86.1 minute in the higher-ubiquinol subgroup.

Discussion

The oxidation of LDL is a gradual reaction, wherein antioxidants are consumed according to different priorities. Besides α-tocopherol, other known antioxidants like ubiquinol occur in very low concentrations in LDL particles. Ubiquinol acts as an effective antioxidant at the very beginning of the peroxidative process. Its level may be reduced by high-dose treatment with an HMG-CoA reductase inhibitor. In the present study, the LDL ubiquinol level decreased during the LT period. The lag time to conjugated-diene formation in ex vivo, isolated LDL was prolonged by α-tocopherol treatment (450 IU/d), regardless of concomitant treatment with lovastatin or placebo. In in vitro studies on copper-induced oxidation of LDL, in which the Cu to LDL particle ratio is at least 3, α-tocopherol acts as an antioxidant. Our finding accords with earlier studies, in which doses of not less than 400 IU/d have lengthened the lag time. The lag time during LT was statistically significantly shorter than that during PT. The results of the AMVN experiments are in accordance with those of metal ion–dependent oxidation: LT accelerated the consumption of reduced α-tocopherol during AMVN-induced oxidation.

In vitro studies using coincubation of LDL and statins have suggested that lovastatin, simvastatin, and fluvastatin themselves might have some direct antioxidative influence on LDL. Mevalonate is the precursor of many nonsteroidal derivatives, such as geranyl and farnesy1 pyrophosphates. They are lipid moieties of prenylated proteins, which may regulate superoxide generation in neutrophils and macrophages. Other molecules that are affected by inhibition of HMG-CoA reductase and that may participate in the defense against oxidative stress are Rho-related...
proteins, like rac 1 and rac 2.\textsuperscript{41} Simvastatin has decreased the formation of superoxide in human monocytes,\textsuperscript{42} and lovastatin has inhibited the oxidizability of LDL by rabbit leukocytes.\textsuperscript{40} Finally, both vitamin E and lovastatin therapy have diminished the number of atherosclerotic lesions in rabbits.\textsuperscript{40,43} In contrast, lovastatin has also been found to potentiate superoxide-radical generation in the atherosclerotic vascular wall in rabbits.\textsuperscript{44} However, it is not conceivable that the shortening of lag time is due to the direct prooxidative effect of lovastatin.\textsuperscript{45}

Lag time, an accepted marker of LDL susceptibility to oxidation, is associated with endothelial function and the severity of coronary atherosclerosis in cross-sectional settings.\textsuperscript{46,47} However, no controlled, prospective clinical trials have been carried out to compare the different measures of LDL antioxidative capacity as surrogates for CHD events. The amount and formation rate of conjugated dienes reflect later phases of LDL oxidation than does the lag time or antioxidant depletion time of AMVN experiments. Effective statin therapy has been reported to slow the oxidation rate and to decrease the maximal production of conjugated dienes.\textsuperscript{17,38,40–52} These phenomena may be secondary to a greater decrease in the lipid moiety than that of the proteins in LDL.\textsuperscript{17,49,53} Later phases of LDL oxidation can also be studied by using excessive, long-lasting oxidation with other measures for LDL peroxidation, like the determination of thiobarbituric acid–reactive substances and trinitrobenzenesulfonic acid,\textsuperscript{38} or LDL malondialdehyde, peroxides, and the total amount of conjugated dienes.\textsuperscript{54} This kind of long-lasting oxidation may also lead not only to peroxidation of the lipids in the LDL particle but also to the degradation of its protein, like the inner part of apo B.\textsuperscript{55} Therefore, the effect of vigorous lovastatin therapy may be simultaneously indifferent or favorable when judged according to oxidation rate or maximal diene formation and unfavorable when based on lag time or antioxidant depletion.

Table 4 compiles the characteristics of earlier studies, wherein the effects of statin treatment on lag time were measured during copper-induced oxidation ex vivo. Two groups studied oxidation of the non-HDL fraction, including VLDL, whereas all of the others used the LDL fraction. In 5 of 7 studies, lag time did not change significantly.\textsuperscript{48–52} Our earlier study was a randomized, placebo-controlled, double-blind trial with a crossover protocol, in which 60 mg daily of lovastatin shortened the lag time by 7%.\textsuperscript{17} However, in an open, uncontrolled setting with 10 subjects, fluvastatin at 40 mg/d was reported to be associated with a lengthening of lag time by 2.5 times.\textsuperscript{39} Fluvastatin has also been studied in a randomized, double-blind, parallel, placebo-controlled trial design, wherein the daily dose of 80 mg did not change the lag time significantly.\textsuperscript{48}

As in most of the earlier studies,\textsuperscript{17,48–52} oxidation tests were performed on frozen samples after the entire clinical phase had been completed. If some oxidation or conformational changes did occur in LDL, then lovastatin made LDL more vulnerable to minimal oxidation than placebo, because the conditions were the same for all samples and the treatment periods were balanced. Also, with this design, the possible interassay variability does not affect the results.

If cholesterol-lowering therapy is effective enough, the nonbeneficial effect of lovastatin on the by-products of cholesterol synthesis (eg, ubiquinol) may become important.\textsuperscript{18} In the present study, serum LDL cholesterol was decreased to levels below those in most of the other statin trials in which antioxidative defense mechanisms were studied (Table 4). LDL was isolated by a rapid procedure to minimize oxidative stress before the analysis. The effect of statin treatment on the antioxidative defense against the very initial phase of LDL oxidation (eg, ubiquinol) may disappear if LDL is isolated more slowly.

The statins may differ in their effect on LDL susceptibility to oxidation. Treatment with pravastatin but not with simvastatin has reduced the malondialdehyde content of the vessel wall, at the same time enhancing endothelium-

![Figure 3. A representative example of the formation of conjugated dienes during copper-induced oxidation of LDL. LDL was isolated by rapid density-gradient ultracentrifugation and desalted on a gel filtration column. The final concentration of CuSO\textsubscript{4} was 1.67 μmol/L. Formation of conjugated dienes was followed by measuring absorbance changes at 234 nm. Treatments are as follows: ○, WO; ●, LT; and ○, PT.](image-url)
TABLE 4. Effect of Statin Treatment on the Lag Time to Conjugated-Diene Formation in Previous Clinical Ex Vivo Studies

<table>
<thead>
<tr>
<th>First Author</th>
<th>Subjects</th>
<th>n/Group</th>
<th>Design</th>
<th>Placebo Controlled?</th>
<th>Therapy</th>
<th>Dose/d</th>
<th>LDL Cholesterol at End</th>
<th>Duration/Period</th>
<th>Lag Time, Minutes</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kleinveld49</td>
<td>Hypercholesterolic</td>
<td>12</td>
<td>Parallel</td>
<td>No</td>
<td>Simvastatin</td>
<td>20→40 mg</td>
<td>4.96 mmol/L</td>
<td>18 wk</td>
<td>80.0→79.8</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td></td>
<td>11</td>
<td></td>
<td></td>
<td>Pravastatin</td>
<td>20→40 mg</td>
<td>3.12 mmol/L</td>
<td>18 wk</td>
<td>78.1→81.5</td>
<td>NS</td>
</tr>
<tr>
<td>Bredie50</td>
<td>Familial combined hyperlipidemic</td>
<td>18</td>
<td>Parallel</td>
<td>No</td>
<td>Simvastatin</td>
<td>20 mg</td>
<td>3.46 mmol/L</td>
<td>3 y</td>
<td>68.5</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td></td>
<td>17</td>
<td></td>
<td></td>
<td>Gemfibrozil</td>
<td>1.2 g</td>
<td>4.59 mmol/L</td>
<td>12 wk</td>
<td>80→200</td>
<td>NS</td>
</tr>
<tr>
<td>Salonen51</td>
<td>Hypercholesterolic</td>
<td>212</td>
<td>Parallel</td>
<td>Yes</td>
<td>Pravastatin</td>
<td>40 mg</td>
<td>4.93 mmol/L</td>
<td>12 wk</td>
<td>74.8→75.3</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td></td>
<td>212</td>
<td></td>
<td></td>
<td>Placebo</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td>73.7→72.8</td>
<td>NS</td>
</tr>
<tr>
<td>Zhang52</td>
<td>IDDM patients with incipient nephropathy</td>
<td>20</td>
<td>Crossover</td>
<td>Yes</td>
<td>Pravastatin</td>
<td>20 mg</td>
<td>2.59 mmol/L</td>
<td>12 wk</td>
<td>95</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td></td>
<td>20</td>
<td></td>
<td></td>
<td>Placebo</td>
<td>...</td>
<td>...</td>
<td>3.28 mmol/L</td>
<td>96</td>
<td></td>
</tr>
<tr>
<td>Hussein53</td>
<td>Hypercholesterolic</td>
<td>10</td>
<td>No controls</td>
<td>No</td>
<td>Fluvastatin</td>
<td>40 mg</td>
<td>4.23 mmol/L</td>
<td>8 wk</td>
<td>74.8→75.3</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td></td>
<td>10</td>
<td></td>
<td></td>
<td>Placebo</td>
<td>...</td>
<td>...</td>
<td>4.93 mmol/L</td>
<td>73.7→72.8</td>
<td>NS</td>
</tr>
<tr>
<td>Leonhardt48</td>
<td>Hypercholesterolic</td>
<td>10</td>
<td>Parallel</td>
<td>Yes</td>
<td>Fluvastatin</td>
<td>80 mg</td>
<td>3.28 mmol/L</td>
<td>24 wk</td>
<td>80→200</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td></td>
<td>20</td>
<td></td>
<td></td>
<td>Placebo</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td>73.7→72.8</td>
<td>NS</td>
</tr>
<tr>
<td>Palomäki17</td>
<td>Hypercholesterolic</td>
<td>27</td>
<td>Crossover</td>
<td>Yes</td>
<td>Lovastatin</td>
<td>60 mg</td>
<td>2.65 mmol/L</td>
<td>6 wk</td>
<td>59.5</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>CHD patients</td>
<td></td>
<td>27</td>
<td></td>
<td></td>
<td>Placebo</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td>4.79 mmol/L</td>
<td>63.5</td>
</tr>
</tbody>
</table>

To further elucidate the clinical relevance of statin treatment on LDL antioxidative capacity, we need more randomized, controlled, double-blind studies to compare statins with each other. It might also be worthwhile to study different patient groups to evaluate whether some of them have LDL that is more vulnerable to oxidation during statin therapy than others. This trial supports the view that the 450-IE daily dose of vitamin E slightly but significantly elevated both apo A-I and HDL cholesterol levels. The association between α-tocopherol and HDL is controversial.7–62 In a study with 24 volunteers, HDL remained stable during all-α-tocopherol therapy of 800 IU (727.3 mg) daily.63,64 The initial HDL level of patients in our study was 21% to 32% lower than in some of the studies showing no change.7,37,63,64 α-Tocopherol might be elementary in the synthesis of apo A-I and HDL but may not have any elevating effect on them if the level of HDL is already sufficient in the cholesterol circulation. HDL is a vehicle for peroxidized lipids, and it may protect LDL from peroxidation.65,66

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