Dietary Antioxidants Inhibit Development of Fatty Streak Lesions in the LDL Receptor–Deficient Mouse

Richard S. Crawford, Elizabeth A. Kirk, Michael E. Rosenfeld, Renée C. LeBoeuf, Alan Chait

Abstract—Oxidized low density lipoprotein (LDL) promotes atherogenesis. Although pharmacological antioxidants such as probucol inhibit both LDL oxidation and atherosclerosis in hyperlipidemic animals, the effects of natural antioxidants such as vitamin E are inconclusive. To further determine the effects of supplemental dietary antioxidants in vivo, we evaluated whether combined dietary antioxidants (0.1% vitamin E, 0.5% β-carotene, and 0.05% vitamin C) inhibit LDL oxidation and fatty streak lesion development in homozygous LDL receptor–null (LDLR−/−) mice fed a high-fat, high-cholesterol diet. An additional group of mice were fed black tea, which has been shown to inhibit LDL oxidation in vitro. After receiving a high-fat, high-cholesterol diet for 8 weeks, the combined antioxidant–supplemented (antioxidant) group (n=18), tea group (n=19), and control group (n=17) had equivalent plasma cholesterol levels. LDL oxidation, as measured by the lag phase of conjugated diene formation, was markedly inhibited in the antioxidant group compared with the tea or control groups [mean lag phases=143±7 (antioxidant), 100±5 (tea), and 84±4 (control) minutes; P<0.0001 antioxidant versus tea or control]. The cross-sectional surface area of fatty streak lesions in the aortic sinus was reduced by 60% in the antioxidant group compared with both the tea and control groups (P<0.0001 antioxidant versus tea or control). There was no difference in lesion area between tea and control groups. Although both LDL oxidation and atherosclerosis were significantly inhibited in the antioxidant group, no correlation between lag phase values and lesion size was observed among individual animals. Furthermore, black tea did not inhibit fatty streak development in LDLR−/− mice. These data suggest that combined natural dietary antioxidants inhibit both LDL oxidation and atherosclerosis in animals with elevated LDL but that inhibition of LDL oxidation alone may not prevent the development of atherosclerosis. (Arterioscler Thromb Vasc Biol. 1998;18:1506-1513.)

Key Words: atherosclerosis ■ antioxidants ■ LDL ■ black tea

Oxidized LDL has been implicated in the development of atherosclerosis.1 Multiple biological properties of oxidized LDL may promote the development of atherosclerotic lesions, including stimulation of monocyte adhesion,2 enhanced cytotoxicity,3 uptake of oxidized LDL by macrophage scavenger receptors leading to the formation of foam cells,4,5 and altered expression of cytokines and growth factors.6–8 Conversely, the development of atherosclerotic lesions in hyperlipidemic rabbits and monkeys can be reduced by antioxidants such as probucol.9,10 Other antioxidants such as BHT11 and N,N'-diphenyl-1,4-phenylenediamine have similar effects in hypercholesterolemic rabbits and apo E–deficient mice.12 However, studies investigating the effects of natural dietary antioxidants such as vitamin E, β-carotene, and vitamin C on the susceptibility of LDL to oxidation and on atherosclerosis have been inconclusive. Studies have shown that vitamin E protects LDL against oxidation.13,14 While some studies demonstrate that vitamin E retards atherosclerotic lesion development in hyperlipidemic animals,15,16 other studies find no antiatherogenic benefit of vitamin E.17,18 Conversely, β-carotene has not been found to inhibit LDL oxidation,13 yet its administration resulted in retardation of atherosclerotic lesion development in 1 study.19 Recent research has focused on flavonoids, a separate group of dietary antioxidants. In a cross-sectional epidemiological study, an inverse relationship between coronary mortality and dietary flavonoid intake was observed in elderly men.20 The most prominent source of flavonoids in this population was black tea. Furthermore, Miura et al21 demonstrated that tea flavonoids inhibit LDL oxidation in vitro. Studies examining the effect of tea consumption on LDL oxidation ex vivo are inconsistent. One recent study showed that tea consumption was associated with a reduction of LDL oxidation ex vivo in humans,22 while another study failed to show any reduction of LDL oxidation ex vivo.23 In a study in rabbits, black tea consumption was associated with a decrease in the susceptibility of LDL toward oxidation but was without effect on atherosclerotic lesion development.24 However, other previous studies using indirect methods of lipoprotein oxidation have demonstrated a correlation between the extent of atherosclerosis and the susceptibility of LDL to oxidation.25–26 Therefore, this study was undertaken to determine whether consumption of a combination of supplemental natural dietary antioxidants (vitamin E, β-carotene, and vitamin C) or black tea could reduce the susceptibility of
TABLE 1. Composition of Experimental Diets*

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Control and Tea Diets</th>
<th>Antioxidant Diet</th>
</tr>
</thead>
<tbody>
<tr>
<td>g/kg Diet</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Purina Mouse Chow, 5015</td>
<td>750.0</td>
<td>750.0</td>
</tr>
<tr>
<td>Casein</td>
<td>75.0</td>
<td>75.0</td>
</tr>
<tr>
<td>Dextrose, monohydrate</td>
<td>17.5</td>
<td>17.5</td>
</tr>
<tr>
<td>Sucrose</td>
<td>16.25</td>
<td>16.25</td>
</tr>
<tr>
<td>Dextrin</td>
<td>16.25</td>
<td>16.25</td>
</tr>
<tr>
<td>Cocoa butter</td>
<td>75.0</td>
<td>75.0</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>12.5</td>
<td>12.5</td>
</tr>
<tr>
<td>Sodium cholate</td>
<td>5.0</td>
<td>5.0</td>
</tr>
<tr>
<td>Cellulose</td>
<td>12.5</td>
<td>12.5</td>
</tr>
<tr>
<td>Mineral mix, AIN-76 (170915)</td>
<td>8.75</td>
<td>8.75</td>
</tr>
<tr>
<td>Choline chloride</td>
<td>1.25</td>
<td>1.25</td>
</tr>
<tr>
<td>mg/kg Diet</td>
<td></td>
<td></td>
</tr>
<tr>
<td>β-Carotene†</td>
<td>0.0</td>
<td>5000</td>
</tr>
<tr>
<td>Ascorbic acid‡</td>
<td>0.0</td>
<td>500</td>
</tr>
<tr>
<td>Vitamin E, IU/kg</td>
<td>&lt;30.0</td>
<td>1000§</td>
</tr>
</tbody>
</table>

*Control and tea diets (TD 95170) and the antioxidant diet (TD 95171) were obtained from Harlan-Teklad. Diets also contained a complement of vitamins adequate to meet mouse nutritional needs, with the exception of vitamin E.
†β-Carotene, trans, crystalline.
‡Ascorbic acid, coated (97.5%).
§Dry vitamin E acetate (500 IU/g; 2 g added per kg of diet).

lipoproteins to oxidation ex vivo and inhibit the development of fatty streak lesions in an atherosclerosis-prone animal model, the LDL receptor (LDLR)–deficient mouse.

Methods

Animal Model

The atherosclerosis-prone animal model used in this study was the homozygous LDLR-null (LDLR–/–) strain of mouse with a C57BL/6j background (Jackson Labs, Bar Harbor, Me). These animals develop marked hypercholesterolemia and early to intermediate atherosclerotic lesions after 6 to 8 weeks on a high-fat, high-cholesterol (HFHC) diet.6 Twenty female LDLR–/– mice (age 7 to 9 weeks) were maintained in a temperature-controlled (25°C) facility with a strict 12-hour light/dark cycle and were given free access to food. Animals were fasted overnight before collection of blood from the retro-orbital sinus into tubes containing EDTA (10 mmol/L) for preparation of plasma. Mice were killed at the end of the study by cervical dislocation, and hearts were obtained for subsequent analyses. This project was approved by the Animal Care and Use Committee of the University of Washington (protocol No. 2140-12).

Test Diets

Mice were fed standard pelleted mouse chow (Wayne Rodent Blox 8604, Teklad) for 2 weeks before the start of the test diets. The mice were randomly divided into 3 equal-size groups (control, tea, and antioxidant groups) and started on the test diets (Table 1). Control and tea groups were fed a semisynthetic HFHC pelleted diet with reduced vitamin E (<30 IU vitamin E/kg diet) and containing 15% fat (cocoa butter), 1.25% cholesterol, and 0.5% cholic acid by weight (TD 95170 Teklad). The antioxidant group received a diet with the same fat, cholesterol, and cholic acid contents as the other 2 groups but it contained additional vitamin E (0.1% by weight of diet, or 200 mg/kg body weight per day), β-carotene (0.5% by weight of diet, or 1000 mg/kg body weight per day), and ascorbic acid (0.05% by weight of diet, or 100 mg/kg body weight per day).

These concentrations were chosen on the basis of previous studies in which supplementary dietary antioxidants were fed to mice.23,24 The animals remained on their respective test diets for 8 weeks.

Animals in the control and antioxidant groups were given water ad libitum. Animals in the tea group were given a black tea infusion ad libitum as their sole source of fluid intake beginning at the time of initiation of the experimental HFHC diet. Tea was introduced gradually by increasing serial concentrations as follows: days 1 and 2, 75% water–25% tea; days 3 and 4, 50% water–50% tea; days 5 and 6, 25% water–75% tea; and day 7, 100% tea. Black tea was brewed every other day by infusing 50 g of black tea leaf (Southern Tea Co) with 4 L distilled water in a Bunnomatic TU-3 tea maker (Bunn Inc). This provided consistent final tea infusions of 1.25%, which are similar to tea brews consumed by humans.25

Plasma Lipid Determination

Animals were fasted overnight and plasma was obtained for total cholesterol and triglyceride levels at baseline and at sacrifice. Plasma total cholesterol was determined by a colorimetric assay (Diagnostic kit No. 236691, Boehringer Mannheim) with cholesterol standards (Preciset No. 125512, Boehringer Mannheim), as described previously.26 Plasma triglycerides were determined after removal of free glycerol (Diagnostic kit No. 450032, Boehringer Mannheim).

LDL Oxidation

For measurement of its susceptibility to oxidation, LDL (d=1.019 to 1.063 g/mL) was isolated from plasma of each animal by density gradient ultracentrifugation.27 In brief, 200 μL of plasma containing 10 mmol/L EDTA was combined with KBr and saline (d=1.006 g/mL) to bring the volume to 1.5 mL and the final density to 1.21 g/mL. Samples were then overlayed with 3.5 mL of saline (d=1.006 g/mL) in 5-mL centrifuge tubes (Beckman OptiSeal, Beckman Instruments) to create a density gradient and spun at 4.16×10^3 g in a Beckman 65.2 near-vertical rotor (Beckman Instruments) at 7°C for 80 minutes. The top 0.5 mL was saved from the centrifuge tube and the LDL fraction was obtained from the next 1.5 mL. This fraction had been previously determined to contain the LDL fraction in pilot experiments in LDLR–/– animals fed a chow diet (Figure 1A). This fraction could contain IDLs in the HFHC diet animals, since there was no clear distinction between VLDL and LDL (Figure 1B). The LDL samples then were passed over Sephacryl-300 gel columns in PBS to remove KBr, EDTA, and residual albumin. Twenty 1-mL fractions from each sample were collected from the columns and analyzed for cholesterol content by using the colorimetric enzymatic assay described previously.30 Fractions from each of the respective samples that contained cholesterol were pooled. Each pooled sample then was quantified for total cholesterol and adjusted to a final cholesterol concentration of 0.130 mmol/L with addition of PBS.

The susceptibility of LDL to oxidation was assessed by determining the lag phase of conjugated diene formation by using a modification of the method of Esterbauer et al.31 Aliquots of each LDL sample from each animal (300 μL) were gently mixed with CuSO4 solution to achieve a final concentration of 5 μmol/L. The appearance of conjugated dienes was measured by continuously monitoring absorbance at 234 nm in a Varian Cary 1-E spectrophotometer (Varian Australia Ltd) for 16 hours at 37°C. In addition to the lag phase, the rate of conjugated diene formation was determined.

Fatty Streak Lesion Quantification

Fatty streak lesions were quantified by evaluation of lesion size in the aortic sinus as described previously34 with minor modifications.35 At sacrifice, hearts and proximal aortas were removed, cleaned of pericardial fat under a dissecting microscope, and fixed in formalin. The hearts then were cut directly under and parallel to the aortic leaflet, and the upper portions were imbedded in OCT medium and frozen at −70°C. Ten-micron-thick sections were cut through the aortic sinus, which is recognized by 3 valve cusps at the junction of the left ventricle and the aorta. Thirty sections per animal were stained for lipid with oil red O and counterstained with Harris’

Crawford et al September 1998 1507
Statistical Analysis

Data are reported as mean±SEM. Statistical differences were determined by ANOVA with SYSTAT software for the Macintosh (version 5.2, SYSTAT Inc). Differences in the plasma variables, including total cholesterol and triglyceride levels, were detected by 2-way ANOVA (diet×time). Differences in variables, including lag phase, rates of conjugated diene formation, and lesion areas, were detected by 1-way ANOVA (diet). Post hoc analyses of significance were made by using Tukey’s test for additivity. In some cases, Student’s t test also was used to compare independent means. Pearson’s correlation coefficient was used to assess correlations. P<0.05 was accepted as statistically significant.

Results

Tolerance of Diets and Fluids

The tea-fed group showed similar fluid intake compared with the 2 water-fed groups. Three animals from the control group, 2 in the antioxidant group, and 1 in the tea group died during the course of the study. No adverse effects from the diets were noted in the remaining mice.

Cholesterol and Triglycerides

There was a >10-fold elevation in total cholesterol levels at 8 weeks in all 3 groups on the HFHC diet compared with baseline (Table 2). Total cholesterol values at 8 weeks were similar among the 3 groups. In the control group, mean triglyceride levels were significantly lower at the end of the study period compared with baseline. Conversely, the final triglyceride levels were significantly higher in both the tea group and antioxidant group compared with baseline values.

Susceptibility of LDL to Oxidative Modification

The susceptibility of lipoproteins to oxidation was measured as the lag phase and rate of conjugated diene formation (Table 3). Owing to the relatively large volume of plasma required for the oxidation assay, lag phases and rates were measured at the end of the study only. Mean lag phases in the tea and antioxidant groups were 19% and 68% longer, respectively, compared with baseline values.

TABLE 2. Plasma Total Cholesterol and Triglyceride Levels (mmol/L) of LDLR−/− Mice Immediately Before and After Being Fed Control, Tea, or Antioxidant Diets for 8 Weeks

<table>
<thead>
<tr>
<th>Diet Group</th>
<th>Total Cholesterol</th>
<th>Triglyceride</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 Weeks</td>
<td>8 Weeks</td>
</tr>
<tr>
<td>Control</td>
<td>7.2±0.2</td>
<td>78.5±4.0*</td>
</tr>
<tr>
<td>Tea</td>
<td>6.3±0.3</td>
<td>87.0±3.7*</td>
</tr>
<tr>
<td>Antioxidant</td>
<td>7.3±0.3</td>
<td>93.5±5.7*</td>
</tr>
</tbody>
</table>

Values are expressed as mean±SEM for 17 to 20 animals. *P<0.0001 compared with time 0. †P<0.01 compared with time 0. ‡P<0.0001 compared with control group.

2-way ANOVA (diet×time). Differences in variables, including lag phase, rates of conjugated diene formation, and lesion areas, were detected by 1-way ANOVA (diet). Post hoc analyses of significance were made by using Tukey’s test for additivity. In some cases, Student’s t test also was used to compare independent means. Pearson’s correlation coefficient was used to assess correlations. P<0.05 was accepted as statistically significant.

TABLE 3. Lag Phase and Rate of Conjugated Diene Formation of LDL Particles From LDLR−/− Mice Fed Control, Tea, or Antioxidant Diets for 8 Weeks

<table>
<thead>
<tr>
<th>Diet Group</th>
<th>Lag Phase, min</th>
<th>Rate, ΔA234/min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>84±4</td>
<td>0.19±0.01</td>
</tr>
<tr>
<td>Tea</td>
<td>100±5</td>
<td>0.20±0.02</td>
</tr>
<tr>
<td>Antioxidant</td>
<td>143±7†</td>
<td>0.14±0.01†</td>
</tr>
</tbody>
</table>

Values are expressed as mean±SEM for 16 to 18 animals. *P<0.0001 compared with tea group. †P<0.0001 compared with control group. ‡P<0.05 compared with tea group.
Figure 2. Fatty streak lesions in aortic sinus. LDLR−/− mice were fed their respective atherogenic test diets for 8 weeks as described in Methods. Ten-micron-thick sections at the aortic sinus were stained for lipid with oil red O and counterstained with hematoxylin. Characteristic lesions in each of 3 groups are depicted with arrows. A, Control group at 40×. B, Control group at 100×. C, Tea group at 40×. D, Tea group at 100×. E, Antioxidant group at 40×. F, Antioxidant group at 100×.
Dietary Antioxidants Inhibit Fatty Streak Lesions

Figure 3. Lesion area of LDLR<sup>−/−</sup> mice after consumption of experimental diets for 8 weeks. Lesion area was determined as described in Methods by using quantitative morphometric analysis of oil red O–stained lesions in the aortic sinus of each animal. Each circle represents an individual animal. Mean lesion area for each group is represented by horizontal bar.

Compared with the control group, Compared with the control group, the prolonged lag phase seen in the tea group failed to reach statistical significance by ANOVA yet was significantly longer (P=0.01) when compared by Student’s t test. These findings are consistent with a reduced susceptibility of LDL to oxidation in the tea group and an even greater reduced susceptibility to oxidation in the antioxidant group.

The mean conjugated diene formation rates were similar in the control and tea groups and ~40% reduced in the antioxidant group. By this criterion, only the antioxidant group had a reduced susceptibility of LDL to oxidation.

Atherosclerotic Lesions Analysis

Previous studies have shown that the LDLR<sup>−/−</sup> mouse model develops atherosclerotic lesions when fed an HFHC diet. In the current study, all 3 groups developed fatty streak lesions in the aortic sinus when fed the HFHC diets (Figures 2 and 3). Characteristic sections from each of the 3 study groups showed the lesions appeared similar in the control and tea groups but were markedly smaller in the antioxidant group (Figure 2). When the lesions were quantified, the control and tea groups showed identical total lesion areas, whereas the antioxidant group had a 60% reduced mean lesion area compared with the other 2 groups (Figure 3).

Consistent with the 60% reduction in lesion size in the antioxidant-fed group, there were significantly fewer cells in the lesions from the antioxidant-treated animals. However, when normalized to lesion area, there were no differences in the cellularity of the lesions in any of the 3 groups (Table 4). Immunocytochemical staining of the lesions with an antibody specific for smooth muscle cells demonstrated that lesions were basically devoid of smooth muscle cells (Figure 4).

Discussion

In this study, combined supplemental dietary antioxidants markedly inhibited fatty streak lesion formation in the LDLR<sup>−/−</sup> mouse. This mouse model was chosen because (1) atherosclerosis develops rapidly in response to the feeding of an atherogenic diet; (2) the major lipoprotein present is LDL, distinct from many other rodent models; and (3) oxidation-specific epitopes have been observed in lesions from this animal model. The marked reduction of fatty streak lesion development observed in the antioxidant group may be related to the reduced susceptibility of LDL to oxidation seen in this group. The lag phase of conjugated diene formation, which measures the resistance of LDL to oxidation, was longer in the antioxidant group compared with the tea group and ~68% greater than that in the control group. Similarly, the mean rate of conjugated diene formation was reduced in the antioxidant group compared with the 2 other groups. The tea group showed a trend toward a prolonged mean LDL lag phase compared with the control group. Although insufficient plasma was available to quantify plasma flavonoid levels, the prolonged mean lag phase suggests that tea flavonoids retain their antioxidant properties, as measured by testing the susceptibility of LDL to oxidation ex vivo. These findings are similar to the results of a recent study in humans. However, despite the prolongation of the mean lag phase in the tea group, there was no difference in mean lesion area between tea and control groups, findings similar to those reported in a recent study in hypercholesterolemic rabbits. In that study, black tea consumption resulted in a 15% prolongation in the mean lag phase without any reduction in atherosclerotic lesion size compared with control animals.

No correlation between lag phase values and fatty streak lesion size was observed among individual animals in the current study, similar to findings in the study by Tijburg et al. This is in contrast to inverse correlations between the individual lag phase of LDL oxidation and extent of atherosclerosis previously reported in both humans and nonhuman primates. Although we are unable to explain the differences between previous studies and our study, the lack of correlation in the current study may point out the limitations of measuring the susceptibility of LDL to oxidation ex vivo. Because LDL is likely to undergo oxidative modification in the interstitial space in the arterial intima rather than intravascularly, measurement of LDL oxidation in vivo is difficult. Attempts have been undertaken to better characterize in vivo LDL oxidation by using antibodies against oxidized LDL in atherosclerotic lesions in both experimental animals and humans. However, this technique is limited, because these antibodies can also detect epitopes on proteins other than LDL and can only be used after sacrifice of experimental animals or on surgical or autopsy specimens in humans.

Therefore, indirect methods for evaluating LDL oxidation, such as that used in the present study, have been

TABLE 4. Analysis of Fatty Streak Lesion Cellularity From LDLR<sup>−/−</sup> Mice Fed Control, Tea, or Antioxidant Diets for 8 Weeks

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Tea</th>
<th>Antioxidant</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total cellularity</td>
<td>834±133.5</td>
<td>1056±84.4</td>
<td>513±86.6*</td>
</tr>
<tr>
<td>Total cellularity/lesion area, ×10^-3</td>
<td>4.2±0.2</td>
<td>4.2±0.2</td>
<td>4.6±0.2</td>
</tr>
</tbody>
</table>

Values are expressed as mean±SEM for 6 animals. *P<0.05 compared with control and tea groups.
used widely. Although several studies have demonstrated a relationship between individual lag phase measurements and the extent of atherosclerotic lesions, other studies showed inhibition of LDL oxidation ex vivo without inhibition of atherosclerosis. It is conceivable that despite the ability to demonstrate inhibition of LDL oxidation ex vivo, insufficient inhibition of oxidation might be occurring in vivo to retard atherosclerosis. Rather, a certain threshold inhibition of oxidation may be required to inhibit atherogenesis. Additionally, although prolongation of the LDL lag phase and protection against lesion development may sometimes be observed concurrently as a result of antioxidant therapy, it is conceivable that some antioxidants can ameliorate atherosclerosis by mechanism other than, or in addition to, inhibition of LDL oxidation. This concept is supported by results from a study in which the antioxidant probucol reduced the extent of atherosclerosis in hypercholesterolemic rabbits, while a probucol analogue, which also inhibited LDL oxidation albeit to a lesser extent than probucol, did not retard atherosclerotic lesion development.

Several factors may have accounted for the lack of reduction in fatty streak formation in the tea group. First, it is likely that the amount of tea consumed had less of an antioxidant effect, as judged by the differences in lag phase and rate of conjugated diene formation compared with the antioxidant cocktail. The dose of tea used was based on previous studies in mice in which tea had been shown to reduce the development of cancer. Yet this dose may have been insufficient to protect against the development of fatty streak lesions in the setting of the atherogenic insult due to very high cholesterol levels that result from feeding of an HFHC diet to LDLR<sup>−/−</sup> mice. It is conceivable that the antioxidant properties of tea might have been able to reduce both LDL oxidation and lesion development in an animal model with milder hyper-

Figure 4. Lack of smooth muscle cells in lesions of control and antioxidant-treated LDLR<sup>−/−</sup> mice. Immunocytochemical staining of frozen sections from control (A) and antioxidant-treated (B) mice with an anti–α-actin-specific antibody demonstrates absence of smooth muscle cells within intima (I) and presence of smooth muscle cells within media (M). L indicates lumen; 870× final magnification.
cholesterolemia and less marked atherosclerosis. Second, both the tea and control groups were fed a semisynthetic diet with reduced vitamin E to maximize any differences that might be due to the antioxidant effects of tea and the dietary antioxidant supplementation that were provided. The vitamin E content in the control and tea diets was <30 IU/kg and was less than half the daily vitamin E recommended in rodent chow.47 Although suboptimal vitamin E intake in the control and tea diets might have led to a vitamin E deficiency, the implications on how this deficiency might have influenced LDL oxidation and fatty streak lesion size are unclear. Several studies have failed to show increased lipid peroxidation products in vivo in animals fed a vitamin E–deficient diet.38–50 It is conceivable that different results might have been obtained had the animals not received a diet deficient in vitamin E.

The extent of fatty streak lesion development was 60% lower in the antioxidant group than in either the control or tea-fed animals. In contrast to previous studies of probucol-treated mice, in the antioxidant group than in either the control or tea-fed animals. In contrast to previous studies of probucol-treated mice, the antioxidants inhibited LDL oxidation and fatty streak lesion size were unclear. Nevertheless, observations in the current study suggest that lesions can be reduced by the use of high-dose antioxidant supplements in these hypercholesterolemic animals.

In this study, the antioxidant diet contained a combination of vitamin C, β-carotene, and vitamin E. It was not possible to determine which of the antioxidants in the cocktail was responsible for the benefits seen in reducing LDL oxidation or fatty streak lesion development. Both vitamin E15,16 and β-carotene19 have been associated with protection against atherosclerosis in experimental animals. In the study by Tijburg et al.,24 rabbits fed vitamin E had a 63% increase in lag phase, animals fed black tea had a 15% increase in lag phase, and animals fed β-carotene had no change in lag phase. None of these interventions had an effect on atherosclerotic lesion size. Therefore, it is possible that a combination of antioxidants is required to protect against lesion development. One study involving rabbits fed a high-fat diet and tea diets might have led to a vitamin E deficiency, the antioxidants inhibited LDL oxidation and fatty streak lesion development in the LDLR−/− mouse and may be important in reducing atherosclerosis in humans with high LDL levels.

Acknowledgments

This research was supported by the Tea Trade Health Research Association (R.C.L., M.E.R., A.C.), National Institutes of Health grants HL 30086 (A.C.) and HL 07028 (R.S.C.), and the University of Washington Clinical Nutrition Research Unit Laboratory Core NIH/NIDDK DK35816. We thank Shari Wang and Pat Suryuktasut-Woo for their expert technical assistance.

References

9. Carew TE, Schwendeman DC, Steinberg D. Antiatherogenic effect of probucol unrelated to its hypocholesterolemic effect: evidence that the antioxidants 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 (WHHL) rabbit. Proc Natl Acad Sci U S A. 1987;84:7725–7729.


Dietary Antioxidants Inhibit Development of Fatty Streak Lesions in the LDL Receptor−Deficient Mouse
Richard S. Crawford, Elizabeth A. Kirk, Michael E. Rosenfeld, Renée C. LeBoeuf and Alan Chait

doi: 10.1161/01.ATV.18.9.1506
Arteriosclerosis, Thrombosis, and Vascular Biology is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1998 American Heart Association, Inc. All rights reserved.
Print ISSN: 1079-5642. Online ISSN: 1524-4636

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://atvb.ahajournals.org/content/18/9/1506

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Arteriosclerosis, Thrombosis, and Vascular Biology can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Arteriosclerosis, Thrombosis, and Vascular Biology is online at:
http://atvb.ahajournals.org//subscriptions/