Vitamin E Combined With Selenium Inhibits Atherosclerosis in Hypercholesterolemic Rabbits Independently of Effects on Plasma Cholesterol Concentrations

Dawn C. Schwenke, Stephen R. Behr

Abstract—Several antioxidants inhibit atherosclerosis. This study investigated the hypothesis that combining vitamin E, a lipophilic antioxidant, with vitamin C, a hydrophilic antioxidant, and/or selenium, a cofactor of peroxidases that detoxify lipid peroxides, would inhibit atherosclerosis more effectively than vitamin E alone. We also considered whether regional variation in inhibition of atherosclerosis by antioxidants would be associated with regional variation in aortic lipophilic antioxidants. Rabbits were fed an atherogenic diet (control) or an atherogenic diet supplemented with vitamin E, vitamins E and C, vitamin E+selenium, vitamins E and C+selenium, or probucol (positive control). Supplements were as follows: vitamin E, 146 IU/d; vitamin C, 791 mg/d; selenium, 22 μg/d; or probucol, 406 mg/d. Vitamin C did not influence atherosclerosis. After 22 weeks of treatment, rank order of aortic atherosclerosis was control > vitamin E (with or without vitamin C) > vitamin E+selenium (with or without vitamin C) > probucol. Antioxidant treatment reduced aortic cholesterol concentrations 21% to 56%, 29% to 86%, and 19% to 75% for the aortic arch, descending thoracic aorta, and abdominal aorta, respectively (P<0.025 to P<0.0003 by ANOVA), with slightly greatly reductions for areas of atherosclerotic lesions. Some treatments reduced plasma cholesterol concentrations, but none altered the distribution of cholesterol among lipoproteins. Corrected for differences in plasma cholesterol concentrations, aortic cholesterol concentrations were reduced up to 72% (P<0.02) by the antioxidant treatments, with equal reductions by vitamin E+selenium and by probucol. Aortic \( \alpha \)-tocopherol standardized by aortic cholesterol as a measure of aortic lipids was lower in the abdominal aorta than in the aortic arch of rabbits not given \( \alpha \)-tocopherol and increased relatively more in the abdominal aorta than in the aortic arch with \( \alpha \)-tocopherol supplementation. The results of this study suggest that vitamin E+selenium inhibited atherosclerosis as effectively as an equally hypocholesterolemic dose of probucol by a mechanism(s) that is in part independent of effects on plasma and lipoprotein cholesterol concentrations. The tendency for greater efficacy of antioxidant treatments in the abdominal aorta than aortic arch may relate to the lower concentrations of \( \alpha \)-tocopherol in the abdominal aorta of unsupplemented rabbits. (Circ Res. 1998;83:366-377.)

Key Words: vitamin E ■ selenium ■ atherosclerosis ■ antioxidant ■ aorta

Several antioxidants, including probucol,1-7 butylated hydroxytoluene,8 and \( N, N' \)-diphenyl-phenylenediamine,9 inhibit atherosclerosis in rabbits. Other independent evidence suggests a number of potential mechanisms by which intraportal oxidation of lipoproteins might initiate and promote atherogenesis,10-12 A number of studies in rabbits have considered the potential for the lipophilic nutrient antioxidant vitamin E to inhibit atherosclerosis.13-21 Other studies have investigated the influence of the hydrophilic antioxidant vitamin C on atherosclerosis in rabbits.22,23 Most of these studies evaluated one or several of these antioxidants individually. However, 1 study investigated inhibition of athero-sclerosis by combined treatment with vitamin E and selenium,13 1 study considered combined treatment with vitamins E and A,24 and 3 studies investigated combined treatment with vitamins E and C.4,7,25 Only the study of vitamin E plus selenium compared the combined treatment with treatment by each supplement separately.

Thus, it is not yet known how vitamin E, vitamin C, and selenium might interact to inhibit atherosclerosis. However, antioxidants are known to interact with one another. For example, vitamin C protects LDL from loss of \( \alpha \)- and \( \gamma \)-tocopherol and inhibits in vitro lipid peroxidation of LDL.26,27 Glutathione decreases the amount of vitamin E required to inhibit peroxidation of microsomal lipids by preserving the microsomal content of vitamin E.28,29 Either vitamin C or glutathione blocks the oxidation of platelet tocopherol.30,31 Glutathione is thought to react with dehydroascorbic acid to regenerate ascorbate.32,33 Selenium has a role as a cofactor of glutathione peroxidase and phospholipid
hydroperoxide glutathione peroxidase, enzymes that detoxify lipid peroxides.\textsuperscript{34–36} Thus, it seems possible that vitamin E, vitamin C, and selenium might interact in vivo to inhibit atherosclerosis more effectively than only one or several of these antioxidants.

The present study investigated the hypothesis that combined treatment with antioxidants that function by different mechanism(s) and within different cellular locations (vitamin E [a lipophilic antioxidant], vitamin C [a hydrophilic antioxidant], and selenium [a cofactor for selenoperoxidases that can detoxify lipid hydroperoxides]) would inhibit atherosclerosis more effectively than treatment with fewer of these antioxidants. Because inhibition of atherosclerosis by antioxidants may be mediated in part by aortic concentrations of antioxidants, we also measured aortic concentrations of lipophilic antioxidants.

In the present study, we report that vitamin E combined with selenium inhibited atherosclerosis more effectively than did vitamin E alone. Vitamin E and selenium combined inhibited atherosclerosis in part by a mechanism(s) that is independent of effects on plasma cholesterol concentrations and, to an extent, similar to inhibition of atherosclerosis by an equally hypocholesterolemic dose of probucol. The antioxidant interventions were more efficacious in the abdominal aorta (an aortic region where \( \alpha \)-tocopherol concentrations were lower and were increased more by \( \alpha \)-tocopherol supplementation) than in the aortic arch.

**Materials and Methods**

**Rabbits**

A total of 72 sexually mature young female New Zealand White rabbits were studied. Rabbits were received from the supplier (Robinson Services, Inc, Winston-Salem, NC) in groups of 6. Rabbits were acclimated to the animal facility for \( \approx \) 1 week, during which time they were fed each day 100 g of a standard cholesterol-free rabbit chow (Prolab, Agway). After acclimation to the animal facility, rabbits were randomly assigned to 6 treatment groups: atherogenic diet only (control group) or atherogenic diet supplemented with vitamin E only (group E), vitamins E and C (EC group), vitamin E plus selenium (ES group), vitamins E and C plus selenium (ESe group), or probucol (Prob group). The composition of the atherogenic diet and the amounts of the antioxidant supplements are shown in Table 1. The amounts of the vitamin E, vitamin C, and selenium supplements were determined with reference to the literature,\textsuperscript{13,15–20,22,23} and were set at levels similar to those that have been shown to inhibit atherosclerosis when studied individually.\textsuperscript{13,15,16,22,23} We used probucol at 40% of the daily dose that we used in a previous study\textsuperscript{1} because we anticipated greater absorption of probucol on the higher fat diet in the present study. During the first 6 days of treatment, rabbits were fed increasing amounts of atherogenic diet blended with decreasing amounts of rabbit chow. Rabbits were fed 55 g of atherogenic diet per day for the remainder of the study, which averaged a total of 152\( \pm \)2 days (\( n = 70 \)), with no difference in treatment time among groups. Mean values for uncontrolled diet (collected every day during the first week and 2 to 3 times per week for the rest of the treatment period) averaged 1 to 3 g/d. One rabbit was lost before baseline measurements could be collected; another had to be removed from the study because of ileal intussusception. The remaining 70 rabbits weighed 2.52\( \pm \)0.03 (mean\( \pm \)SEM) and 2.94\( \pm \)0.04 kg before and after treatment, respectively, with no difference among groups either before or after treatment.

**TABLE 1. Composition of Atherogenic Diet**

<table>
<thead>
<tr>
<th>Component</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Casein</td>
<td>100 g</td>
</tr>
<tr>
<td>( \alpha )-Methionine</td>
<td>1 g</td>
</tr>
<tr>
<td>Sucrose</td>
<td>233 g</td>
</tr>
<tr>
<td>Cellulose</td>
<td>70 g</td>
</tr>
<tr>
<td>Corn oil</td>
<td>5 g</td>
</tr>
<tr>
<td>Butter, dairy</td>
<td>55.5 g</td>
</tr>
<tr>
<td>Cholesterol, USP</td>
<td>0.48 g</td>
</tr>
<tr>
<td>Salt mix</td>
<td>20 g</td>
</tr>
<tr>
<td>Magnesium oxide</td>
<td>1.2 g</td>
</tr>
<tr>
<td>Potassium citrate</td>
<td>13.8 g</td>
</tr>
<tr>
<td>Vitamin mix</td>
<td>10 g</td>
</tr>
<tr>
<td>Choline bitartrate</td>
<td>1.0 g</td>
</tr>
<tr>
<td>Total</td>
<td>510.98 g</td>
</tr>
<tr>
<td>Antioxidant supplements</td>
<td></td>
</tr>
<tr>
<td>Vitamin E</td>
<td>1375 IU</td>
</tr>
<tr>
<td>Vitamin C</td>
<td>7.5 g</td>
</tr>
<tr>
<td>Selenium</td>
<td>214 ( \mu )g</td>
</tr>
<tr>
<td>Probucol</td>
<td>3.8 g</td>
</tr>
</tbody>
</table>

Twenty grams of the salt mix contained 4.1 g calcium, 0.9 g phosphorus, 2.4 g potassium, 150 mg magnesium, 65 mg sulfur, 0.55 g sodium, 1.9 g chloride, 7.1 mg copper, 11.3 mg iodine, 50 mg iron, 25 mg manganese, 2.8 mg zinc, 3.7 mg cobalt, 0.2 mg aluminum, and 9.0 mg fluoride. Ten grams of the vitamin mix contained 7500 IU vitamin A palmitate, 1450 IU vitamin D3, 25 IU vitamin E acetate, 5 mg menadione sodium bisulfite, 0.1 mg biotin, 15 \( \mu \)g cyanocobalamin, 5 mg folic acid, 25 mg nicotinic acid, 15 mg calcium pantothenate, 5 mg pyridoxine hydrochloride, 5 mg riboflavin, 5 mg thiamine hydrochloride, and 500 mg inositol. Values in parentheses are daily doses.

*This value for selenium was confirmed by atomic absorption spectrometry and was \( \approx \) 2.5 times that which we assayed in several batches of cholesterol-free rabbit chow (Prolab, Agway), which was fed to the rabbits before they began treatment with special diets. The selenium content of the unsupplemented atherogenic diet was about one-tenth that of the selenium supplement. A selenium requirement has not been established for rabbits.\textsuperscript{71}

**Plasma and Lipoprotein Lipids**

Plasma concentrations of cholesterol and triglyceride were measured while rabbits were consuming rabbit chow, at intervals during treatment with atherogenic diet (cholesterol, every 2 weeks; triglyceride, every 4 weeks), and at the end of the study. Plasma was obtained from blood samples collected after an overnight fast. These samples were collected into 0.01 vol of a solution of 0.4 mol/L disodium EDTA and 0.4% sodium azide, pH 7.4.

Lipoproteins were isolated from plasma collected after an overnight fast before treatment with atherogenic diet, after 12 weeks of treatment, and at the end of the 22-week study. Blood was collected into a cocktail containing protease inhibitors and EDTA to prevent oxidation. Plasma was mixed with the serine protease inhibitor phenylmethylsulfonyl fluoride (final concentration, 0.5 mmol/L). Plasma was adjusted to 1.065 g/mL with KBr and centrifuged at 288,000 g for 24 hours in an SW41 rotor (Beckman Instruments, Inc) to separate HDL (density fraction [d] > 1.060 g/mL) from apoipoprotein B–containing lipoproteins. VLDL, IDL, and LDL were isolated from the d<1.060 g/mL plasma fraction by a modification of the step gradient described by Terpstra et al. Infranatant lipoprotein fraction (4.35 mM) adjusted to 1.080 g/mL with KBr was overwashed with 3.75 mL of 1.060 g/mL density solution and 3.9 mL of 1.00 g/mL density solution and centrifuged at 288,000 g for 13.5 hours. VLDL (d<1.006), IDL (1.006<d<1.020), and LDL (1.020<d<1.060) were isolated by tube slicing. For samples collected from rabbits before treatment with atherogenic diet, choles-
terol was measured in both d<1.060 (HDL) and d<1.060 (VLDL+IDL+LDL) fractions, but separation of VLDL, IDL, and LDL was not performed. Cholesterol concentrations in plasma, lipoprotein fractions, and plasma triglyceride concentrations were determined by enzymatic methods in the Centers for Disease Control and Prevention–standardized Lipid Laboratory of the Wake Forest University School of Medicine, Winston-Salem, NC.

Aortic Sampling

At the end of treatment, rabbits were exsanguinated after being deeply anesthetized with ketamine hydrochloride and xylazine (60 mg and 6 mg/kg body wt, respectively). The heart together with the aorta extending to the iliac bifurcation was removed. The aorta was separated from the heart at the aortic valve. The thoracic and abdominal aorta were separated 2 mm distal to the celiac bifurcation. Each aortic segment was cleaned of adventitial tissue, opened longitudinally, and pinned flat. Aortas were photographed under conditions that maximized discrimination between macroscopically normal and atherosclerotic areas of aorta before and after separating the aortic arch from the descending thoracic aorta at the level of the diaphragm scar. During these procedures, arterial samples were maintained at 4°C whenever possible. Aortic samples were weighed and frozen at −70°C under argon protected from light until analysis.

Aortic Atherosclerosis

Aortic atherosclerosis was determined both as aortic surface areas involved with atherosclerotic lesions and by aortic cholesterol concentrations. Surface areas of entire aortic segments and areas of atherosclerotic lesions were determined by planimetry of photographic enlargements. Lipids were extracted from aortic samples with 2:1 (vol:vol) chloroform:methanol in the presence of the internal standards needed for assay of lipidic antioxidants (below). The resulting extracts were washed with water, Total and nonesterified cholesterol concentrations were determined by aliquots of these lipid extracts by enzymatic methods as described. Each assay included a reference standard (level 2, Solomon Park). Mean intra-assay coefficients of variation for assays of total and nonesterified cholesterol were 4.8% and 3.3%, respectively; corresponding interassay coefficients of variation were 1.5% and 1.4%, respectively. Esterified cholesterol concentrations were calculated as the differences between the measured total and nonesterified cholesterol concentrations.

Standardized aortic total cholesterol concentrations for individual animals were calculated by dividing aortic total cholesterol concentrations (μmol/g) by the aortic exposure to plasma cholesterol concentrations during dietary treatment [mmol/L]×days]. Aortic exposure to plasma cholesterol concentrations during treatment was determined by the area under the curve of the time-varying concentration of cholesterol in plasma. Aortic cholesterol concentrations expressed in this way allow comparison of the antioxidant treatments independent of differences in plasma cholesterol concentrations.

Plasma and Aortic Concentrations of Nutrient Antioxidants and Probucol

Plasma concentrations of α- and γ-tocopherol, selenium, and probucol were measured while rabbits were consuming rabbit chow, every 4 weeks during treatment with atherogenic diet, and at the end of the study. Although dietary retinol (vitamin A) did not differ between groups, we also measured plasma retinol at these times to determine whether the supplementary vitamin E might complete for absorption of vitamin A and thus reduce plasma vitamin A. Blood samples for these purposes were collected into 0.01 vol of a solution of 0.4 mol/L disodium EDTA and 0.4% sodium azide, pH 7.4. Plasma samples for determination of selenium concentrations were frozen at −20°C until analysis by atomic absorption spectroscopy at Ross Laboratory. Plasma samples for analysis of concentrations of α- and γ-tocopherol, probucol, and retinol were overlaid with argon and frozen at −70°C and protected from light until analysis. Lipophilic antioxidants in plasma and aorta were extracted into hexane respectively, in the presence of tocol as an internal standard for vitamin E, MDL 27 272 (an analogue of probucol) for probucol, and retinol palmitate for retinol. Concentrations of α-tocopherol, γ-tocopherol, probucol, and retinol were determined by HPLC with a modification of the method of Elinder and Waldhuis with the mobile phase modified to acetonitrile: tetrahydrofuran:methanol:ammonium acetate (1:5%, 68.4:22.0:6.8:2.8 (vol:vol:vol:vol). These assays were performed in a core laboratory of the Wake Forest University School of Medicine Comprehensive Cancer Center.

Vitamin E is lipophilic, and differences in aortic α-tocopherol and probucol among groups and between aortic regions could reflect differences in aortic lipid. Nonesterified cholesterol and cholesterol ester account for most of the lipid in atherosclerotic aortas and, together with phospholipid, account for almost all of the lipid in atherosclerotic aortas. Also, phospholipid in atherosclerotic aortas is highly correlated with aortic total cholesterol. Therefore, aortic concentrations of α-tocopherol and probucol in individual animals were standardized by aortic total cholesterol as a measure of aortic total lipid. Standardizing concentrations of antioxidants in this way both allows comparison of aortic data for antioxidants among treatments independent of differences in aortic cholesterol concentrations and facilitates comparison of data for plasma and aortic antioxidants.

Statistical Methods

Differences among treatment groups were investigated by ANOVA. After a significant ANOVA, the following comparisons were made to test our original hypothesis: control versus vitamin E, effect of addition of vitamin C (E and ESe groups compared with EC and ESe groups), effect of addition of selenium (E and EC groups compared with ESe and ECSe groups), and control versus probucol. We also investigated whether vitamin C or selenium had different effects in the presence and the absence of the other nutrient (interaction effect); none was found for any of the parameters measured (P>0.2). Additional comparisons motivated by the data were made with the use of the Scheffé criteria. A value of P<0.05 was considered significant.

Results

Plasma Lipids, Nutrients, and Antioxidants Before Atherogenic Diet

Plasma cholesterol and triglyceride concentrations before feeding the atherogenic diet (Table 2) were low and did not differ among treatment groups. Plasma concentrations of selenium, α-tocopherol, and retinol did not differ among groups. Neither γ-tocopherol nor probucol was detected in the plasma of any rabbit before treatment with the atherogenic diet.

Plasma Lipids, Nutrients, and Antioxidants During Treatment With Atherogenic Diet

The atherogenic diet substantially increased plasma cholesterol and α- and γ-tocopherol concentrations (Tables 2 and 3). Dietary supplementation with vitamin E reduced plasma cholesterol concentrations 17%, increased plasma α-tocopherol concentrations 7.3-fold, and reduced plasma γ-tocopherol concentrations 95%. Adding vitamin C to treatment with vitamin E or the treatment with vitamin E plus selenium had no effect on concentrations of any plasma constituent measured. Adding selenium to a diet supplemented with vitamin E or with vitamins E and C increased plasma concentrations of selenium 18% but had no influence on any other plasma constituent measured. Dietary supplementation with probucol reduced plasma cholesterol concentrations 40% and plasma γ-tocopherol 49%. None of the dietary supplements influenced plasma triglyceride or retinol concentrations.
TABLE 3. Mean Plasma Lipids and Antioxidants During Treatment

<table>
<thead>
<tr>
<th>Diet Group</th>
<th>n</th>
<th>Cholesterol, mmol/L</th>
<th>Triglyceride, mmol/L</th>
<th>Selenium, µmol/L</th>
<th>α-Tocopherol, µmol/L</th>
<th>γ-Tocopherol, µmol/L</th>
<th>Probucol, µmol/L</th>
<th>Retinol, µmol/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>12</td>
<td>1.65±0.26</td>
<td>0.26±0.03</td>
<td>1.95±0.09</td>
<td>3.39±0.49</td>
<td>2.90±0.25</td>
<td></td>
<td></td>
</tr>
<tr>
<td>E</td>
<td>11</td>
<td>1.76±0.18</td>
<td>0.28±0.02</td>
<td>2.01±0.08</td>
<td>6.17±2.35</td>
<td>3.36±0.45</td>
<td></td>
<td></td>
</tr>
<tr>
<td>EC</td>
<td>12</td>
<td>1.40±0.13</td>
<td>0.27±0.02</td>
<td>2.05±0.06</td>
<td>3.81±0.64</td>
<td>3.02±0.29</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ESe</td>
<td>11</td>
<td>1.34±0.13</td>
<td>0.31±0.06</td>
<td>2.01±0.06</td>
<td>3.23±0.52</td>
<td>2.95±0.29</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ECSe</td>
<td>12</td>
<td>1.42±0.13</td>
<td>0.35±0.04</td>
<td>2.04±0.08</td>
<td>3.68±0.50</td>
<td>3.14±0.28</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Prob</td>
<td>12</td>
<td>1.37±0.16</td>
<td>0.27±0.04</td>
<td>2.03±0.06</td>
<td>2.58±0.34</td>
<td>3.20±0.57</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

γ-Tocopherol and probucol were not detected in any plasma samples. Values are mean±SEM for the indicated number of animals (n). P values for ANOVA among groups were >0.2 for all plasma constituents.

Lipoproteins
About half of the cholesterol in plasma of rabbits fed a cholesterol-free diet was in the HDL fraction (Figure, top). After 12 and 22 weeks of treatment with the atherogenic diet, about half of the cholesterol was present in the LDL fraction, with relatively little in the VLDL, IDL, and HDL fractions (Figure, middle and bottom). This is in marked contrast to the distribution of cholesterol among lipoproteins that is found in rabbits fed chow supplemented with cholesterol.43 There was no difference among groups for distribution of cholesterol among lipoprotein fractions.

Areas of Atherosclerotic Lesions
ANOVA indicated highly significant differences among groups for areas of atherosclerotic lesions in the aortic arch, descending thoracic aorta, abdominal aorta, and total aorta (Table 4). When added to treatment with vitamin E or vitamin E plus selenium, vitamin C had no influence on areas of atherosclerotic lesions. Rank order for areas of atherosclerotic lesions for all aortic regions were as follows: control>vitamin E (with or without vitamin C)>vitamin E plus selenium (with or without vitamin C)>. Probucol treatment reduced atherosclerotic lesion areas 61%, 92%, 84%, and 81% for the aortic arch, descending thoracic aorta, abdominal aorta, and total aorta, respectively. Areas of atherosclerotic lesions for animals treated with vitamin E plus selenium tended to be reduced compared with lesion areas for animals treated with vitamins E and C plus selenium. Post hoc testing indicated no difference between areas of atherosclerotic lesions for rabbits treated with probucol or with vitamin E plus selenium.

Aortic Cholesterol Concentrations
ANOVA revealed significant differences in total, nonesterified, and esterified cholesterol concentrations among treatment groups for descending thoracic aorta, abdominal aorta, and total aorta (Table 5). For the aortic arch, differences

TABLE 3. Mean Plasma Lipids and Antioxidants During Treatment

<table>
<thead>
<tr>
<th>Diet Group</th>
<th>Cholesterol, mmol/L</th>
<th>Triglyceride, mmol/L</th>
<th>Selenium, µmol/L</th>
<th>α-Tocopherol, µmol/L</th>
<th>γ-Tocopherol, µmol/L</th>
<th>Probucol, µmol/L</th>
<th>Retinol, µmol/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>19.7±0.8</td>
<td>0.73±0.11</td>
<td>1.83±0.06</td>
<td>26±2</td>
<td>0.39±0.08</td>
<td>ND</td>
<td>2.70±0.14</td>
</tr>
<tr>
<td>E (11)</td>
<td>16.3±1.5</td>
<td>0.72±0.09</td>
<td>1.88±0.04</td>
<td>216±22</td>
<td>0.02±0.02</td>
<td>ND</td>
<td>3.16±0.12</td>
</tr>
<tr>
<td>EC (12)</td>
<td>17.4±1.0</td>
<td>0.71±0.07</td>
<td>1.85±0.05</td>
<td>237±20*</td>
<td>0.05±0.02</td>
<td>ND</td>
<td>2.97±0.15</td>
</tr>
<tr>
<td>ESe (11)</td>
<td>15.8±1.1</td>
<td>0.56±0.05</td>
<td>2.17±0.05*</td>
<td>208±26*</td>
<td>0.05±0.04</td>
<td>ND</td>
<td>2.73±0.12</td>
</tr>
<tr>
<td>ECSe (12)</td>
<td>15.1±1.5</td>
<td>0.60±0.06</td>
<td>2.22±0.04*</td>
<td>191±16*</td>
<td>0.002±0.002</td>
<td>ND</td>
<td>3.09±0.16</td>
</tr>
<tr>
<td>Prob (12)</td>
<td>11.9±1.0</td>
<td>0.78±0.10</td>
<td>1.88±0.05</td>
<td>24±2</td>
<td>0.20±0.06</td>
<td>105±14</td>
<td>3.00±0.17</td>
</tr>
</tbody>
</table>

P value (ANOVA) 0.0007 0.43 0.0000 0.0000† 0.0000 0.0000 0.16
P values

Control vs E 0.05 ... 0.46 0.0000 0.0000 1.0 ...
Effect of Selenium 0.23 ... 0.0000 0.20 0.82 1.0 ...
Control vs Prob 0.0000 ... 0.48 0.53 0.005 0.0000 ...
ECSe vs Prob 0.06 ... 0.0000 0.0000 0.0028 0.0000 ...

ND indicates not detected. Mean values during treatment were calculated from mean values for individual animals during treatment. Mean values for individual animals during treatment were determined from the area under the corresponding plasma curves derived from measurements made before treatment and every 2 weeks after beginning treatment (cholesterol) or before treatment and after 4, 8, 12, 16, 20, and 22 weeks of treatment (all other values). Values are mean±SEM for the indicated number of animals (in parentheses). There was no interaction between vitamin C and selenium treatment for any plasma constituent (P=0.4). There was no effect of vitamin C (comparison of E and ESe groups with EC and ECSe groups) on any plasma constituent (P>0.7).

†P<0.005 compared with control (t test with Scheffé adjustment).
‡Data transformed to logarithms.
E and EC groups compared with ESe and ECSe groups.
among groups were significant for total and nonesterified cholesterol, with a trend for esterified cholesterol. When added to treatment with vitamin E or vitamin E plus selenium, vitamin C had no influence on aortic cholesterol concentrations. Rank order for aortic concentrations of total, nonesterified, and esterified cholesterol were control, vitamin E (with or without vitamin C), vitamin E plus selenium (with or without vitamin C), probucol. Compared with treatment with vitamin E (with or without vitamin C), treatment with vitamin E plus selenium (with or without vitamin C) significantly reduced total, esterified, and nonesterified cholesterol concentrations for the descending thoracic aorta, consistent with results for areas of atherosclerotic lesions. Compared with treatment with vitamin E (with or without vitamin C), treatment with vitamin E plus selenium (with or without vitamin C) also resulted in significant or borderline significant reductions in nonesterified cholesterol in the abdominal aorta, aortic arch, and total aorta and in total cholesterol in the total aorta. Probucol reduced aortic total cholesterol 56%, 86%, 75%, and 71% for the aortic arch, descending thoracic aorta, abdominal aorta, and total aorta, respectively. Reductions in esterified and nonesterified cholesterol were similar. As for areas of atherosclerotic lesions, probucol reduced aortic total, esterified, and nonesterified cholesterol concentrations relatively more than did combined treatment with vitamins E and C plus selenium. Aortic cholesterol concentrations for animals treated with vitamin E plus selenium tended to be reduced compared with animals treated with vitamins E and C plus selenium. Probucol reduced aortic cholesterol concentrations for the descending thoracic aorta, consistent with results for areas of atherosclerotic lesions. Compared with treatment with vitamin E (with or without vitamin C), treatment with vitamin E plus selenium (with or without vitamin C) also resulted in significant or borderline significant reductions in nonesterified cholesterol in the abdominal aorta, aortic arch, and total aorta and in total cholesterol in the total aorta. Probucol reduced aortic total cholesterol 56%, 86%, 75%, and 71% for the aortic arch, descending thoracic aorta, abdominal aorta, and total aorta, respectively. Reductions in esterified and nonesterified cholesterol were similar. As for areas of atherosclerotic lesions, probucol reduced aortic total, esterified, and nonesterified cholesterol concentrations relatively more than did combined treatment with vitamins E and C plus selenium. Aortic cholesterol concentrations for animals treated with vitamin E plus selenium tended to be reduced compared with animals treated with vitamins E and C plus selenium. Post hoc testing indicated no difference between rabbits treated with probucol or with vitamin E plus selenium for aortic total, esterified, or nonesterified cholesterol concentrations. Among the treatments, only probucol reduced the percentage of aortic cholesterol esterified, and this was observed only for the descending thoracic aorta (not shown).

Aortic Cholesterol Concentrations Standardized by Mean Plasma Cholesterol Concentration During Treatment

ANOVA indicated significant differences among treatment groups for aortic total cholesterol concentrations standardized by aortic exposure to plasma cholesterol concentrations during treatment for descending thoracic and abdominal aortas. Rank order for standardized aortic total cholesterol concentrations (Table 6) was generally similar to that for aortic total cholesterol concentrations (Table 5), but differences among treatment groups were reduced. Standardized aortic total cholesterol accumulation was inhibited significantly by probucol for descending thoracic and abdominal aortas. The addition of selenium to vitamin E (with or without vitamin C) resulted in increased inhibition of standardized total cholesterol for the descending thoracic aorta. Importantly, when adjusted for differences in plasma cholesterol concentrations in this way, inhibition of aortic total cholesterol by probucol, vitamin E plus selenium, and vitamins E and C plus selenium was equivalent. Qualitatively similar results were obtained for comparisons among groups for both aortic nonesterified and esterified cholesterol standardized by plasma cholesterol concentrations (not shown).

Aortic Concentrations of \( \alpha \)-Tocopherol and Probucol

ANOVA indicated a significant difference among groups for aortic concentrations of \( \alpha \)-tocopherol and probucol (Table 7). Aortic concentrations of \( \alpha \)-tocopherol were elevated in all groups treated with vitamin E. The addition of vitamin C to treatment with vitamin E or vitamin E plus selenium had no influence on aortic concentrations of \( \alpha \)-tocopherol. The addition of selenium to treatment with vitamin E or vitamins E and C reduced concentrations of \( \alpha \)-tocopherol for the descending thoracic aorta. However, aortic \( \alpha \)-tocopherol standardized by aortic cholesterol was not influenced by
selenium (Table 8). Probucol treatment did not influence aortic concentrations of α-tocopherol.

Concentrations of α-tocopherol were higher for the aortic arch than for the abdominal aorta (Table 7). Aortic α-tocopherol standardized by aortic cholesterol was also increased in the aortic arch compared with the abdominal aorta for rabbits not given supplementary α-tocopherol (Table 8). Supplementation with α-tocopherol increased standardized aortic α-tocopherol relatively more for the abdominal aorta than for the aortic arch, so that differences between these aortic regions were eliminated. In rabbits fed diets not supplemented with α-tocopherol, aortic α-tocopherol standardized by aortic cholesterol was less than or equal to plasma α-tocopherol standardized by plasma cholesterol. In contrast, for rabbits fed diets supplemented with α-tocopherol, standardized aortic α-tocopherol was almost twice as high as standardized plasma α-tocopherol.

**Discussion**

The present study had several goals: (1) to determine whether the lipophilic antioxidant vitamin E inhibits atherosclerosis in rabbits fed a diet that promotes elevation of plasma LDL, (2) to consider whether atherosclerosis would be further inhibited if additional antioxidant nutrients that act by different mechanisms and/or cellular locations were combined with vitamin E, (3) to consider whether the complete nutrient antioxidant combination (vitamin E, vitamin C, and selenium) would inhibit atherosclerosis as effectively as the potent antioxidant drug probucol, and (4) to consider whether regional variation in inhibition of atherosclerosis by antioxidants was associated with regional variation in aortic concentrations of vitamin E.

The principal findings of the present study were as follows: Addition of selenium to a diet supplemented with vitamin E alone or vitamins E and C further reduced areas of atherosclerotic lesions and aortic concentrations of total, esterified, and nonesterified cholesterol for the descending thoracic aorta, the aortic region that was most influenced by antioxidant treatment. Nonesterified cholesterol was also significantly reduced for the aortic arch, abdominal aorta, and total aorta. There were similar trends of borderline significance for total cholesterol concentrations and areas of atherosclerotic lesions for the total aorta. In contrast, addition of vitamin C to treatment with vitamin E (with or without selenium) did not influence any measure of aortic atherosclerosis in any aortic region. Probucol treatment reduced several measures of atherosclerosis relatively more than did combined treatment with vitamins E and C plus selenium. When aortic cholesterol concentrations were adjusted for differences in plasma cholesterol concentrations during treatment, inhibition of atherosclerosis by probucol remained significant, as was the further inhibition of atherosclerosis by selenium added to vitamin E (with or without vitamin C). Importantly, inhibition of atherosclerosis by probucol, vitamin E plus selenium, and vitamins E and C plus selenium was equivalent when adjusted for differences in plasma cholesterol concentrations during treatment. The antioxidant interventions were more effective in the abdominal aorta (an aortic region where α-tocopherol concentrations were lower and were increased more by α-tocopherol supplementation) than in the aortic arch. Finally, these antioxidant treatments effectively reduced atherosclerosis in rabbits in which the distribution of cholesterol among lipoprotein fractions was very similar to that in humans.53

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**Table 4. Areas of Atherosclerotic Lesions (Percent of Aortic Surface)**

<table>
<thead>
<tr>
<th>Aortic Region</th>
<th>Control (12)</th>
<th>E (11)</th>
<th>EC (12)</th>
<th>ESe (11)</th>
<th>ECSe (12)</th>
<th>Prob (12)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aortic Arch</td>
<td>66.8±8.3</td>
<td>46.3±8.3</td>
<td>40.3±7.9</td>
<td>30.9±5.4</td>
<td>35.6±8.8</td>
<td>25.4±7.8</td>
</tr>
<tr>
<td>Thoracic Aorta</td>
<td>37.3±8.6</td>
<td>25.4±9.3</td>
<td>21.4±6.1</td>
<td>6.6±2.0</td>
<td>11.9±4.5</td>
<td>3.0±2.0</td>
</tr>
<tr>
<td>Abdominal Aorta</td>
<td>37.1±7.1</td>
<td>21.9±6.4</td>
<td>24.9±6.8</td>
<td>14.3±3.6</td>
<td>16.7±4.8</td>
<td>6.1±1.8</td>
</tr>
<tr>
<td>Total Aorta</td>
<td>43.4±7.4</td>
<td>28.3±7.2</td>
<td>26.7±6.2</td>
<td>14.1±2.8</td>
<td>18.3±5.3</td>
<td>8.2±2.6</td>
</tr>
</tbody>
</table>

P value (ANOVA)* 0.0067 0.0001 0.0005 0.0003

P values

- Control vs E 0.38
- Effect of Selenium† 0.12
- Control vs Prob 0.003
- ESe vs Prob 0.25

Values are mean ± SEM for the indicated number of animals (in parentheses). There was no interaction between vitamin C and selenium treatment for any aortic region (P>0.7). There was no effect of vitamin C (comparison of E and ESe groups with EC and ECSe groups) for areas of atherosclerotic lesions in any aortic region (P>0.4). Post hoc analysis indicated no difference between probucol group and ESe group (P>0.23). ANOVA, including data for all aortic regions transformed to logarithms, indicated significant differences in areas of atherosclerotic lesions among aortic regions for each diet group individually (all P<0.001), with most of this difference due to greater values for aortic arch (P<0.003).

*Data transformed to logarithms.
†E and EC groups compared with ESe and ECSe groups.
| Diet Group | Aortic Region | | | | | \hline
| | Total cholesterol, \(\mu\text{mol/g}\) | Aortic Arch | Thoracic Aorta | Abdominal Aorta | Total Aorta | \hline
| Control (12) | 70.7±11.4 | 41.2±12.3 | 35.5±7.6 | 49.9±10.4 | \hline
| E (11) | 47.6±9.4 | 29.4±12.1 | 22.3±5.1 | 33.6±9.4 | \hline
| EC (12) | 56.0±8.9 | 26.2±6.5 | 28.8±5.8 | 37.2±6.9 | \hline
| ESe (11) | 34.2±6.7 | 8.7±1.3 | 15.0±2.4 | 18.3±3.0 | \hline
| ECSe (12) | 47.8±11.3 | 15.4±5.0 | 19.1±4.1 | 27.2±6.8 | \hline
| Prob (12) | 31.0±8.5 | 5.8±1.6 | 8.7±1.7 | 14.7±3.6 | \hline
| P value (ANOVA)* | 0.022 | 0.0003 | 0.0002 | 0.0021 | \hline
| P values | | | | | \hline
| Control vs E | 0.17 | 0.15 | 0.095 | 0.14 | \hline
| Effect of Se† | 0.15 | 0.029 | 0.092 | 0.063 | \hline
| Control vs Prob | 0.0011 | 0.0000 | 0.0000 | 0.0001 | \hline
| ECSe vs Prob | 0.17 | 0.067 | 0.015 | 0.098 | \hline
| Esterified cholesterol, \(\mu\text{mol/g}\) | | | | | \hline
| Control (12) | 43.9±9.1 | 23.8±9.1 | 18.1±4.8 | 29.3±7.8 | \hline
| E (11) | 25.0±5.2 | 14.2±6.8 | 9.5±2.6 | 16.6±5.2 | \hline
| EC (12) | 32.6±5.6 | 13.9±4.1 | 12.5±2.7 | 19.9±4.2 | \hline
| ESe (11) | 18.9±4.8 | 3.3±0.8 | 6.2±1.5 | 8.9±2.0 | \hline
| ECSe (12) | 28.9±8.0 | 7.6±3.2 | 8.8±2.4 | 15.1±4.6 | \hline
| Prob (12) | 20.2±6.4 | 2.1±1.2 | 4.0±1.2 | 8.4±2.7 | \hline
| P value (ANOVA)* | 0.068 | 0.0014 | 0.0036 | 0.013 | \hline
| P values | | | | | \hline
| Control vs E | ... | 0.12 | 0.063 | 0.10 | \hline
| Effect of Se† | ... | 0.043 | 0.17 | 0.11 | \hline
| Control vs Prob | ... | 0.0001 | 0.0000 | 0.0007 | \hline
| ECSe vs Prob | ... | 0.13 | 0.075 | 0.22 | \hline
| Nonesterified cholesterol, \(\mu\text{mol/g}\) | | | | | \hline
| Control (12) | 27.8±2.7 | 17.4±3.5 | 17.4±2.9 | 20.6±2.9 | \hline
| E (11) | 22.6±4.4 | 15.2±5.4 | 12.8±2.4 | 17.0±4.3 | \hline
| EC (12) | 23.5±3.1 | 12.4±2.5 | 16.3±3.1 | 17.2±2.7 | \hline
| ESe (11) | 15.3±2.1 | 5.4±0.6 | 8.7±0.9 | 9.4±1.0 | \hline
| ECSe (12) | 18.9±3.6 | 7.9±1.8 | 10.3±1.7 | 12.1±2.3 | \hline
| Prob (12) | 10.7±2.2 | 3.7±0.4 | 4.7±0.4 | 6.3±0.9 | \hline
| P value (ANOVA)* | 0.0014 | 0.0001 | 0.0000 | 0.0001 | \hline
| P values | | | | | \hline
| Control vs E | 0.27 | 0.21 | 0.15 | 0.21 | \hline
| Effect of Se† | 0.069 | 0.010 | 0.030 | 0.017 | \hline
| Control vs Prob | 0.0001 | 0.0000 | 0.0000 | 0.0000 | \hline
| ECSe vs Prob | 0.038 | 0.052 | 0.0028 | 0.023 | \hline

Values are mean±SEM for the indicated number of animals (in parentheses). There was no interaction between vitamin C and selenium treatment for any aortic region (P>0.6). There was no effect of vitamin C (comparison of E and ESe groups with EC and ECSe groups) for any form of cholesterol in any aortic region (P>0.4). Post hoc analysis indicated no difference between probucol group and ESe group (P>0.2). ANOVA, including data for all aortic regions transformed to logarithms, indicated significant differences in aortic cholesterol (total, nonesterified, and esterified) concentrations among aortic regions for each diet group individually (all P<0.01), with most of the difference due to greater values for the aortic arch (P<0.01).

*Data transformed to logarithms.
†E and EC groups compared with ESe and ECSe groups.
Regional Variation in Aortic Antioxidants and Atherosclerosis

In rabbits, the increased susceptibility of the aortic arch to atherosclerosis compared with the abdominal aorta is well established.1,6,19,20,54–56 The only report that provides comparative data for antioxidants in aortic arches and abdominal aortas of rabbits is for probucol and a structurally related antioxidant.6 That study showed concentrations of probucol to be increased for atherosclerotic lesions in the aortic arch compared with lesions in the abdominal aorta.6 Consistent

<table>
<thead>
<tr>
<th>TABLE 6. Aortic Total Cholesterol Concentrations Standardized by Area Under the Plasma Cholesterol Concentration Curve During Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Diet Group</strong></td>
</tr>
<tr>
<td>Control (12)</td>
</tr>
<tr>
<td>E (11)</td>
</tr>
<tr>
<td>EC (12)</td>
</tr>
<tr>
<td>ESe (11)</td>
</tr>
<tr>
<td>ECSe (12)</td>
</tr>
<tr>
<td>Prob (12)</td>
</tr>
</tbody>
</table>

| P values (ANOVA)* | 0.38 | 0.013 | 0.037 | 0.072 |

Aortic cholesterol concentrations standardized by the area under the plasma cholesterol concentration curve during treatment of individual rabbits are presented in μL plasma/g per day. Mean values for areas under the plasma cholesterol concentration curve during treatment in (mmol/L) × days, were as follows: control 3062 ± 144; E, 2506 ± 226; EC, 2690 ± 154; ESe, 2386 ± 194; ECSe, 2309 ± 232; and Prob, 1727 ± 189. Values are mean ± SEM for the indicated number of animals (in parentheses). There was no interaction between vitamin C and selenium treatment for any aortic region (P > 0.5). There was no effect of vitamin C (comparison of E and ESe groups with EC and ECSe group) for any form of cholesterol in any aortic region (P > 0.4). Post hoc analysis indicated no difference between probucol group and ESe group (P > 0.25).

*Data transformed to logarithms.
†E and EC groups compared with ESe and ECSe groups.

<table>
<thead>
<tr>
<th>TABLE 7. Aortic α-Tocopherol and Probucol Concentrations</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Diet Group</strong></td>
</tr>
<tr>
<td>Control (12)</td>
</tr>
<tr>
<td>E (11)</td>
</tr>
<tr>
<td>EC (12)</td>
</tr>
<tr>
<td>ESe (11)</td>
</tr>
<tr>
<td>ECSe (12)</td>
</tr>
<tr>
<td>Prob (12)</td>
</tr>
</tbody>
</table>

| P value (ANOVA)‡ | 0.0000 | 0.0000 | 0.0000 | 0.0000 |

<table>
<thead>
<tr>
<th>P values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control vs E</td>
</tr>
<tr>
<td>Effect of Se§</td>
</tr>
<tr>
<td>Control vs Prob</td>
</tr>
<tr>
<td>ECSe vs Prob</td>
</tr>
</tbody>
</table>

ND indicates not detected. Values are mean ± SEM (in nmol/g aorta) for the indicated number of animals (in parentheses). There was no interaction between vitamin C and selenium treatment for either aortic region (P > 0.27). There was no effect of vitamin C (comparison of E and ESe groups with EC and ECSe groups) on α-tocopherol in either aortic region (P > 0.33). P < 0.005 compared with corresponding value for aortic arch. †P < 0.005 compared with control (t test with Scheffé adjustment). ‡Data for α-tocopherol transformed to logarithms. §E and EC groups compared with ESe and ECSe groups.
Interestingly, more for the abdominal aorta than for the aortic arch. The molar ratio of vitamin E to total arterial polyunsaturated fatty acid is probably about twice the molar ratio of vitamin E to polyunsaturated fatty acid hydroperoxide production. Given the composition of atherosclerotic arteries, the molar ratio of vitamin E to total arterial polyunsaturated fatty acid is probably about twice the molar ratio of vitamin E to total arterial cholesterol. In the present study, the molar ratio of α-tocopherol to cholesterol for the aortic arch of control rabbits was 1.2:1000, whereas it was only half this value for the abdominal aorta (Table 8). In comparison, these ratios were 19:1000 or greater for both aortic regions of rabbits supplemented with vitamin E. Therefore, it seems likely that aortic vitamin E concentrations are sufficient to prevent significant oxidation for both aortic regions of rabbits supplemented with vitamin E.

### Inhibition of Atherosclerosis by Vitamin E

Two studies in rabbits reported significant inhibition of atherosclerosis by vitamin E, whereas a relatively larger number of studies reported vitamin E to have no effect, whereas another reported inhibition of atherosclerosis by 74%. In comparison, our results showed a tendency for vitamin E to reduce areas of atherosclerotic lesions (41%,  and total (37%, ) and esterified (48%, ) cholesterol in the abdominal aorta.

### Inhibition of Atherosclerosis by Probucol

Many studies have demonstrated inhibition of atherosclerosis by probucol. In most studies, plasma cholesterol concentrations also tended to be reduced, although not always significantly. No previous report has described inhibition of aortic cholesterol accumulation by probucol after adjustment of arterial data for differences in plasma cholesterol concentrations. However, 2 studies in rabbits fed cholesterol attempted to compensate for the hypocholesterolemic effect of probucol by adjusting dietary cholesterol. One of those
studies found probucol to have no effect on aortic cholesterol. However, the other study reported probucol treatment to reduce total cholesterol in the aortic arch 49%. In comparison, we found aortic total cholesterol adjusted for differences in plasma cholesterol concentrations to be reduced 32% in aortic arch and to be reduced 55% to 77% for other aortic regions.

**Combined Influence of Vitamin C and Vitamin E on Atherosclerosis**

No previous study has reported on the inhibition of atherosclerosis in rabbits by vitamin E compared with the combination of vitamins E and C. On the basis of early reports, we anticipated vitamin C to reduce atherosclerosis. We expected to observe better inhibition of atherosclerosis by vitamin C combined with vitamin E than with vitamin E alone. However, vitamin C did not further influence either aortic cholesterol or areas of atherosclerotic lesion when combined with vitamin E (with or without selenium). Furthermore, vitamins E and C combined did not significantly inhibit atherosclerosis compared with the control. In comparison, one study reported vitamins E and C combined to inhibit atherosclerosis in rabbits, whereas another study in rabbits observed a small but not significant inhibition of atherosclerosis by vitamins E and C combined. A recent study provided qualitative data for inhibition of atherosclerosis by vitamin E and C combined.

**Combined Influence of Vitamin E and Selenium on Atherosclerosis**

An earlier study reported that areas of atherosclerotic lesions in the total aorta were reduced 49% by selenium and 63% by vitamin E combined with selenium and were slightly (25%) but not significantly reduced by vitamin E. Our results (68% and 35% reduction of areas of atherosclerotic lesions for total aorta by vitamin E and selenium combined and vitamin E alone, respectively, and significantly greater inhibition of atherosclerosis when selenium was added to vitamin E [with or without vitamin C]; Table 4) are consistent with those results. The present study extends previous observations by providing data for the combined influence of vitamin E and selenium on aortic cholesterol concentrations and by demonstrating that the additional inhibitory action of selenium on aortic cholesterol accumulation was in part independent of differences in plasma cholesterol concentrations (Table 6).

**Model for Interactive Inhibition of Atherosclerosis by Antioxidants**

The lipophilic antioxidant vitamin E is thought to be the major chain-breaking antioxidant in cellular membranes and lipoproteins. In addition to inhibiting oxidation and diminishing cellular response to oxidized LDL, vitamin E has other effects that could influence atherosclerosis. In vitro, vitamin E blocked the stimulation of smooth muscle cell proliferation induced by platelet-derived growth factor and serum. This effect was observed at 50 μmol/L α-tocopherol, about one quarter of the plasma α-tocopherol concentrations for vitamin E supplemented rabbits in the present study (Table 3). Together with data in Table 8, these data might suggest that aortic concentrations of α-tocopherol may have been sufficient to inhibit smooth muscle proliferation, possibly contributing to the inhibition of atherosclerosis by vitamin E in other studies and the tendency in that direction in the present study.

In vitro studies have shown that vitamin C will regenerate vitamin E or otherwise preserve vitamin E levels. In the present study, plasma concentrations of vitamin E were not altered by vitamin C. However, vitamin E was provided in excess to supplemented rabbits, possibly masking any regeneration of vitamin E by vitamin C. Alternatively, it is possible that rabbits, which are a species that does not require vitamin C, have sufficient vitamin C for maximal regeneration of vitamin E even without supplementation. Other work showed that vitamin E and vitamin C combined (each at 10 μmol/L) inhibited apoptosis mediated by lipopolysaccharide, whereas either of these vitamins alone was less effective. If vitamin E and C combined were to inhibit apoptosis in arteries in vivo, the net effect could be greater cellular accumulation and enhanced atherosclerosis compared with vitamin E alone.

Data suggest that selenium-dependent peroxidases, including phospholipid hydroperoxide glutathione peroxidase, could prevent the decomposition of phospholipid and cholesterol ester hydroperoxides to damaging free radicals thus reducing oxidation. Selenium may also contribute to increased antioxidant defense as a cofactor of glutathione peroxidase. Thus, these selenium-dependent peroxidases could be viewed to serve as backup antioxidant activities when vitamin E levels are not sufficient to prevent lipid peroxidation. However, as discussed above, it is probable that aortic levels of α-tocopherol in α-tocopherol–supplemented rabbits were adequate to prevent most, if not all, lipid peroxidation. Nonetheless, in a subset of these rabbits, we found aortic concentrations of some phospholipids, oxidized phospholipids, and lysophospholipids to be highly correlated with aortic atherosclerosis (N. Leitinger, D.C. Schwenke, A.D. Watson, G. Subbanagounder, K.F. Faull, A.M. Fogelman, J.A. Berliner, unpublished data, 1998), suggesting the possibility that reduced lipid peroxidation may play a role in the enhanced inhibition of atherosclerosis by vitamin E plus selenium compared with vitamin E alone. However, selenium has other effects, including effects on phospholipase A2 activity, prostacyclin release and production of platelet-activating factor by endothelial cells, and lymphocyte proliferation. Selenium is also a cofactor for thyroxine 5'-deiodinase, an enzyme involved in growth hormone expression. Further work will be needed to elucidate the mechanism(s) by which selenium enhances the inhibition of atherosclerosis by vitamin E.

In summary, we found the combination of vitamin E and selenium (with or without vitamin C) to inhibit atherosclerosis in hypercholesterolemic rabbits more effectively than vitamin E alone and equally as well as probucol. Importantly, the benefit conferred by selenium was independent of effects on plasma lipids and lipoproteins. The mechanism(s) accounting for the protective effect of selenium remains to be determined.
Acknowledgments

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References

1. Carew TE, Schwenke DC, Steinberg D. Antiatherogenic effect of probucol unrelated to its hypocholesterolemic effect: evidence that antioxidant in vivo can selectively inhibit low density lipoprotein degradation in macrophage-rich fatty streaks and slow the progression of atherosclerosis in the Watanabe heritable hyperlipidemic rabbit. Proc Natl Acad Sci U S A. 1987;84:7725–7729.


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Vitamin E Combined With Selenium Inhibits Atherosclerosis in Hypercholesterolemic Rabbits Independently of Effects on Plasma Cholesterol Concentrations
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