

A randomized, single-blind, placebo-controlled trial of the effects of 200 mg α -tocopherol on the oxidation resistance of atherogenic lipoproteins¹⁻³

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ABSTRACT Supplementation with high doses of α -tocopherol has increased the oxidation resistance of LDL in many clinical trials. There have been only a few placebo-controlled trials in healthy persons of α -tocopherol doses usually contained in dietary supplements. We carried out a single-blind, placebo-controlled, randomized trial to examine the effect of 200 mg *RRR*- α -tocopheryl acetate/d on the oxidation resistance of atherogenic lipoproteins (VLDL+LDL including intermediate-density lipoproteins) in 40 smoking men. VLDL+LDL oxidation resistance was assessed as conjugated dienes after copper induction and hemin degradation after hydrogen peroxide induction. Also, the LDL total peroxyl-radical trapping antioxidant parameter (LDL TRAP) and plasma malondialdehyde were measured at baseline and after 2 mo of supplementation. Plasma *RRR*- α -tocopherol concentrations were measured at 2-h intervals for 12 h at baseline and after 2 mo of supplementation. Compared with placebo, 200-mg *RRR*- α -tocopheryl acetate supplementation elevated plasma and VLDL+LDL α -tocopherol concentrations, LDL TRAP, and oxidation resistance of VLDL+LDL. Plasma α -tocopherol increased by 88% ($P < 0.0001$), VLDL+LDL α -tocopherol increased by 90% ($P < 0.0001$), and LDL TRAP by 58% ($P < 0.0001$). The time to the start of oxidation (lag time) was prolonged by 34% when assessed with a copper-induced method and by 109% when assessed with a hemin + hydrogen peroxide-induced method; the time to maximal oxidation was prolonged by 21% (copper-induced method) in the vitamin E-supplemented group. Changes in plasma α -tocopherol, lipid-standardized α -tocopherol, and VLDL+LDL α -tocopherol correlated significantly with changes in LDL TRAP, lag time, and time to maximal oxidation. Differences in changes between groups in the area under the curve for plasma α -tocopherol were significant ($P < 0.009$). Our results suggest that 200 mg oral *RRR*- α -tocopheryl acetate/d had a clear effect on the in vitro oxidation of VLDL+LDL in smoking men. *Am J Clin Nutr* 1998;68:1034-41.

KEY WORDS Vitamin E, lipid peroxidation, low density lipoprotein, very-low-density lipoprotein

INTRODUCTION

There is a growing body of evidence concerning the atherogenicity of oxidized LDL (1-3). Despite the many studies about the oxidative hypothesis of atherosclerosis and the preventive

role of antioxidants, more precise information is still needed to fully understand their role in vivo.

Lipid peroxidation is the free radical-mediated oxidation of membrane lipids preceded by a chain reaction that is influenced by antioxidants and prooxidants. It is suggested that radical-mediated lipid oxidation proceeds by a similar mechanism in isolated LDLs and VLDLs (4) even though the oxidation of VLDL+LDL, which are atherogenic (5), has not been studied as much as the oxidation of LDL alone. The best known prooxidants are the reactive free radicals present in the gas and tar phases of cigarette smoke (6) and catalytic transition metals such as copper, iron, and mercury. Redox active transition metals, including copper, have been detected in human atherosclerotic lesions (7). However, the most convincing evidence for the atherogenicity of oxidized LDLs and preventive effects of antioxidants comes from animal studies. Studies in WHHL rabbits and cholesterol-fed rabbits have shown the effect of the synthetic antioxidants probucol (8-10) and butylated hydroxytoluene (BHT) (11) in the prevention of lipid peroxidation and of atherogenesis. However, these compounds possess side effects and are not suitable for humans (12, 13).

LDLs and VLDLs contain several antioxidative vitamins that can protect atherogenic lipoproteins against oxidative modification. The quantitatively most important antioxidant present in LDLs is α -tocopherol, followed by retinyl stearate, γ -tocopherol, β -carotene, and lycopene (14). As measured by the capability to prevent oxidative modification of LDLs in high radical flow conditions in vitro, the most important and active antioxidant is also thought to be α -tocopherol (15), which generally has no side effects even at high doses (16, 17).

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²Supported by grants from Ferrosan A/S, Søborg, Denmark; the Yrjö Jahnsson Foundation, Helsinki; and the Austrian Science Foundation (project F709), Vienna. The tocopherol tablets and placebo were kindly supplied by Ferrosan A/S.

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Received November 25, 1997.

Accepted for publication April 14, 1998.

Antioxidant supplementation has increased the resistance of LDLs to oxidative modification in several studies (6, 18–27). Nevertheless, only a few groups of investigators have studied the effect of doses usually contained in α -tocopherol supplements on atherogenic lipoprotein oxidation in healthy humans. In all of these studies, small numbers of subjects were supplemented and most studies had no randomized control group.

The purpose of this randomized, single-blind, placebo-controlled study was to examine the effect of supplementing smoking men with 200 mg *RRR*- α -tocopheryl acetate (the ester of the most active isomer of the vitamin E family) on the oxidation resistance of VLDL+LDL, including intermediate-density lipoproteins, and the plasma concentration of *RRR*- α -tocopherol in a separate substudy.

SUBJECTS AND METHODS

Study subjects

The present study was part of the Multiple Antioxidant Supplementation Intervention Study, a randomized, single-blind, parallel, placebo-controlled, 2-mo trial of the effect of antioxidant supplementation on various pharmacokinetic and efficacy indexes of oxidative stress and lipid peroxidation in smoking men (28–30). Sixty healthy men aged 47 ± 6 y ($\bar{x} \pm$ SD) who smoked regularly > 10 cigarettes/d and who had no specific contraindications [regular intake of any drug with antioxidative properties or acetylsalicylic acid, a body mass index (BMI; in kg/m^2) > 31 , type 1 diabetes, or severe disease] were recruited by newspaper advertisements from eastern Finland. All subjects completed the trial and provided informed, written consent. The Research Ethics Committee of the University of Kuopio approved the study protocol and the study was conducted according to the Good Clinical Practice procedures and the Declaration of Helsinki (31).

Two-month supplementation study

To assess the effect of α -tocopherol and α -tocopherol plus ascorbic acid supplementation on atherogenic lipoprotein (VLDL+LDL) oxidation resistance, subjects were randomly assigned to 1 of 3 groups (20 men in each group) to receive 200 mg *RRR*- α -tocopheryl acetate/d (100 mg twice a day; vitamin E group), 200 mg *RRR*- α -tocopheryl acetate/d (100 mg twice a day) plus 500 mg slow-release ascorbic acid/d (250 mg twice a day) in one tablet, or placebo for 2 mo. Subjects were instructed to take the tablets with meals and milk to achieve better absorption. Treatment with *RRR*- α -tocopheryl acetate alone resulted in plasma concentrations of α -tocopherol that were ≈ 3 times higher than concentrations after treatment with α -tocopheryl acetate plus slow-release ascorbic acid (28). Because of this discrepancy in plasma α -tocopherol concentrations, the combination group was disregarded for data analysis. The plasma concentration of α -tocopherol, malondialdehyde, vitamin C, coenzyme Q-10 (coQ10), and β -carotene; the total antioxidative capacity of LDL [total peroxy-radical trapping antioxidant parameter (LDL TRAP)]; and the oxidation resistance and oxidation susceptibility of VLDL+LDL were measured in 40 men at the beginning and at the end of a 2-mo supplementation period.

Twelve-hour substudy of short-term changes in plasma α -tocopherol

Five men from each group were randomly chosen for a 12-h substudy that was carried out at baseline and after 2 mo of *RRR*-

α -tocopheryl acetate supplementation to assess the plasma concentration of α -tocopherol and the direct effect on lipoprotein oxidation resistance of one tablet containing *RRR*- α -tocopheryl acetate. Subjects were asked to fast for 12 h before, not to eat food containing vitamin E (roe, cod-liver oil, and nuts) for 1 d before, and not to consume alcohol for 2 d before the baseline and termination visits. After the first blood sample (day 0 sample) was drawn in the morning, the breakfast containing bread, margarine, salami, cheese, coffee or tea, and milk was served with the dose of 100 mg *RRR*- α -tocopheryl acetate or placebo. Blood samples for α -tocopherol and vitamin C measurements were obtained every 2 h and samples for the determination of LDL TRAP and VLDL+LDL oxidation induced by hemin+hydrogen peroxide were obtained 6 h after the day 0 sample was drawn. The last sample for α -tocopherol measurement was taken 12 h after the day 0 sample was drawn; thereafter, the subjects took the second dose of *RRR*- α -tocopheryl acetate or placebo and resumed their normal eating habits. Lunch (the same as breakfast plus white rice and meat stew) was served after the second blood sample was drawn and dinner (same as lunch) was served after the fifth sample was drawn. A sweet cookie was served after the fourth sample was drawn. Subjects were not allowed to eat anything else during the substudy. Smoking and drinking coffee, tea, or water were allowed during the study.

To confirm the VLDL+LDL oxidation results of the substudy in the LDL fraction, 5 men were asked to participate in a second substudy. The men received 200 mg *RRR*- α -tocopheryl acetate/d (100 mg twice a day) for 2 wk. In this second substudy, blood samples for determination of α -tocopherol and VLDL+LDL and LDL oxidation susceptibility were drawn 6 and 12 h postdosing. Otherwise, the study followed the protocol of the first substudy.

Plasma samples

Blood samples were obtained by venipuncture after a 12-h fast and collected into vacuum tubes (Venoject; Terumo, Leuven, Belgium). Blood for lipoprotein fractionation and LDL TRAP, plasma malondialdehyde, and coQ-10 measurements were drawn into EDTA-containing tubes and placed in an ice bath. Plasma was separated by centrifugation at $3500 \times g$ for 15 min at 4°C and frozen in aliquots at -80°C . Lipoprotein fractionation and atherogenic lipoprotein oxidation measurements were done from frozen plasma within 1 wk after blood was drawn. Samples for the second substudy were handled and measured immediately. Blood for α -tocopherol, vitamin C, and β -carotene measurements were collected in tubes containing lithium and heparin and plasma samples were stored at -80°C until used. Plasma vitamin concentrations in blood samples taken at baseline and 2 mo postdosing were measured in the same batch after the study ended.

Isolation of VLDL+LDL

VLDL+LDL were isolated as detailed previously (32) from fresh, EDTA-containing plasma by ultracentrifugation based on density-gradient ultracentrifugation (potassium bromide gradient). All solutions were made in metal-free water (Super Q Plus; Millipore, Bedford, MA). The tubes were centrifuged in an XL-90 ultracentrifuge (Beckman, Palo Alto, CA) with a 50.4 Ti rotor (Beckman) at $110300 \times g$ for 23 h at 4°C . The top layer from the tube (density: $< 1.063 \text{ kg}/\text{L}$) was collected with a Pasteur pipette and assayed immediately for susceptibility to oxidation.

Isolation of LDL

LDL was separated from fresh EDTA-containing plasma by short, single-step ultracentrifugation. The plasma samples were adjusted to a density of 1.24 kg/L by using potassium bromide and were layered underneath a solution with a density of 1.006 kg/L. The tubes were centrifuged for 2.5 h at 10°C and $417\,000 \times g$ with an Optima TLX ultracentrifuge and a TLA-100.4 rotor (Beckman). The LDL fraction was seen in the middle of the tube and was removed with a needle; the total volume was 800 μ L.

VLDL+LDL or LDL oxidation by copper chloride and hemin+hydrogen peroxide

The *in vitro* oxidation of VLDL+LDL was assessed by induction with either copper (32, 33) or hemin + hydrogen peroxide (20). Briefly, for copper-induced VLDL+LDL oxidation, EDTA and other compounds with a low molecular weight were removed chromatographically with small gel-filtration columns. Copper chloride was added to start the formation of conjugated dienes, which was assessed spectrophotometrically at 234 nm. Lag time to maximal oxidation rate (lag time), maximum reaction velocity (V_{max}), maximum absorbance (A_{max}), and the time to maximal oxidation (A_{max} time) were determined from the reaction kinetics as described previously (32). Hemin+hydrogen peroxide-induced VLDL+LDL oxidation was assayed photometrically at 405 nm in a microtiter plate reader. Lag time and the V_{max} of hemin degradation were determined as described previously (20).

Determination of α -tocopherol

Heparin-treated plasma and the VLDL+LDL fraction were extracted with ethanol and hexane for determination of α -tocopherol by reversed-phase HPLC (32, 34). Because plasma vitamin E has strong associations with serum concentrations of both triacylglycerols (0.45, $P < 0.001$) and LDL cholesterol (0.53, $P < 0.001$), we also used lipid-standardized vitamin E in the present analysis (32).

Determination of LDL TRAP

LDL TRAP was measured from precipitated LDL as described previously (29) with a model 1251 luminometer (Bio-Orbit, Turku, Finland).

Determination of plasma malondialdehyde

Malondialdehyde was measured from frozen, EDTA-containing plasma after thiobarbituric acid (TBA) reaction by using a slight modification of the HPLC method described by Rabl et al (35). The plasma samples were thawed immediately before the assay and 100 μ L was mixed with 100 μ L water, 300 μ L of 0.15 mol phosphoric acid/L, 10 μ L BHT 0.2% methanolic solution, and 100 μ L 0.6% TBA and incubated at 95°C for 60 min. The chromogen was extracted with 1.25 mL butanol-1 and analyzed by HPLC with fluorometric detection (excitation wavelength: 525 nm; emission wavelength: 550 nm). The malondialdehyde-TBA adduct was calibrated with tetramethoxypropane standard solutions and processed as were the plasma samples.

Other measurements

Serum LDL cholesterol was precipitated by using polyvinyl sulfate (Boehringer Mannheim, Mannheim, Germany) and calculated as the difference between total and supernatant cholesterol. The serum HDL-cholesterol concentration was determined after

precipitation with magnesium chloride dextran sulfate. Serum total cholesterol (Kone Diagnostics, Espoo, Finland) and triacylglycerol (Boehringer Mannheim) concentrations were determined enzymatically with an autoanalyzer (Kone Specific, Kone Ltd, Espoo, Finland). Plasma ascorbic acid (29) and dehydroascorbic acid (36), β -carotene and lycopene (34), and coQ-10 (30) concentrations were determined by HPLC as described previously.

Statistics

The statistical analyses were carried out by using SPSS statistical software (version 5.0; SPSS Inc, Chicago). Differences in changes between the placebo and vitamin E groups were tested with Wilcoxon's rank-sum test. To separate the effect of α -tocopherol from that of serum lipids, lipid-standardized α -tocopherol concentrations were used in the statistical analysis. The standardization procedure was described in detail previously (37). The association between 2-mo changes in α -tocopherol (plasma, VLDL+LDL, and lipid standardized) and indicators of blood antioxidative capacity were evaluated by using Pearson's correlation coefficients. The area under the curve (AUC) for plasma α -tocopherol from zero to the last observed point was used in the first substudy. It was calculated according to the trapezoidal rule (38) after the baseline value obtained before the first dose was subtracted from the values obtained after supplementation. Wilcoxon's rank-sum test was used to compare the heterogeneity of the AUC.

RESULTS

Two-month supplementation study

The study participants in both groups were healthy, middle-aged (mean: 47 y) men who were heavy smokers (mean: 21 cigarettes/d). Their smoking habits, serum triacylglycerol, and serum total, LDL-, and HDL-cholesterol concentrations did not change significantly during the 2 mo of supplementation (Table 1). At baseline, the LDL-cholesterol concentration was higher in the placebo group than in the vitamin E group, but not significantly so (Table 1).

Mean baseline concentrations of plasma and VLDL+LDL α -tocopherol were similar in both groups (Table 2). Two months of *RRR*- α -tocopheryl acetate supplementation (200 mg/d) significantly increased α -tocopherol concentrations in both plasma and in VLDL+LDL as compared with placebo (Table 2). During supplementation, the mean plasma α -tocopherol concentration increased from 29.6 to 55.5 μ mol/L (by 88%; 95% CI: 73%, 102%) and VLDL+LDL α -tocopherol increased from 18.3 to 34.7 μ mol/L (by 90%; 95% CI: 68%, 111%) in the vitamin E group. There were no significant changes in either plasma or VLDL+LDL α -tocopherol in the placebo group and no significant changes in the other plasma antioxidants (ascorbic acid, dehydroascorbic acid, coQ-10, β -carotene, and lycopene) in either group during the supplementation period. There was no significant change in plasma malondialdehyde during the 2-mo supplementation period in either group (Table 2); however, the increase in plasma malondialdehyde correlated significantly with the increase in plasma and VLDL+LDL vitamin E concentrations (Table 3).

RRR- α -tocopheryl acetate supplementation significantly elevated LDL TRAP (by 58%; 95% CI: 42%, 73%) compared with placebo (Table 2). In all subjects, the increase in LDL TRAP cor-

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RRR- α -tocopheryl acetate supplementation significantly elevated LDL TRAP (by 58%; 95% CI: 42%, 73%) compared with placebo (Table 2). In all subjects, the increase in LDL TRAP cor-

TABLE 1

Baseline values and changes in smoking habits and in serum total cholesterol, LDL-cholesterol, HDL-cholesterol, and triacylglycerol concentrations in smoking men before and after supplementation with α -tocopherol or placebo for 2 mo¹

	Vitamin E		Placebo		P for difference in change between groups ²
	Baseline	Change	Baseline	Change	
Smoking (cigarettes/d)	20.5 \pm 1.1 ³	0 \pm 0	21.5 \pm 2.0	0 \pm 0.4	1.000
Total cholesterol (mmol/L)	5.48 \pm 0.30	0.02 \pm 0.09	5.78 \pm 0.21	-0.21 \pm 0.11	0.108
LDL cholesterol (mmol/L)	3.59 \pm 0.32 [19]	0.01 \pm 0.15 [19]	4.11 \pm 0.21	-0.25 \pm 0.13	0.097
HDL cholesterol (mmol/L)	1.07 \pm 0.07	0.04 \pm 0.03	1.01 \pm 0.04	0.08 \pm 0.05	0.871
Triacylglycerol (mmol/L)	1.89 \pm 0.25	0.24 \pm 0.39	1.66 \pm 0.18	-0.30 \pm 0.18	0.465

¹n = 20, except where otherwise indicated in brackets.

²Wilcoxon's rank-sum test.

³ $\bar{x} \pm$ SEM; n = 20.

related significantly with increases in plasma α -tocopherol ($r = 0.70$), lipid-standardized plasma α -tocopherol ($r = 0.73$), and VLDL+LDL α -tocopherol ($r = 0.66$) (Table 3).

Lipid peroxidation susceptibility, measured as copper- and hemin+hydrogen peroxide-induced VLDL+LDL oxidation, decreased significantly during the 2-mo supplementation period in the vitamin E group. As assessed by copper-induced oxidation, lag time increased by 34% (95% CI: 23%, 45%) and Amaxtime increased by 21% (95% CI: 11%, 31%). As shown in Table 3, increases in both lag time and Amaxtime were associated with increases in α -tocopherol. Lag time increased by 109% (95% CI: 50%, 169%) when the oxidation susceptibility of VLDL+LDL was induced by hemin+hydrogen peroxide (Table 2). Also, Vmax—measured as hemin+hydrogen peroxide-induced VLDL+LDL oxidation—decreased by 30% (95% CI: 3%, 57%) after 2 mo of RRR-

α -tocopheryl acetate supplementation. The decrease in Vmax was associated with an increase in plasma α -tocopherol (Table 3).

Substudy of short-term changes in plasma α -tocopherol

Substudy 1

At baseline, mean concentrations of α -tocopherol, VLDL+LDL α -tocopherol, reduced ascorbic acid, and dehydroascorbic acid; LDL TRAP; and Vmax and lag time as assessed by hemin + hydrogen peroxide-induced VLDL+LDL oxidation were similar in the vitamin E and placebo groups. The maximal plasma α -tocopherol concentration was achieved in the vitamin E group \approx 10 h after the first supplement (Figure 1). Ten hours after the first dose of RRR- α -tocopherol, the plasma α -tocopherol concentration had increased by 33% in the vitamin E

TABLE 2

Baseline values and changes in plasma α -tocopherol and indicators of blood antioxidative capacity in smoking men before and after supplementation with α -tocopherol or placebo for 2 mo¹

	Vitamin E		Placebo		P for difference in change between groups ²
	Baseline	Change	Baseline	Change	
Plasma α -tocopherol (μ mol/L)	29.6 \pm 2.0 ³	25.9 \pm 2.1	29.3 \pm 1.2	-1.3 \pm 0.8	<0.0001
Lipid-standardized α -tocopherol	1.01 \pm 0.03	0.55 \pm 0.05	1.00 \pm 0.03	-0.13 \pm 0.01	<0.0001
VLDL+LDL α -tocopherol (μ mol/L)	18.3 \pm 1.7	16.4 \pm 1.8	17.9 \pm 1.0	-1.3 \pm 0.7	<0.0001
Plasma AA (μ mol/L)	57.2 \pm 4.8	-2.5 \pm 4.9	59.3 \pm 4.3 [18]	-4.5 \pm 5.6 [18]	0.483
Plasma DHA (μ mol/L)	1.82 \pm 0.74 [19]	1.19 \pm 1.06 [19]	1.95 \pm 0.61 [15]	0.59 \pm 1.27 [15]	0.656
Plasma coQ-10 (μ mol/L)	1.16 \pm 0.12	0 \pm 0.05	0.89 \pm 0.07	-0.01 \pm 0.06	0.850
VLDL+LDL coQ-10 (μ mol/L)	1.15 \pm 0.10	-0.07 \pm 0.07	1.02 \pm 0.07	-0.03 \pm 0.06	0.598
Plasma β -carotene (μ mol/L)	0.23 \pm 0.03	-0.01 \pm 0.02	0.27 \pm 0.03	-0.01 \pm 0.02	0.704
Plasma lycopene (μ mol/L)	0.17 \pm 0.03	0.03 \pm 0.02	0.21 \pm 0.03	-0.00 \pm 0.02	0.255
LDL TRAP (pmol/nmol cholesterol)	13.3 \pm 0.9 [19]	7.7 \pm 1.0 [19]	12.3 \pm 0.8	-0.2 \pm 0.7	<0.0001
Plasma malondialdehyde (μ mol/L)	0.40 \pm 0.03	0.03 \pm 0.04	0.40 \pm 0.03	-0.05 \pm 0.03	0.090
Copper-induced VLDL+LDL oxidation					
Lag time (min)	82 \pm 2 [19]	28 \pm 4 [19]	74 \pm 2 [19]	1 \pm 2 [19]	<0.0001
Amaxtime (min)	145 \pm 6 [19]	30 \pm 7 [19]	134 \pm 6 [19]	-2 \pm 6 [19]	0.001
Vmax (mabs/min)	9.1 \pm 0.4 [19]	-0.9 \pm 0.5 [19]	10.6 \pm 0.6 [19]	0 \pm 0.5 [19]	0.255
Amax (abs)	0.568 \pm 0.018 [19]	-0.004 \pm 0.026 [19]	0.597 \pm 0.014 [19]	0.005 \pm 0.020 [19]	0.896
Hemin+hydrogen peroxide-induced VLDL+LDL oxidation					
Lag time (min)	137 \pm 13	150 \pm 39	113 \pm 9	5 \pm 10	<0.0001
Vmax (mabs/min)	0.7209 \pm 0.068	-0.2169 \pm 0.094	0.8425 \pm 0.050	0.0078 \pm 0.044	0.0023

¹n = 20, except where otherwise indicated in brackets. AA, reduced ascorbic acid; DHA, dehydroascorbic acid; coQ-10, coenzyme Q-10; TRAP, total peroxyl-radical trapping antioxidant parameter; Amaxtime, time to maximal oxidation; Vmax, maximum reaction velocity; Amax, maximum absorbance; abs, absorbance; mabs, milliabsorbance.

²Wilcoxon's rank-sum test.

³ $\bar{x} \pm$ SEM.

TABLE 3
Pearson's coefficients for correlations between 2-mo changes in plasma α -tocopherol, lipid-standardized plasma α -tocopherol, and VLDL+LDL α -tocopherol concentrations and indicators of blood antioxidative capacity¹

	Plasma α -tocopherol	Lipid-standardized plasma α -tocopherol	VLDL+LDL α -tocopherol
Copper-induced VLDL+LDL oxidation			
Lag time	0.65 ²	0.46 ⁴	0.57 ²
Amaxtime	0.55 ²	0.28	0.56 ²
Vmax	-0.29	-0.05	-0.29
Amax	-0.10	0.05	-0.06
Hemin + hydrogen peroxide-induced VLDL+LDL oxidation			
Lag time	-0.02	-0.07	0.04 ²
Vmax	-0.42 ²	-0.21	-0.46 ²
LDL TRAP	0.70 ²	0.73 ²	0.66 ²
Plasma malondialdehyde	0.36 ⁴	0.25	0.38 ⁴

¹*n* = 38. Amaxtime, time to maximal oxidation; Vmax, maximum reaction velocity; Amax, maximum absorbance; TRAP, total peroxy-radical trapping antioxidant parameter.

²*P* < 0.001.

³*P* < 0.005.

⁴*P* < 0.05.

group and decreased by 9% in the placebo group. The difference in the change between the 2 groups was significant (*P* = 0.009), even when the increase was assessed by using the AUC (Table 4). After the 2-mo supplementation period, the fasting plasma α -tocopherol concentration had increased by 74% in the vitamin E group and the AUC was significantly different between groups (Table 4). As shown in Figure 1, plasma vitamin E concentrations increased over the first 10 h of the baseline period and in the first 8 h of the supplementation period.

Mean (\pm SD) reduced ascorbic acid concentrations in plasma before the first dose of α -tocopherol were 50.8 ± 8.8 μ mol/L in

the vitamin E group and 54.1 ± 23.7 μ mol in the placebo group. There were no significant differences in plasma total, reduced, or dehydroascorbic acid AUC values between the vitamin E and placebo groups either at baseline or the 2-mo visit (Table 4).

LDL TRAP α -tocopherol equivalency as well as VLDL+LDL oxidation susceptibility and resistance measured by hemin + hydrogen peroxide were determined at baseline and 6 h after the first dose of RRR- α -tocopheryl acetate. The mean lag time was prolonged at 6 h from 151 to 221 min (by 46%) and the mean Vmax decreased by 19%. In the placebo group there was only a small change (Table 4). However, the change

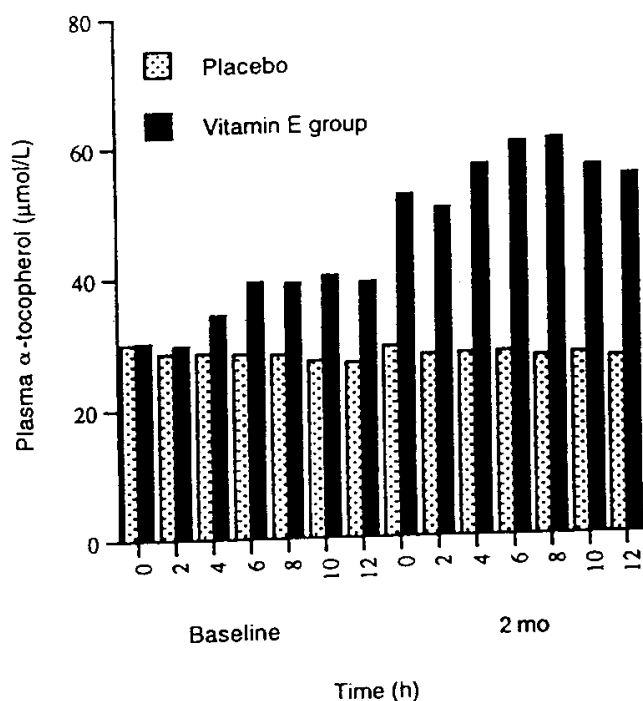


FIGURE 1. Changes in mean α -tocopherol concentrations in plasma after the first 100-mg dose of RRR- α -tocopheryl acetate or placebo and after 2 mo of supplementation.

TABLE 4

Area under the curve (AUC) for plasma α -tocopherol and ascorbic acid concentrations and 6-h changes in lipoprotein resistance to oxidation in the 12-h study¹

	Vitamin E	Placebo	<i>P</i> for difference between groups ²
AUC (0–12 h), baseline (μ mol·h/L)			
Plasma α -tocopherol	73	-21	0.009
Plasma reduced ascorbic acid	-79	-131	0.465
Plasma dehydroascorbic acid	22	11	0.465
Plasma total ascorbic acid	-57	-120	0.347
AUC (0–12 h), 2-mo (μ mol·h/L)			
Plasma α -tocopherol	291	-24	0.009
Plasma reduced ascorbic acid	108	-123	0.175
Plasma dehydroascorbic acid	3	11	0.917
Plasma total ascorbic acid	112	-112	0.175
Lag time change (0–6 h) in hemin + hydrogen peroxide-induced VLDL+LDL oxidation			
Baseline (min)	70	28	0.530
2 mo (min)	273	28	0.047
LDL TRAP change (0–6 h)			
Baseline (pmol/nmol cholesterol)	3.4	0.6	0.047
2 mo (pmol/nmol cholesterol)	2.3	-1.1	0.251

¹ \bar{x} ; *n* = 5. For AUC, the initial value was subtracted from each value for the baseline and 2-mo visit. TRAP, total peroxy-radical trapping antioxidant parameter.

²Wilcoxon's rank-sum test.

in lag time did not differ significantly between groups. After 2 mo of vitamin E supplementation, the mean fasting lag time was 319 min and increased significantly to 424 min (by 33%) at 6 h. The change from baseline in LDL TRAP differed significantly between the 2 groups at 6 h; it increased by 26% in the vitamin E group and by 5% in the placebo group. After 2 mo of vitamin E supplementation, the difference in change for LDL TRAP between the 2 groups at 6 h was not significant.

Substudy 2

At baseline, lag time assessed by copper-induced VLDL+LDL oxidation increased by 6% and lag time assessed by hemin + hydrogen peroxide-induced VLDL+LDL oxidation increased by 20%. At 12 h, lag time assessed by copper-induced VLDL+LDL oxidation increased by 19%. After 2 wk of supplementation, the mean lag times of VLDL+LDL oxidation were 25% higher with the copper-induced method and 45% higher with the hemin + hydrogen peroxide-induced method than values determined before the first dose was given. The mean lag time in LDL oxidation was 13% higher than that before the first dose after 2 wk of supplementation. There was no change in lag time, regardless of the method used, during the 12-h substudy after 2 wk of supplementation. The correlation in lag times between LDL and VLDL+LDL fractions, when results from baseline measurements were pooled ($n = 15$), was 0.60 ($P = 0.02$). When all lag time results were pooled ($n = 30$), the correlation between fractions was 0.37 ($P = 0.04$).

DISCUSSION

In the present randomized, single-blind, parallel, placebo-controlled trial, a 200-mg dose of the natural isomer of vitamin E (*RRR*- α -tocopheryl acetate; 100 mg twice a day) for 2 mo increased plasma and VLDL+LDL α -tocopherol concentrations, the total antioxidative capacity of LDL, and the oxidation resistance of atherogenic plasma lipoproteins in smoking men. Even though the plasma vitamin E concentration increased by 88%, LDL TRAP rose by only 58% and the oxidation resistance of VLDL+LDL by only 34% when measured with the copper-induced method. However, when oxidation resistance was measured after induction with hemin + hydrogen peroxide, the lag time increased by 109%. Supplementation did not affect plasma concentrations of reduced ascorbic acid, dehydroascorbic acid, coQ-10, and β -carotene, or serum concentrations of triacylglycerols and HDL and LDL cholesterol, which all influence the susceptibility of LDL to oxidation *in vitro*.

The favorable effect of vitamin E on plasma lipoprotein oxidation resistance agrees with the findings of many previous trials concerning vitamin E supplementation. However, many of these studies had only a few, relatively young and healthy subjects, who were given larger doses of synthetic vitamin E than the doses given in our study and therefore the results are not comparable with ours. The aim of our study was to test the hypothesis that in a well-controlled study, subjects who smoked heavily and therefore were exposed to chronic free radical stress could benefit from a 2-mo dose of the natural isomer of α -tocopherol acetate usually contained in dietary supplements.

Dieber-Rotheneder et al (18) supplemented healthy, nonsmoking subjects with 101–805 mg *RRR*- α -tocopherol/d for 21 d. They showed that the oxidation resistance of LDL could be increased by vitamin E supplementation. However, in their study

they had only 2 subjects in each supplemented group; therefore, they were not able to make proper statistical analyses to determine the minimum dose of α -tocopherol needed to increase the oxidation resistance.

Princen et al (23) gave 6 healthy, nonsmoking volunteers 1000 mg *all-rac*- α -tocopheryl acetate/d for 7 d and found that the oxidation resistance increased and the oxidation rate decreased significantly. In their study there was no control group and they used the synthetic form of vitamin E rather the natural form, which we used. Jialal and Grundy (21) supplemented 12 subjects with 727 mg *all-rac*- α -tocopherol/d and 12 subjects received placebo for 12 wk. They showed that α -tocopherol supplementation resulted in an increase in plasma and LDL α -tocopherol concentrations and in a decreased susceptibility of LDL to oxidation.

On the basis of the dose-response study by Jialal et al (39), the minimum dose of α -tocopherol needed to increase the resistance of LDL to oxidation is ≈ 364 mg/d. Devaraj et al (27) compared *RRR*- α -tocopherol with *all-rac*- α -tocopherol, also dose-responsively, and found that the lag phases of oxidation were significantly prolonged with doses ≥ 268 mg/d (400 IU/d). However, Princen et al (24) observed that supplementation with as little as 25 mg vitamin E/d led to a significant increase in the resistance of LDL to oxidation, but only after intakes of 400 and 800 mg vitamin E/d was the V_{max} significantly reduced. Their study was a sequential study in which they continued to increase the dose of vitamin E in the same 20 subjects from 25 to 800 mg/d for 12 wk at 2-wk intervals. There were no washout periods between different sequential doses and there was no control group. Wander et al (26) observed in their sequential study that supplements as low as 100 mg α -tocopheryl acetate/d increased the resistance of LDL to oxidation when fish-oil supplements were used. They studied 48 women taking or not taking hormone replacement therapy in a crossover study. Each of the 4 periods lasted 5 wk and was followed by a 4-wk washout interval. During each period, all subjects were given a 15-g supplement of fish oil and either placebo or 100, 200, or 400 mg *RRR*- α -tocopheryl acetate/d.

Bowry and Stocker (40) proposed that α -tocopherol can also be a prooxidant in isolated LDL. The elevation of plasma malondialdehyde during the α -tocopherol supplementation in this study, though not statistically significant, provided some support for the theory that α -tocopherol makes lipoproteins more reactive to radical oxidants in low radical flux conditions (41). Also, the increase in plasma malondialdehyde had a positive, though weak, correlation with the elevation of plasma α -tocopherol. However, the suggested point at which α -tocopherol switches from a prooxidant to an antioxidant for copper-initiated LDL oxidation is reached at copper-LDL ratios of $\approx 3:1$ (42); in our study this ratio was 33:1.

The elevation of plasma (by 88%) α -tocopherol concentration in the present study was of the same magnitude as achieved in previous studies (18, 24, 39), which used doses of α -tocopherol similar to those we used. In our study, we also measured VLDL+LDL α -tocopherol, whereas others measured α -tocopherol in just LDL. We used the combined fraction VLDL+LDL instead of LDL in oxidation resistance measurements for reasons explained previously (32). Also, it has been shown that radical-mediated lipid oxidation proceeds via a similar mechanism in isolated LDL and VLDL (4), and not only modified LDL but also modified IDL and VLDL have been suggested to be atherogenic (5). In our substudy, the correlation between LDL and VLDL+LDL oxidation susceptibility measurements at baseline

was high. However, after 2 wk of *RRR*- α -tocopheryl acetate supplementation, the correlation between measurements got smaller. This may have been a consequence of differential incorporation of α -tocopherol into different lipoproteins and transfer of α -tocopherol between lipoproteins. Consistent with this, Traber et al (43, 44) found that by 24 h after supplementation VLDL is preferentially enriched with *RRR*- α -tocopherol, and the subsequent catabolism of VLDL allows transfer of *RRR*- α -tocopherol to LDL and HDL. The plasma concentrations of reduced ascorbic acid, coQ-10, and β -carotene were also measured to determine whether α -tocopherol preserves other antioxidants. However, there were no significant changes in either the vitamin E or placebo group.


In our substudy, the maximum elevation in plasma concentrations of α -tocopherol was reached \approx 10 h after the first 100-mg dose of vitamin E. Earlier, Dimitrov et al (45) reported that the plasma concentration of *all-rac*-tocopherol peaked 12–24 h after ingestion of a single dose of 440, 880, or 1320 mg vitamin E. Ferslew et al (46) supplemented 12 healthy, male volunteers with 400 mg *RRR*- α -tocopherol twice a day and estimated that the time to reach maximum concentration was 13.5 h. This difference in results may have been because the absorption of vitamin E is influenced by the intake of dietary fat (47) or because we measured the plasma concentration of α -tocopherol for only 12 h. We gave standardized meals to our subjects, but did not evaluate the effect of dietary fat on the plasma response to vitamin E supplementation. Similarly, in both studies, there were large individual variations in plasma concentrations of α -tocopherol after a single dose of supplement.

Because no standardized oxidation resistance measurement is available, we used 4 different methods, which concerned different phases of the lipid peroxidation process. Both transition metal-derived radicals (copper chloride and hydrogen peroxide) and peroxy radicals [(2,2-azobis(2,4-dimethylvaleronitrile)] were used as lipid peroxidation initiators. Measured lipid peroxidation indicators were conjugated dienes, hemin degradation, or radicals producing luminescence. Also, plasma malondialdehyde was measured as a product of further advanced lipid peroxidation. We used the same techniques in our earlier studies (32, 48–50). All measurements were done in conditions of high radical flux because those in low radical stress are not reproducible. Our measurements have been standardized and their reproducibility has been maximized for large numbers of specimens.

On the basis of the present data, the susceptibility of lipoproteins is influenced by α -tocopherol more when the oxidation reaction is started by hemin + hydrogen peroxide (iron) than by copper. It has also been suggested that iron is a more physiologic prooxidant than is copper. However, the measurement variability of the hemin + hydrogen peroxide method is larger.

It is clear that there are several mechanisms in the LDL particle that scavenge peroxy radicals. TRAP is a fast method to assess the total capacity of the LDL particle to scavenge these radicals. In the present study we observed that 200 mg *RRR*- α -tocopheryl acetate/d increased the total capacity of LDL to scavenge peroxy radicals by more than half. Changes in plasma and lipid-standardized α -tocopherol also correlated significantly with the change in LDL TRAP. These results agree with those of Smith et al (51), who showed that \approx 80% of the antioxidant capacity of LDL is accounted for by α -tocopherol in the samples.

In conclusion, our study showed that 200 mg *RRR*- α -tocopherol elevated plasma and VLDL+LDL α -tocopherol and

increased both the antioxidative capacity and the oxidation resistance of atherogenic lipoproteins. Our data support the notion that *RRR*- α -tocopherol is an effective antioxidant in the protection of atherogenic lipoproteins against oxidation, at least in high radical flux conditions. The effects of vitamin E supplementation on antioxidative capacity and oxidation resistance on the one hand, and on the plasma malondialdehyde concentration on the other hand, were in opposite directions and thus inconsistent. For this reason, further randomized, placebo-controlled supplementation trials are needed. 

We thank the late Herman Esterbauer for the malondialdehyde analyses in Graz, Austria; our laboratory staff for performing the blood analyses; Kimmo Ronkainen for data analysis; and our public health nurses for subject management.

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