The Effect of α-Tocopherol Supplementation on LDL Oxidation

A Dose-Response Study

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Abstract Because much data have accrued to support the concept that oxidatively modified LDL (Ox-LDL) can promote atherogenesis, the role of antioxidants in decreasing LDL oxidation has assumed great importance. High-dose α-tocopherol supplementation in humans decreases the susceptibility of LDL to oxidation. Hence, the aim of the present study was to ascertain the minimum dose of α-tocopherol that would decrease the susceptibility of LDL to oxidation. The effect of α-tocopherol in doses of 60, 200, 400, 800, and 1200 IU/d on copper-catalyzed LDL oxidation was tested in a randomized placebo-controlled study over 8 weeks. There were eight subjects in each group. Oxidation of LDL was monitored by measuring the formation of conjugated dienes and lipid peroxides by the thiobarbituric acid–reacting substances (TBARS) assay over an 8-hour time course at baseline and after 8 weeks of supplementation. Neither placebo nor any of the doses of α-tocopherol resulted in any side effects or exerted an adverse effect on the plasma lipoprotein profile. However, there was a dose-dependent increase in plasma and lipid-standardized α-tocopherol levels with increasing doses of α-tocopherol supplementation. LDL α-tocopherol appeared to follow a similar trend. When the time-course curves of LDL oxidation and the kinetics of LDL oxidation were examined, there was no significant effect at 8 weeks compared with baseline in the groups that received placebo or α-tocopherol 60 or 200 IU/d. However, in the groups that received at least 400 IU/d α-tocopherol, there was a decreased susceptibility of LDL to oxidation, as shown by the mean levels in the time-course curves, prolongation in the lag phase, and a decrease in the oxidation rate. Furthermore, both plasma and LDL α-tocopherol correlated significantly with the lag phase of oxidation and inversely with the oxidation rate. The results of the present study show that the minimum dose of α-tocopherol needed to significantly decrease the susceptibility of LDL to oxidation is 400 IU/d. (***Arterioscler Thromb Vasc Biol.* 1995;15:190-198.**)

Key Words • lipid peroxidation • α-tocopherol • LDL oxidation • atherosclerosis • antioxidants

Much data have accrued to support the concept that oxidatively modified LDL (Ox-LDL) can promote atherogenesis.[1,2] Ox-LDL could promote atherogenesis in several ways: by uptake by the scavenger receptor mechanism, which would result in cholesterol ester accumulation[3]; by its cytotoxic effect, which could promote endothelial cell dysfunction and evolution of the fatty streak to the more advanced lesion[4]; and by its chemotactic effect on monocytes and its inhibitory effect on macrophage migration, which could result in accumulation of macrophages in the artery wall.[5,6] It could also promote atherogenesis by altering gene expression in the arterial wall, such as induction of leukocyte adhesion molecules on monocytes and induction of monocyte chemotactic protein–1 and interleukin-1.[7-9] Ox-LDL can adversely affect the coagulation pathway by inducing tissue factor synthesis[10]; it has also been shown that products of oxidized LDL can impair endothelium-derived relaxation factor–mediated vasorelaxation.[11,12] Furthermore, several lines of evidence support the in vivo existence of oxidized LDL. Data have been presented for the occurrence of a modified form of LDL with many physical, chemical, and biological properties of Ox-LDL in arterial lesions.[13] Antibodies against epitopes on oxidized LDL recognize material in atherosclerotic lesions but not normal arteries.[14-16] Circulating antibodies against epitopes of oxidized LDL have been demonstrated in the plasma of humans,[16] and in fact, the titer of this antibody correlates independently with the progression of carotid artery stenosis.[17] In addition, the susceptibility of LDL to oxidation (the lag phase of oxidation) is correlated with the severity of coronary atherosclerosis as shown by angiography.[18] However, the most persuasive data are the findings that antioxidant supplementation will inhibit the progression of atherosclerosis in animal models.[19-22] Thus, a clinical trial in humans with antioxidants is crucial in validating the oxidized LDL hypothesis. Most of the antioxidants tested in animal models to date, probucol, butylated hydroxytoluene, and *N,N'*-diphenylphenylenediamine, carry potential side effects that preclude their utility in human clinical trials.[22-24] However, the dietary micronutrients with antioxidant properties such as ascorbate, α-tocopherol, and β-carotene may provide an alternative approach to protect LDL against oxidative modification and prevention of atherosclerosis. The most consistent data with respect to micronutrient antioxidants and atherosclerosis appear to relate to α-tocopherol (vitamin E), which is the major antioxidant in LDL.[25] Several lines of evidence support an inverse relationship between α-tocopherol and atherogenesis.

Received August 2, 1994; revision accepted November 8, 1994.

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Low levels of α-tocopherol have been shown in epidemiological studies to be related to an increased frequency of cardiovascular disease mortality.[26,27] Case-control studies have shown lower levels in patients with angina pectoris compared with control subjects,[28] and both men and women in the highest compared with the lowest quintile of vitamin E intake have a significantly lower relative risk of coronary heart disease.[29,30] Numerous studies have now documented that α-tocopherol can inhibit LDL oxidation in vitro.[31,32] In addition, high-dose α-tocopherol supplementation can decrease the susceptibility of LDL to oxidation in human subjects.[33-36] Furthermore, it appears that supplementation with ascorbate and β-carotene, in addition to high-dose α-tocopherol, does not appear to confer an additional benefit in decreasing the susceptibility of LDL to oxidation.[36,37] The doses of α-tocopherol used in these studies greatly exceed the RDA.[38] Hence, it is important to establish the minimum dose of α-tocopherol that would decrease the susceptibility of LDL to oxidation. Accordingly, the present study was designed to examine the dose-response effect of α-tocopherol on the susceptibility of LDL to oxidation.

Methods

This study was approved by the Institutional Review Board of the University of Texas Southwestern Medical Center. All subjects gave informed consent. The study design was that of a placebo-controlled, randomized trial. Healthy men had to fulfill all the following criteria to be entered into the study: nonsmokers; not on any vitamin supplements for at least 6 months before entry; alcohol intake <1 oz/d; normal plasma glucose, hepatic, and renal function tests; no evidence of malabsorption, pancreatic, or biliary disease; and no acute medical condition for at least 3 months prior to entry. A total of 48 subjects were selected. Fasting blood samples were obtained at baseline for complete blood count and plasma glucose, protein, hepatic, and renal function tests; these were all assayed in a standardized hospital laboratory.

Blood (120 mL) was also obtained for lipid and lipoprotein profiles; plasma α-tocopherol, ascorbate, and β-carotene levels; and for LDL isolation. The samples for LDL isolation were collected in tubes containing EDTA (1 mg/mL). All blood samples were collected on ice, and the plasma was separated by low-speed centrifugation at 4°C. Thereafter, the participants were randomly assigned to receive either placebo (soybean oil) or α-tocopherol capsules at dosages of 60, 200, 400, 800, or 1200 IU/d for 8 weeks. The supplement was in the form of DL-α-tocopherol, and all capsules were provided by Hoffmann-La Roche Inc. All six groups were studied in parallel. They were advised to maintain their usual diet and activities during the 8 weeks and to report any side effects immediately to the investigators. The subjects returned to the clinic at 8 weeks. They continued to take the α-tocopherol capsules until the day on which blood samples were obtained. At each visit a clinical examination was performed, and blood samples were obtained as described above for the baseline period. Samples for plasma ascorbate were deproteinized with ice-cold 10% metaphosphoric acid and centrifuged, and the supernatant was purged with nitrogen and stored below −20°C in foil-covered tubes.

The plasma lipid and lipoprotein levels were assayed by using Lipid Research Clinics methodology, except that cholesterol and triglyceride levels were determined enzymatically.[39] The concentrations of α-tocopherol and β-carotene were measured in plasma and LDL following extraction by reverse-phase high-performance liquid chromatography.[40] The plasma levels of both α-tocopherol and β-carotene were standardized to total plasma lipids as described.[41] The LDL concentrations were expressed per milligram LDL protein. Plasma ascorbate levels were determined spectrophotometrically after derivatization with 2,4-dinitrophenylhydrazine.[42]

Plasma fatty acids at baseline and 8 weeks were measured by gas-liquid chromatography after extraction and transmethylation.[43] An internal standard of 17:0 was added to all samples; fatty acid standards were obtained from NuChek Prep. Data are expressed in millimoles per liter for 14:0, 16:0, 18:0, 18:1, 18:2, 18:3, and 20:4.

LDL (d=1.019 to 1.063 g/mL) was isolated by preparative ultracentrifugation in NaBr-NaCl solutions containing 1 mg/mL EDTA as described.[44] The isolated LDL was extensively dialyzed against three exchanges (4, 4, and 2 L) of saline-EDTA (150 mmol/L NaCl, 1 mmol/L EDTA, pH 7.4) at 4°C over 24 hours. Thereafter the LDL was filtered and stored at 4°C under nitrogen until protein was measured by the method of Lowry et al[45] on the same day using bovine serum albumin as the standard. Stock LDL solutions obtained at 8 weeks were diluted with the NaCl-EDTA dialysis buffer such that the protein concentration did not vary from baseline levels by more than 0.5 mg/mL. LDL oxidation was undertaken after an overnight dialysis against 1 L phosphate-buffered saline (PBS), pH 7.4, at 4°C. Thus, oxidation studies were performed within 48 hours of LDL isolation by ultracentrifugation. LDL (200 μg protein/mL) was oxidized in a cell-free system using 5 μmol/L copper in PBS at 37°C.[33] The time course of oxidation was studied over an 8-hour period. Each time point was set up in triplicate. At 0, 0.5, 1, 1.5, 2, 3, 5, and 8 hours, oxidation was arrested by refrigeration and the addition of 200 μmol/L EDTA and 40 μmol/L BHT.

Two indices of oxidation were used in this study. The lipid peroxide content of oxidized LDL was measured by a modification of the thiobarbituric acid–reactive substances (TBARS) assay of Buege and Aust.[46] TBARS activity was expressed as malondialdehyde equivalents using freshly diluted 1,1,3,3-tetramethoxypropane as the standard. The amount of conjugated dienes formed during LDL oxidation was determined by measuring the absorbance of LDL against a PBS blank at 234 nm following a 1:4 dilution of the samples in PBS.[47] We have shown that dilution of an oxidized LDL sample to 1:2, 1:4, and 1:8 displays linearity and excellent recovery; the data are expressed as the increase in conjugated dienes over baseline (ΔA234).[48] The rate of LDL oxidation was determined from the propagation phase of the time-course curve using a spline function. The lag phase was obtained by drawing a tangent to the slope of the propagation phase and extrapolating it to the horizontal axis.[48] The lag time constitutes the interval from zero time to the intersection point.

Statistical Methods

Results are expressed as mean±SD or for skewed data as median (range). ANOVA with the Student-Newman-Keuls multiple range test was used to assess differences between groups at baseline. Paired t tests were used to determine differences within each group between baseline and 8 weeks for plasma lipid and lipoprotein levels (except triglycerides) and LDL oxidation kinetic parameters. For skewed data such as plasma triglycerides and antioxidant levels, the Wilcoxon signed rank test was used to compare baseline and week 8 measurements.[49,50] Comparison of baseline and 8-week time-course curves within each group were made by using two-factor repeated-measures analysis of variance. Where the week×hour interaction was significant, comparisons at each time point were made using paired t tests. The relationships between percent change in plasma and LDL α-tocopherol concentrations and lag phase and oxidation rates were determined using Spearman rank correlation. For α-tocopherol levels and oxidation kinetic parameters, percent change from baseline was computed for each subject and is reported as median because of skewness. The level of significance was α=.05. Analyses were performed using BMDP programs 3D, 2V, and 3S (BMDP Statistical Software, Inc).

TABLE 1. Group Characteristics and Lipid and Lipoprotein Profile

	α-Tocopherol Dosage					
	0 IU/d	60 IU/d	200 IU/d	400 IU/d	800 IU/d	1200 IU/d
Age, y	32.1±8.3	32.3±6.7	31.8±7.2	31.1±7.3	35.6±5.6	33.4±3.9
BMI, kg/m^2	26.8±4.6	23.8±2.3	24.4±3.5	24.1±2.6	24.5±3.8	24.3±1.6
Total cholesterol, mmol/L						
Week 0	4.63±0.56	4.86±0.43	4.46±0.72	4.77±0.88	5.51±1.22	5.21±0.77
Week 8	4.93±0.58	4.80±0.80	4.78±0.98	5.00±0.71	5.81±1.14	5.30±0.81
Triglycerides, mmol/L						
Week 0	1.12±0.46 (1.08)	1.20±0.39 (1.06)	1.27±0.47 (1.18)	1.44±0.52 (1.28)	1.50±0.79 (1.05)	1.48±0.42 (1.35)
Week 8	1.14±0.38 (1.14)	1.28±0.67 (1.11)	1.20±0.42 (1.17)	1.56±1.18 (1.15)	1.36±0.79 (1.01)	1.62±0.85 (1.20)
LDL cholesterol, mmol/L						
Week 0	3.06±0.71	3.26±0.49	3.01±0.67	3.03±0.75	3.76±0.98	3.62±0.64
Week 8	3.28±0.72	3.25±0.66	3.28±0.80	3.21±0.49	4.11±1.01	3.64±0.80
HDL cholesterol, mmol/L						
Week 0	1.19±0.40	1.18±0.17	1.03±0.19	1.22±0.23	1.15±0.20	1.07±0.16
Week 8	1.27±0.53	1.11±0.25	1.09±0.16*	1.18±0.29	1.19±0.22	1.09±0.17

BMI indicates body mass index. Data are presented as mean±SD; median for triglycerides is shown in parentheses. BMI is defined as weight in kilograms divided by the square of the height in meters. Each group had eight subjects.
*P=.04, week 0 versus week 8; all other comparisons were nonsignificant.

Results

Table 1 shows the characteristics of the subjects at baseline. Both age and body mass index were similar across the six groups. There were no changes in diets, activity levels, or weights while the subjects were on supplementation. In addition, none of the subjects reported any adverse effects of supplementation, nor did routine laboratory values change for any subject. Table 1 also displays the plasma lipid and lipoprotein profiles of the study participants at baseline and at 8 weeks. All groups had similar baseline lipid and lipoprotein concentrations. No significant differences were seen within each group between levels at baseline and after 8 weeks of supplementation, except for a marginally significant increase in HDL cholesterol in the group that received 200 IU/d.

The fatty acid profiles for the subjects are shown in Table 2. The concentrations of the fatty acids measured were not significantly different by ANOVA among the groups at either baseline or 8 weeks. In addition, paired t tests revealed no significant differences within any group during the study except for linoleic acid levels in the group that took 200 IU/d.

Table 3 shows the concentrations of plasma and LDL antioxidants. No differences were seen between baseline and 8 weeks for plasma ascorbate or β-carotene within any group, regardless of whether the plasma β-carotene concentration was lipid standardized. The group supplemented with 60 IU/d α-tocopherol had a rise in LDL β-carotene at 8 weeks that was marginally significant (P=.047) relative to baseline; there were no other differences in LDL β-carotene. All supplemented groups

TABLE 2. Plasma Fatty Acid Profiles for Study Participants at 0 and 8 Weeks

	α-Tocopherol Dosage					
	0 IU/d	60 IU/d	200 IU/d	400 IU/d	800 IU/d	1200 IU/d
14:0						
Week 0	0.09±0.07 (5)	0.08±0.02 (7)	0.12±0.06 (8)	0.16±0.10 (8)	0.12±0.06 (8)	0.12±0.07 (8)
Week 8	0.12±0.08 (4)	0.16±0.14 (8)	0.10±0.07 (8)	0.14±0.08 (8)	0.08±0.03 (7)	0.13±0.07 (7)
16:0						
Week 0	2.00±0.57	2.02±0.10	2.42±0.50	2.81±0.85	2.59±0.75	2.53±0.63
Week 8	2.31±0.35	2.46±0.62	2.37±0.67	2.91±1.06	2.56±0.68	2.61±0.86
18:0						
Week 0	0.66±0.14	0.65±0.11	0.70±0.07	0.81±0.19	0.78±0.21	0.67±0.18
Week 8	0.71±0.11	0.71±0.13	0.74±0.12	0.82±0.24	0.79±0.20	0.77±0.22
18:1						
Week 0	1.93±0.66	1.89±0.17	2.45±0.66	2.51±0.72	2.60±0.85	2.61±0.45
Week 8	2.00±0.14	2.38±0.91	2.18±0.69	2.78±1.42	2.53±1.01	2.89±1.13
18:2						
Week 0	2.84±0.55	3.12±0.58	3.20±0.37	3.45±0.86	3.48±0.72	3.08±0.50
Week 8	3.10±0.41	3.46±0.84	3.65±0.52*	3.61±1.17	3.62±0.84	2.98±0.44
18:3						
Week 0	0.05±0.03	0.04±0.02	0.05±0.02	0.04±0.03	0.07±0.04	0.08±0.04
Week 8	0.05±0.02	0.04±0.02	0.04±0.01	0.04±0.02	0.08±0.03	0.06±0.01
20:4						
Week 0	0.65±0.15	0.63±0.10	0.67±0.09	0.60±0.17	0.67±0.23	0.67±0.28
Week 8	0.67±0.14	0.66±0.17	0.66±0.10	0.63±0.11	0.71±0.23	0.59±0.20

Numbers in parentheses indicate sample size. Values are mean±SD and are given in millimoles per liter.
*P=.005, week 0 versus week 8; all other comparisons were nonsignificant.

TABLE 3. Effect of Various Doses of α-Tocopherol on Plasma and LDL Antioxidant Levels

	α-Tocopherol Dosage					
	0 IU/d	60 IU/d	200 IU/d	400 IU/d	800 IU/d	1200 IU/d
Plasma α-tocopherol, μmol/L						
Week 0	23±10	28±4	31±8	18±3	22±8	22±5
Week 8	24±5	44±11†	57±20†	42±16†	56±22†	78±28†
Median change, %	14	61	80	97	153	236
LDL α-tocopherol, nmol/mg protein						
Week 0	9±2	9±2	11±4	8±1	7±3	8±3
Week 8	10±2	15±4*	21±5*	14±4‡	18±12†	22±12*
Median change, %	9	80	116	96	151	158
Plasma ascorbate, μmol/L						
Week 0	55±34	69±27	71±16	58±19	59±14	69±15
Week 8	61±35	70±33	77±23	64±23	68±14	68±19
Plasma β-carotene, μmol/L						
Week 0	0.3±0.2	0.3±0.1	0.3±0.2	0.3±0.1	0.3±0.1	0.3±0.2
Week 8	0.4±0.3	0.4±0.4	0.3±0.1	0.3±0.1	0.3±0.1	0.4±0.2
LDL β-carotene, nmol/mg protein						
Week 0	0.13±0.08	0.12±0.03	0.15±0.10	0.06±0.03	0.08±0.03	0.11±0.06
Week 8	0.20±0.13	0.16±0.08*	0.16±0.08	0.07±0.04	0.08±0.04	0.11±0.06

Data are presented as mean±SD. Comparisons were made by using the Wilcoxon signed rank test.
*$P<.05$, †$P<.01$, week 0 versus week 8.
‡$P=.06$, n=6, week 0 vs week 8.

showed significant increases in plasma and lipid-standardized plasma α-tocopherol levels (plasma, $P<.01$ for 60 to 1200 IU/d; lipid-standardized plasma, $P<.05$ for 60 IU/d, $P<.01$ for 200 to 1200 IU/d). Plasma α-tocopherol increased from 60.5% in the 60 IU/d group to 235.8% in the 1200 IU/d group. Plasma lipid-standardized α-tocopherol levels increased in a similar fashion. For the five doses of α-tocopherol (60, 200, 400, 800, and 1200 IU/d), the increases were 48.3%, 80.4%, 110.9%, 145.3%, and 260.3%, respectively. Due to small sample size (n=6) (there was insufficient LDL available in two subjects to assay for α-tocopherol), LDL α-tocopherol in the 400 IU/d group did not reach statistical significance ($P=.063$); otherwise, there were significant increases at 8 weeks over baseline (60, 200, and 1200 IU/d, $P<.05$; 800 IU/d, $P<.01$; Table 3). The increments in LDL α-tocopherol were not as marked with increasing doses compared with the responses in plasma.

The time courses of LDL oxidation showed significant changes at 8 weeks versus baseline only for the groups that took at least 400 IU/d. For both TBARS and conjugated dienes, there were no significant differences for the placebo, 60 IU, or 200 IU groups (Fig 1), whereas the differences between time-course curves were highly significant for the 400 to 1200 IU/d groups (ANOVA, week×hour interaction, $P<.001$). In addition, time point comparisons revealed significantly lower means in the ≥400 IU/d groups (Fig 2). The kinetics of LDL oxidation (lag phase and oxidation rate) were also computed from the time-course curves. In the groups that received placebo or 60 or 200 IU/d α-tocopherol, there were no significant differences in lag phase or oxidation rate. However, there were significant changes for the 400, 800, and 1200 IU/d groups for both the TBARS and conjugated dienes assays (Table 4). The lag phase of LDL oxidation as measured by the TBARS assay increased 16% at 400 IU/d, 36% at 800 IU/d, and 64% at 1200 IU/d (all $P<.01$ within groups). The TBARS oxidation rate decreased 34% at 400 IU/d, 50% at 800 IU/d, and 55.7% at 1200 IU/d dosages ($P<.05$ for 400 and 1200 IU/d; $P<.01$ for 800 IU/d). The lag phase for the conjugated dienes assay showed a similar pattern, with increases of 27.9%, 36.9%, and 62.9%, respectively, in the groups that received 400, 800, and 1200 IU/d. While the oxidation rate at 400 IU/d was not significantly decreased ($P=.06$), both 800 and 1200 IU/d resulted in significant reductions in the oxidation rate. As can be seen in Fig 3, there was a wide interindividual variation in the response to similar doses of α-tocopherol, as assessed by the lag phase of oxidation for the conjugated diene assay. This was most evident for the groups that received 200 or 800 IU/d of α-tocopherol. Similar interindividual variability was seen with the lag phase computed from the TBARS data (results not shown).

There were no significant differences in maximum TBARS activity and conjugated dienes formed between baseline and 8 weeks in any of the studied groups.

Spearman's rank correlation coefficients between plasma and LDL α-tocopherol concentration and lag phase and rate of LDL oxidation are presented in Table 5. Both plasma and LDL α-tocopherol correlated significantly with the lag phase determined from both the TBARS and conjugated dienes time-course curves. Also, there were significant inverse correlations between plasma and LDL α-tocopherol and the oxidation rate determined from both the TBARS and conjugated dienes data.

Discussion

Numerous lines of evidence support a relation between low levels of α-tocopherol and increased risk of atherosclerosis and the hypothesis that supplementation may be potentially beneficial. Some studies have suggested that α-tocopherol supplementation can moderately reduce the progression of atherosclerosis in animal models.[20,51-54] A plausible mechanism for this beneficial effect of α-tocopherol is by decreasing the oxidative susceptibility of LDL. Since high doses of α-tocopherol can decrease the susceptibility of LDL to oxidation in human volunteers,[33-36] the aim of the present study was

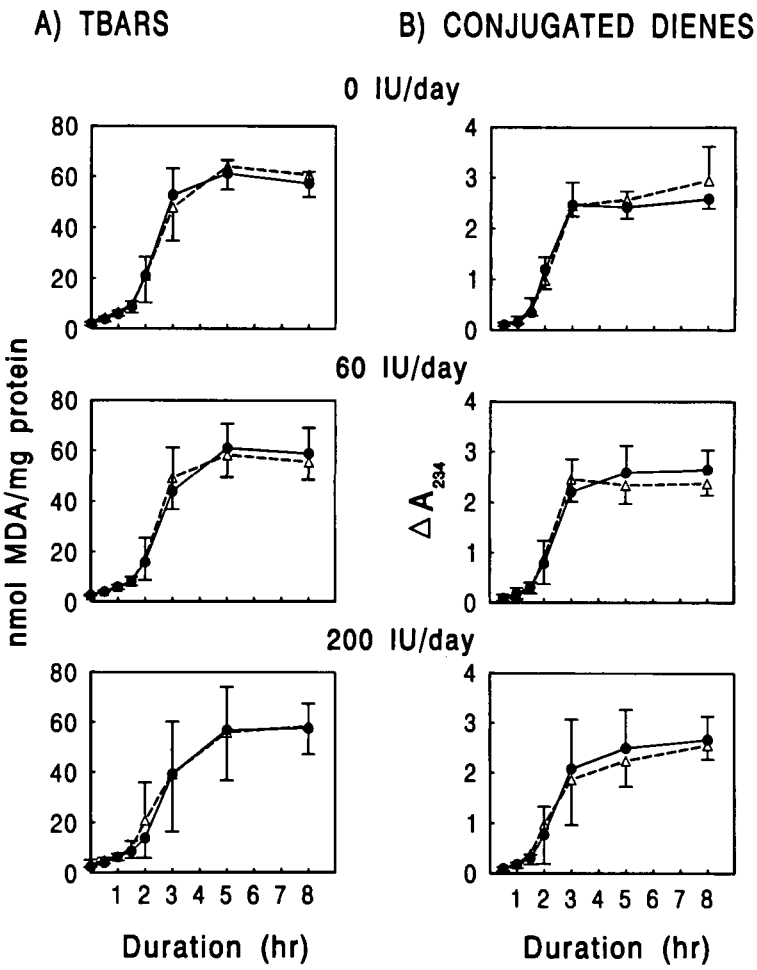

FIG 1. Comparison of time-course curves of LDL oxidation in the groups receiving 0, 60, and 200 IU/d α-tocopherol. At baseline (△) and 8 weeks (●), LDL was isolated and subjected to copper-catalyzed oxidation as described in "Methods." Oxidation was stopped at the time points shown, and samples were assayed for thiobarbituric acid–reactive substances (TBARS) activity (A) and the formation of conjugated dienes (B). Data are presented as mean±SD. No differences were seen in the curves between baseline and 8 weeks for either analysis in these three groups. MDA indicates malondialdehyde.

to ascertain the minimum dose of α-tocopherol that would decrease the susceptibility of LDL to oxidation by conducting a placebo-controlled dose-response study. In this study, healthy male volunteers were given dosages of α-tocopherol ranging from 0 to 1200 IU/d for 8 weeks. None of the subjects experienced any side effects as determined by clinical examination or routine laboratory analysis. Furthermore, in none of the groups receiving α-tocopherol was there a deleterious effect on the plasma lipid and lipoprotein profile. These findings are in accord with the literature[33-36] and contrast with findings with other antioxidants such as probucol,[55] which can alter the lipoprotein profile by lowering HDL cholesterol levels. In addition, α-tocopherol supplementation did not affect the circulating concentrations of ascorbate and β-carotene. However, α-tocopherol supplementation resulted in significant increases in plasma and LDL concentrations of α-tocopherol. While 60 IU/d resulted in a 60.5% increment in plasma α-tocopherol levels, 1200 IU/d produced a 235.8% increase in plasma α-tocopherol concentrations. Similar findings were observed for lipid-standardized levels and to some extent for LDL α-tocopherol concentrations.

The effect of α-tocopherol supplementation on LDL oxidative susceptibility was measured over an 8-hour time course. Two different indices of oxidative modification were used, the formation of conjugated dienes and lipid peroxides (measured as TBARS activity), to obtain a better appreciation of the effect of α-tocopherol on LDL oxidation. It is clear that the mean levels of conjugated dienes and TBARS at 8 weeks were significantly lower than at baseline in the groups supplemented with at least 400 IU α-tocopherol per day. The groups supplemented with less than 400 IU/d α-tocopherol showed no significant changes at 8 weeks in the time-course curves compared with baseline.

To gain more insight on the effect of α-tocopherol supplementation on the susceptibility of LDL to oxidation, the duration of the lag phase and oxidation rate were computed from the time-course data.[48] It is evident that only doses of at least 400 IU α-tocopherol per day had significant effects on the kinetics of LDL oxidation. The lag phase of oxidation was significantly prolonged by doses ≥400 IU/d, as measured by both indices of oxidation. In agreement with the findings of Rifici and Khachadurian,[56] the present study, conducted in a larger number of subjects, shows high interindividual variability in responses to similar doses of α-tocopherol. The lag phase correlates inversely with the severity of clinical atherosclerosis[18]; thus, prolongation of the lag phase with α-tocopherol could prove beneficial. Furthermore, the oxidation rate was decreased after supplementation with ≥400 IU/d as manifested by the formation of conjugated dienes and TBARS. The findings of significant positive correlations between plasma and LDL α-tocopherol and the lag phase of oxidation as well as significant inverse correlations between plasma and LDL α-tocopherol and the oxidation rate clearly demonstrate that α-tocopherol decreased the susceptibility of LDL to oxidation at doses ≥400 IU/d. Thus the major novel observation as it relates to antioxidants and LDL oxidation

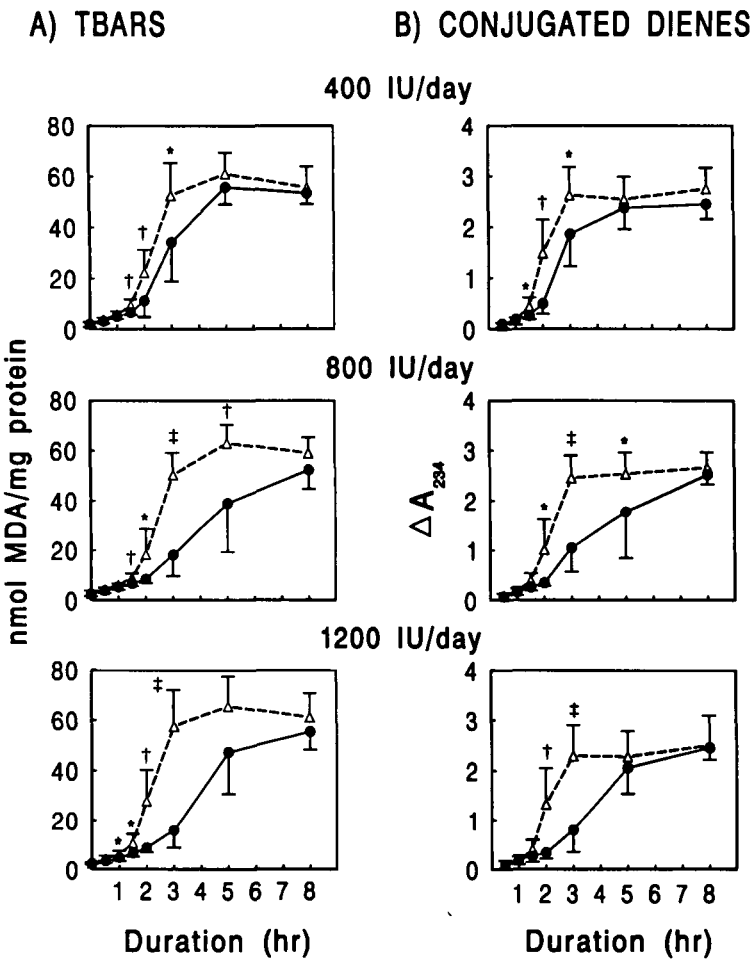

FIG 2. Time-course curves of LDL oxidation in the groups receiving 400, 800, and 1200 IU/d α-tocopherol. Data are presented as mean±SD. The differences between baseline (△) and 8 weeks (●) in these groups were significant by two-way ANOVA (week×hour interaction, $P<.001$). Point-by-point analyses also showed significant differences in all three groups. TBARS indicates thiobarbituric acid–reactive substances; MDA, malondialdehyde. *$P<.05$; †$P<.01$; ‡$P<.001$.

is that this is the first dose-response study to show by statistical analysis that there is a significant protection of LDL with doses ≥400 IU/d of α-tocopherol.

Dieber-Rotheneder et al[35] examined LDL oxidation after supplementation with varying doses of α-tocopherol for 21 days, but this study was severely handicapped by the small number of subjects (n=2) in each supplemented group. This did not allow them to undertake statistical analyses to determine the minimum dose of α-tocopherol needed to decrease the susceptibility of LDL to oxidation. The findings in the present study that α-tocopherol, in doses ≥400 IU/d, decreases the susceptibility of LDL to oxidation may explain in part why, in the recent publication of the Alpha-Tocopherol, Beta-Carotene Cancer Prevention Study Group, no benefit on cardiovascular disease was seen in male smokers.[57] The low dose of α-tocopherol used

TABLE 4. Effect of Various Doses of α-Tocopherol on LDL Oxidation Kinetics

	α-Tocopherol Dosage					
	0 IU/d	60 IU/d	200 IU/d	400 IU/d	800 IU/d	1200 IU/d
TBARS						
Lag phase, h						
Week 0	1.45±0.22	1.54±0.13	1.55±0.54	1.39±0.12	1.49±0.22	1.35±0.16
Week 8	1.44±0.17	1.68±0.37	1.91±0.71	1.74±0.32*	2.39±1.11*	2.31±0.58‡
Oxidation rate, nmol MDA/mg protein/h						
Week 0	35.0±7.6	41.3±8.3	33.8±18.5	43.8±10.6	41.3±8.3	43.8±11.9
Week 8	36.3±5.2	40.0±13.1	33.8±13.0	28.8±8.3†	21.3±6.4†	22.5±11.6†
Conjugated dienes						
Lag phase, h						
Week 0	1.53±0.18	1.63±0.21	1.69±0.39	1.40±0.19	1.58±0.23	1.43±0.20
Week 8	1.43±0.17	1.70±0.39	1.79±0.48	1.78±0.23†	2.84±1.36*	2.41±0.34‡
Oxidation rate, ΔA_{234}/h						
Week 0	2.2±0.6	2.1±0.4	2.1±1.0	2.4±0.7	2.4±0.6	2.3±0.6
Week 8	2.2±0.3	2.1±0.4	1.8±0.8	1.8±0.5	1.0±0.4†	0.9±0.2‡

TBARS indicates thiobarbituric acid–reacting substances; MDA, malondialdehyde. Data are presented as mean±SD. Comparisons were made using paired *t* tests.
*$P<.05$, †$P<.01$, ‡$P<.001$, week 0 versus week 8.

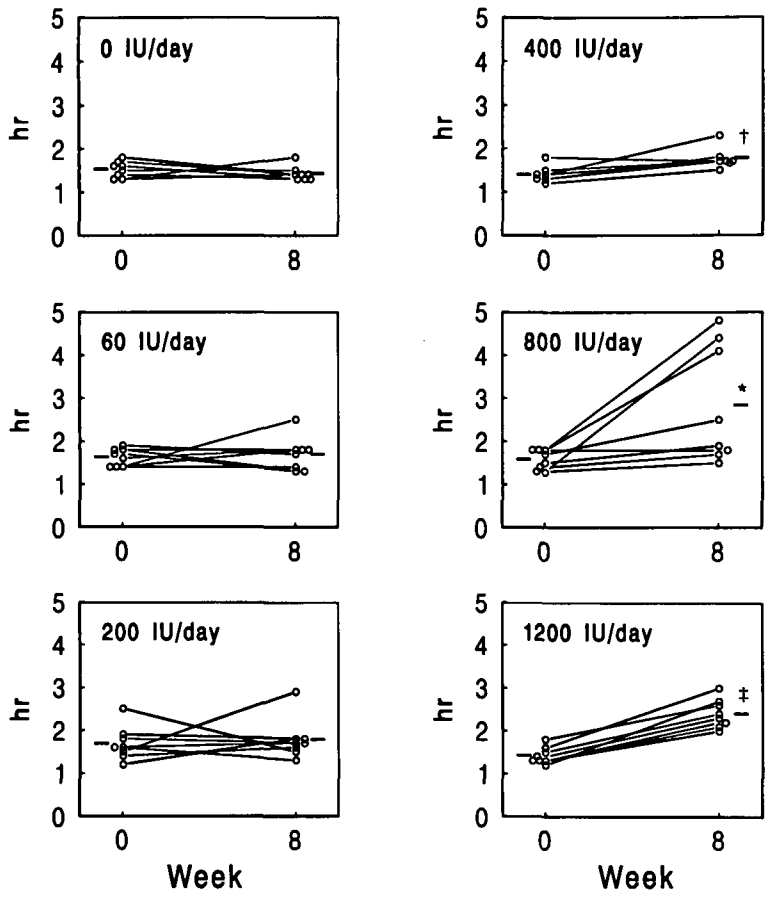

FIG 3. Line plots of changes in the duration of the conjugated dienes lag phase after supplementation with various doses of α-tocopherol. In addition to the absolute levels in each subject at baseline and 8 weeks, the mean values for the respective groups (bar) are shown. Wide interindividual variations in response to α-tocopherol can be seen, particularly in the groups receiving 200 and 800 IU/d. *$P<.05$; †$P<.01$; ‡$P<.001$.

in this study (50 IU/d) has not been shown to decrease the susceptibility of LDL to oxidation.

Most studies that have examined the effect of α-tocopherol on LDL oxidation, including those from this laboratory,[33,37] have used doses ranging from 800 to 1600 IU/d.[34-36] The findings in this study agree with these studies that α-tocopherol decreases the susceptibility of LDL to oxidation although present results suggest that 400 IU/d α-tocopherol can decrease the susceptibility of LDL to oxidation. Abbey et al[58] report that 200 IU/d α-tocopherol could decrease the oxidative susceptibility of LDL. However, the authors used a combined supplement that also included ascorbate and β-carotene. The authors point out in their discussion that although multiple regression analysis supported the hypothesis that α-tocopherol was the major antioxidant, β-carotene was also correlated with the change in α-tocopherol, and in turn the oxidizability of LDL. They suggest that ascribing the antioxidant effect to α-tocopherol alone should be treated with caution. Since these investigators did not use a parallel group that received α-tocopherol alone, their study design does not allow them to ascribe the decreased oxidation of LDL solely to α-tocopherol.

By decreasing LDL oxidation in normal volunteers, α-tocopherol prevented a proatherogenic biological effect, ie, cytotoxicity to porcine aortic endothelial cells.[59] Also, two groups have demonstrated a preservation of endothelium-dependent vasodilation by α-tocopherol supplementation in rabbits rendered hypercholesterolemic through cholesterol feeding[60,61]; α-tocopherol also inhibited in vitro lipoprotein oxidation in both studies. α-Tocopherol has other antiatherogenic effects in addition to inhibition of LDL oxidation, eg, reducing platelet aggregation and adhesion.[62,63] Furthermore, α-tocopherol can decrease smooth muscle cell proliferation by inhibiting protein kinase C activity.[64] Thus, it is possible that α-tocopherol could exert an antiatherogenic effect at lower dosages if these dosages can significantly affect platelet function and smooth muscle cell proliferation. It would be difficult to assess the effect of α-tocopherol supplementation on smooth muscle cell proliferation in human subjects; however, examining the

TABLE 5. Spearman Rank Correlation Coefficients of α-Tocopherol Levels and LDL Oxidation Kinetics

	TBARS, % Change		Conjugated Dienes, % Change	
α-Tocopherol, % Change	Lag Phase	Oxidation Rate	Lag Phase	Oxidation Rate
Plasma	0.60‡	−0.53‡	0.59‡	−0.63‡
Plasma, lipid standardized	0.55‡	−0.52‡	0.57‡	−0.61‡
LDL	0.48‡	−0.30*	0.44†	−0.44†

Correlations were performed on the percent change in α-tocopherol levels, lag phase, and oxidation rate from 48 subjects. *$P=.05$, †$P<.01$, ‡$P<.001$.

effect of α-tocopherol on smooth muscle cell proliferation in experimental models of atherosclerosis could prove very instructive. Insight could also be gained by examining the dose-response effects on monocytes and macrophages by assaying the release of cytokines and growth factors from human mononuclear cells, such as interleukin-1 and platelet-derived growth factor, which appear to stimulate smooth muscle cell proliferation.[65] Reports on the inhibitory effect of α-tocopherol on platelet aggregation appear to conflict.[62,66-68]

In conclusion, the results of the present study show that in a randomized placebo-controlled dose-response design, the minimum dose of α-tocopherol needed to significantly decrease the susceptibility of LDL to copper-catalyzed oxidation is 400 IU/d. Future studies should concentrate on dosages of at least 400 IU/d to ascertain the effects of α-tocopherol supplementation on cardiovascular end points in primary and secondary prevention trials.

Acknowledgments

This work was supported by research grants from the American Heart Association (Texas Affiliate), Hoffman-La Roche, and General Clinical Research Center grant No. M01-RR00633. We thank Dr S.M. Grundy for support, Dr A. Bendich for her assistance with study design and critical discussions, and Nancy Cade for manuscript preparation.

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