

A vitamin E concentrate rich in tocotrienols had no effect on serum lipids, lipoproteins, or platelet function in men with mildly elevated serum lipid concentrations¹⁻³

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ABSTRACT

Background: Tocotrienols, lipid-soluble antioxidants with vitamin E activity, have been reported to lower LDL-cholesterol concentrations and platelet aggregation in men, but results are contradictory.

Objective: To examine in detail the effects of a vitamin E concentrate rich in tocotrienols on serum lipoproteins and on platelet function in men at risk for cardiovascular disease.

Design: In this randomized, double-blind, placebo-controlled parallel trial, 20 men received daily for 6 wk 4 capsules, each containing 35 mg tocotrienols and 20 mg α -tocopherol; 20 other men received 4 capsules daily, each providing 20 mg α -tocopherol. All men had concentrations of serum total cholesterol between 6.5 and 8.0 mmol/L or lipoprotein(a) concentrations > 150 mg/L.

Results: Compliance was confirmed by changes in serum tocopherol and tocotrienol concentrations. Serum LDL cholesterol in the tocotrienol group was 4.80 mmol/L before and 4.79 mmol/L after intervention, and increased from 4.70 to 4.86 mmol/L in the placebo group (95% CI for the difference: -0.54, 0.19 mmol/L; $P = 0.333$). Also, changes in HDL cholesterol, triacylglycerol, lipoprotein(a), and lipid peroxide concentrations did not differ between the groups. After adjustment for differences in initial values, no effects were found on collagen-induced platelet aggregation velocity, maximum aggregation, or thromboxane B₂ formation in citrated whole blood. ATP release, however, was lower in the tocotrienol group. Urinary thromboxane B₂ and 11-keto-thromboxane B₂ concentrations and coagulation and fibrinolytic measures did not change.

Conclusion: The tocotrienol supplements used had no marked favorable effects on the serum lipoprotein profile or on platelet function in men with slightly elevated lipid concentrations. *Am J Clin Nutr* 1999;69:213-9.

KEY WORDS Tocotrienols, cholesterol, HDL cholesterol, triacylglycerols, aggregation, coagulation, fibrinolysis, lipid peroxides, humans, thromboxane B₂, platelets

INTRODUCTION

Tocopherols and tocotrienols are 2 classes of compounds with vitamin E activity. Each group comprises 4 different isomers—indicated by α , β , γ , or δ —which all have different biological

activities. Tocopherols are present in most vegetable oils and are more common in the diet than tocotrienols, which are found at relatively high concentrations in palm oil and rice bran oil (1, 2).

Epidemiologic studies suggest that, probably because of their antioxidative capacity, tocopherols may protect against cardiovascular disease (3). Tocotrienols also have antioxidant activity (4) and (unlike the tocopherols) lower plasma concentrations of atherogenic LDL cholesterol in various animal species (5, 6). This is probably done by posttranscriptional suppression of the key enzyme in cholesterol synthesis, 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMG-CoA reductase; 6). Effects of tocotrienols on LDL-cholesterol concentrations in humans, however, are contradictory (7-11).

Disagreement also exists about the effects of tocotrienols on platelet aggregation. Qureshi et al (7) concluded that administration of tocotrienols reduced in vitro platelet aggregation. These results, however, were not supported by a study by Wahlqvist et al (9). In view of these controversial findings, we decided to test the effects of a tocotrienol-rich fraction from palm oil on serum lipids and lipoproteins and on platelet function in men with mildly elevated serum total cholesterol or lipoprotein(a) [Lp(a)] concentrations.

SUBJECTS AND METHODS

Subjects

Forty-one subjects aged 21-61 y were recruited from among men of the general population of Maastricht; they had been screened in 1989 for a large survey concerning cardiovascular diseases (principal investigator at that time: D Kromhout, Department of Epidemiology, National Institute of Public Health and the Environment, Bilthoven, Netherlands). Only men with a serum total cholesterol concentration between 6.5 and 8.0

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mmol/L (251 and 309 mg/dL), with no history of cardiovascular disease, and who did not use medication known to interfere with any of the variables to be measured, were invited to enter the study. In addition, 5 men with Lp(a) concentrations >150 mg/L who were screened at our department for participation in another dietary study (12) were willing to participate. Subjects with slightly elevated serum total cholesterol or Lp(a) concentrations were recruited because previous studies had suggested that effects of tocotrienols—if any—were most pronounced in these men (7, 12).

Two weeks before the start of the study, these 46 men were invited to undergo a medical examination. Four were excluded because they used drugs incompatible with the study protocol and one because of commitments outside the area during the study. None had proteinuria or glucosuria. One subject withdrew before the start of the study; thus, 40 men entered the study. The experimental protocol and aim of the study were fully explained to the subjects and the protocol was approved by the Medical Ethical Committee of Maastricht University. Written, informed consent was obtained from all men. Subjects did not receive any reward and were free to withdraw from the study at any time.

Experimental design

The study was a randomized, double-blind, placebo-controlled, parallel trial. Before the start of the study, subjects were randomly assigned to receive capsules containing either 240 mg palm olein, 20 mg α -tocopherol, and 40 mg tocotrienols, or 280 mg palm olein and 20 mg D-L- α -tocopherol. Palm olein is made by winterization of palm oil and contains $\approx$ 40% palmitic acid, 4% stearic acid, 43% oleic acid, and 11% linoleic acid by weight (1). α -Tocopherol was added to the placebo oil so that the only difference between the 2 types of capsules was their tocotrienol content. Capsules were supplied by the Palm Oil Research Institute of Malaysia.

Subjects were asked to take 4 capsules daily for 6 wk, one with every meal and one before retiring for the night. Capsules were handed out on 5 different occasions for periods ranging between 6 and 13 d. Unused capsules were returned and counted. Venous blood was sampled 2 wk before the start of the study (preexperimental), on days 1, 35, and 42 of the study, and 9 wk after the study had ended (poststudy). Body weights of subjects without shoes or heavy clothing were recorded at every visit. Within a period of 2 wk, 4–6 subjects entered the study daily from Tuesday to Friday. Each day, the number of subjects in the tocotrienol group and the placebo group was the same or differed by one.

Subjects were urged not to change their usual diet and pattern of activities and were not allowed to take drugs known to interfere with lipoprotein concentrations or platelet function. They recorded in diaries any signs of illness, medication used, and deviations from the protocol. Between weeks 3 and 4 of the study, subjects were instructed by a dietitian to record their food intake on 2 working days and 1 weekend day. Food records were coded and the composition of the diets was calculated by using the 1989 edition of the Netherlands Nutrient Database (13).

Blood sampling

Before the start of the study, one nonfasting blood sample was taken into a vacuum tube between 0830 and 1500. Serum was obtained for analysis of total cholesterol. During the study, blood was sampled between 0830 and 1030 with the subject in a recumbent position. Nine weeks after the study, another blood sample

was taken to examine whether blood values had returned to baseline. Subjects were instructed to consume food items free of fat and alcohol after 2000 and not to eat after 2300 on the day preceding blood sampling. On the morning itself, subjects were fasting and were not allowed to smoke. A forearm vein was punctured under minimal stasis by using a 19-gauge butterfly venisystem (Abbott BV, Amstelveen, Netherlands). The first 3 mL blood was collected into a tube filled with 0.10 mL of a 15% (by wt) EDTA solution (Sherwood Medical, Ballymoney, United Kingdom) and used for platelet counting. The next 9 mL was collected into a pre-cooled Monovette tube (Sarstedt, Nümbrecht, Germany) containing 1 mL of an ice-cold sodium citrate solution (109 mmol/L, pH 7.2–7.4). Plasma for measurement of fibrinolytic variables was obtained by centrifugation for 15 min at $3000 \times g$ and 4°C . For the measurement of platelet aggregation and ATP release in whole blood, 2.7 mL blood was sampled in a prewarmed (40°C) syringe filled with 0.3 mL citrate solution. Then a second Monovette tube with 1 mL citrate solution was filled with 9 mL blood for analysis of coagulation variables and lipid peroxides.

To obtain platelet-free plasma, blood was first centrifuged for 10 min at $3000 \times g$ and room temperature. The plasma was then transferred into a plastic tube and centrifuged again for another 30 min at $12000 \times g$. Finally, blood was collected in two 10-mL tubes. Blood in the first tube was allowed to clot for 1 h at room temperature for analysis of serum lipid, lipoprotein, and vitamin E concentrations. The second tube was placed in a water bath at 37.5°C for exactly 1 h and used for thromboxane B_2 (TxB_2) measurements. Serum was obtained by centrifugation for 15 min at $3000 \times g$ and room temperature. Small aliquots of the serum and plasma were immediately frozen in liquid nitrogen and stored at -80°C .

Urine sampling

One day before the study started, subjects discarded all urine excreted before bedtime. Urine voided during the night and the first urine passed after getting up was collected into a clean plastic container and defined as “morning urine.” On days 41 and 42, morning urine was also collected into 2 separate containers. In addition, the rest-of-the-day sample on day 41 was collected. Urine passed on the morning of day 41 and during the rest of the day was considered one complete 24-h collection. Containers were handed in on days 1 and 42. After being weighed, urine samples were put in small plastic bottles without preservative and stored at -20°C for analysis of thromboxane A_2 (TxA_2) metabolites.

Analytic methods

Serum was analyzed enzymatically for total and HDL cholesterol after precipitation of non-HDL cholesterol with heparin-manganese (Monotest Cholesterol; Boehringer Mannheim GmbH, Mannheim, Germany) and for triacylglycerols (Roche Diagnostica, Hoffmann-La Roche, Basel, Switzerland) on an autoanalyzer (Cobas Bio, Hoffmann-La Roche). The CV for control sera was 0.5% for total cholesterol, 2.6% for HDL cholesterol, and 2.2% for triacylglycerol. Accuracy was checked by analyzing commercially available serum samples. The mean bias of target values was -0.4% for total cholesterol, 6.4% for HDL cholesterol, and -2.2% for triacylglycerols. The LDL-cholesterol concentration was calculated with the equation of Friedewald et al (14). Serum Lp(a) concentrations were analyzed by an enzyme-linked immunosorbent assay (TintElize Lp[a]; Biopool,



Umea, Sweden). A control serum sample was analyzed in each run. The average Lp(a) concentration for this pool sample was 245 mg/L and the interassay and intraassay CVs were both 5.3%. Plasma concentrations of lipid peroxides on day 1 and day 42 were estimated with a test kit (Determiner LPO; Kamiya Biomedical Company, Thousand Oaks, CA).

Sera from day 1, day 42, and 9 wk after the study were analyzed for the various tocopherols and tocotrienols as described previously (15). Five hundred microliters of plasma was mixed with the same amount of distilled water and with 1 mL ethanol. After this sample was vortex mixed and 2 mL hexane added, the mixture was vortex mixed again and centrifuged for 10 min at $2000 \times g$ at room temperature. The hexane phase was removed and the residue was extracted again with hexane. The 2 hexane phases were mixed, dried under nitrogen, and redissolved in 500 μ L hexane. Tocopheryl acetate was used as an external standard. The composition of the capsules was analyzed in a similar way. All samples from one subject were analyzed within one run. However, serum total cholesterol samples obtained before the start of the study and 9 wk after the study had ended were analyzed in separate runs.

Platelet aggregation and ATP release

The blood and the coagulant were mixed gently and 1 mL was transferred immediately by pipette into 2 prewarmed aggregometer cuvettes (37°C; Sarstedt) that had been shortened slightly to fit the thermostatic cuvette holder of the whole blood lumi-aggregometer (model 500 Chronolog; Chronolog Corporation, Havertown, PA). The cuvettes already contained a polytetrafluoroethylene-coated stir bar for stirring at 1000 rpm and 100 μ L luciferase-luciferin reagent for quantifying the release of ATP from platelets. After the mixture was preincubated and calibrated for 5 min, aggregation was induced by adding 10 μ L of a diluted collagen suspension (Collagen Horm; Hormon Chemie, Munich, Germany) freshly prepared each week from a stock solution and a dilution buffer. The final concentration of collagen in the first aggregation cuvette was 0.45 mg/L and in the second cuvette was 0.60 mg/L. After the mixture was aggregated for 15 min, 10 μ L of an ATP solution (133 μ mol/L; Sigma, St Louis) was added to the cuvette. The luminescence response to this standard dose was used to calculate the amount of ATP released by the platelets when activated with collagen. After the mixture was aggregated for 15 min, a sample was taken for the measurement of TxB₂ concentrations.

Other measurements

TxB₂ concentrations in serum or plasma samples were analyzed by radioimmunoassay (EI Du Pont de Nemours & Co, Boston). Factor VII coagulant activity (FVIIc) was expressed relative to that of a pool of plasma from healthy volunteers and fibrinogen concentrations were measured with a one-stage clotting test (16). Enzyme-linked immunosorbent assays were used to analyze concentrations of modified antithrombin III (Asserchrom ATM, Diagnostica Stago, Asnières-sur-Seine, France), prothrombin fragment F1+2 (Enzygnost F1+2; Behringwerke AG, Marburg, Germany), plasminogen activator inhibitor type 1 (PAI-1; Coaliza PAI-1, Chromogenix AB, Mölndal, Sweden), and D-dimers (Coaliza D-dimer; Chromogenix AB, Mölndal, Sweden). Urinary concentrations of TxB₂ and 11-keto-TxB₂ were analyzed by Novo Nordisk A/S (Copenhagen) with an enzyme immunoassay technique (17). Finally, urinary creatinine

concentrations were analyzed by using a colorimetric assay (Boehringer Mannheim).

Statistical methods

Responses were calculated as the change between values at the end (means of days 35 and 42) and the start of the study (day 1). Data were checked for normality by using the Shapiro-Wilk test (18). Responses of serum Lp(a), plasma peroxides, and plasma β -tocopherol and α -, γ -, and δ -tocotrienol were not normally distributed. Differences in effects between the 2 groups were examined by using a two-sided unpaired *t* test or the Mann-Whitney *U* test (19). Normally distributed data are presented as means $\pm$ SDs and other variables as median values. For reasons of uniformity, all tocopherol and tocotrienol concentrations were treated as nonnormally distributed data. *P* values <5% were considered statistically significant. Qureshi et al (7) reported changes in total cholesterol concentrations and whole blood platelet aggregation of 18% after tocotrienol supplementation. The power of our study to detect such effects was >80%.

RESULTS

Subjects and compliance

All participants completed the trial successfully and none reported an event that might have affected the results. The men in the tocotrienol group were 29–60 y of age (mean: 47 y), whereas their body mass indexes (in kg/m²) ranged from 19.9 to 36.6 (mean: 25.1). Those in the placebo group were 34–60 y of age (mean: 45 y) and had body mass indexes of 20.8–29.6 (mean: 25.3). Subjects in the tocotrienol group used, on average, 3.9 capsules/d (range: 3.2–4.1 capsules/d), and those in the placebo group used 3.7 capsules/d (range: 3.0–4.0 capsules/d). Body weight over the 42 d of the study decreased by 0.4 ± 1.0 kg (range: -2.8 to 1.4 kg) in the tocotrienol group and increased by 0.1 ± 1.0 kg (range: -1.5 to 1.9 kg) in the placebo group. None of these variables differed significantly between groups. The average daily energy intake was 10.9 MJ in the tocotrienol group and 12.5 MJ in the placebo group (*P* = 0.05). Nutrient intakes, however, were not different between the 2 groups (Table 1). The only difference between the 2 types of capsules was the amount of tocotrienols. According to chemical analysis, 4 tocotrienol capsules provided 83 mg tocopherol and 139 mg tocotrienols, whereas the placebo capsules supplied 84 mg tocopherol. Thus, the tocopherol content of each capsule was consistent with the value provided by the manufacturer, but the tocotrienol content was 5 mg less.

Compliance was examined by analysis of tocotrienol and α -tocopherol concentrations in serum (Table 2). The increase in both groups in α -tocopherol concentrations was accompanied by decreases in β -, γ -, and δ -tocopherol. Changes in tocopherol concentrations between groups, however, did not differ significantly, as was expected because of the similarity in the tocopherol composition of the 2 types of capsules. Compliance was confirmed by changes in both α - and γ -tocotrienol concentrations, which were significantly greater in the tocotrienol group. The increased intake of δ -tocotrienol, however, was not reflected in serum.

Serum lipid and lipoproteins

Serum lipid concentrations on day 1 of the study were not significantly different between the 2 groups (Table 3). During

TABLE 1

Mean daily nutrient and supplement intake of tocopherols and tocotrienols during the study

	Tocotrienol group	Placebo group
Energy (MJ/d)	10.9 ± 2.4 ¹	12.5 ± 2.6 ²
Protein (% of energy)	14.4 ± 2.5	13.9 ± 2.5
Fat (% of energy)	35.8 ± 7.4	36.7 ± 6.2
Saturated	14.5 ± 2.9	15.9 ± 3.1
Monounsaturated	14.0 ± 3.4	13.5 ± 2.4
Polyunsaturated	6.9 ± 2.2	6.8 ± 2.5
Carbohydrates (% of energy)	42.6 ± 6.1	41.2 ± 5.3
Alcohol (% of energy)	7.1 ± 6.2	7.7 ± 7.1
Cholesterol (mg/MJ)	29.7 ± 12.0	31.0 ± 7.7
Capsules (mg)		
α-Tocopherol	82.8	84.3
α-Tocotrienol	40.4	ND
β-Tocotrienol	5.2	ND
γ-Tocotrienol	68.4	ND
δ-Tocotrienol	25.2	ND

¹ $\bar{x} \pm \text{SD}$. Twenty men received 4 capsules daily for 6 wk containing either a tocotrienol-rich palm oil concentrate or a placebo oil. Nutrient intake was calculated from a 3-d food record, whereas the composition of the capsules was determined chemically. ND, not detectable.

²Significantly different from the tocotrienol group, $P = 0.05$.

the study, serum total cholesterol concentrations increased by 0.16 mmol/L in the tocotrienol group and by 0.19 mmol/L in the placebo group (95% CI for the difference in changes of -0.03 mmol/L between the 2 groups: $-0.39, 0.35$ mmol/L; $P = 0.902$). Changes in serum LDL cholesterol (95% CI for the difference: $-0.54, 0.19$ mmol/L; $P = 0.333$), HDL cholesterol (95% CI for the difference: $-0.07, 0.14$ mmol/L; $P = 0.495$) and triacylglycerols (95% CI for the difference: $-0.10, 0.62$ mmol/L; $P = 0.155$) also did not differ significantly between the 2 groups. Nine weeks after the study had ended, the lipid and lipoprotein concentrations relative to those at the start of the study were not significantly different between the 2 groups.

Lp(a) concentrations were not affected by the supplements and the median change was 1 mg/L in both groups ($P = 0.808$). Changes were not related to initial Lp(a) concentrations. Median plasma lipid peroxide concentrations at the start of the study were 1.50 $\mu\text{mol/L}$ in the tocotrienol group and 1.87 $\mu\text{mol/L}$ in the placebo group. After supplementation, plasma lipid peroxide concentrations had decreased 0.19 $\mu\text{mol/L}$ in the tocotrienol group and 0.58 $\mu\text{mol/L}$ in the placebo group ($P = 0.490$ for the difference).

Platelet aggregation and ATP release

At a final collagen concentration of 0.45 mg/L, the 0.15- Ω/min increase in aggregation velocity (V_a) in the tocotrienol group was significantly less than the change of 1.68 Ω/min in the placebo group (95% CI for the difference: $-2.74, -0.31$ Ω/min ; $P = 0.015$), whereas changes in the maximum aggregation (I_{max}) were not significantly different between the 2 groups (95% CI for the difference: $-8.8, 0.6$ Ω ; $P = 0.087$). Changes in the ATP release velocity (V_r ; 95% CI for the difference: $-0.79, -0.04$ nmol/min; $P = 0.033$) and maximum ATP release (R_{max} ; 95% CI for the difference: $-0.64, -0.08$ nmol; $P = 0.012$) were also significantly different between groups. The difference between the 2 groups in TxB_2 formation after 15 min of aggregation was also significant (95% CI for the difference: $-9.3, -0.4$ $\mu\text{g/L}$;

TABLE 2Effect of tocotrienol supplementation on serum tocopherol and tocotrienol concentrations¹

	Tocotrienol group	Placebo group
α-Tocopherol		
Baseline	36.13 (24.75 to 52.57)	39.19 (25.98 to 59.09)
Change	15.93 (-10.19 to 26.00)	12.96 (-3.69 to 26.45)
Poststudy	38.24 (29.02 to 54.87)	42.07 (24.89 to 55.47)
β-Tocopherol		
Baseline	0.61 (0.37 to 1.06)	24.6 (0.38 to 1.42)
Change	-0.25 (-0.50 to -0.10)	-10.8 (-0.60 to -0.05)
Poststudy	0.55 (0.33 to 1.85)	24.8 (0.32 to 1.31)
γ-Tocopherol		
Baseline	2.91 (1.31 to 10.34)	3.47 (0.34 to 8.25)
Change	-2.18 (-4.50 to -0.94)	-2.24 (-5.26 to 0.96)
Poststudy	2.32 (0.20 to 13.27)	4.26 (0.95 to 7.83)
δ-Tocopherol		
Baseline	0.12 (0.00 to 0.72)	0.10 (0.00 to 0.64)
Change	-0.05 (-0.53 to 0.17)	-0.05 (-0.38 to 0.10)
Poststudy	0.08 (0.00 to 1.01)	0.09 (0.03 to 0.41)
α-Tocotrienol		
Baseline	0.00 (0.00 to 0.03)	0.00 (0.00 to 0.05)
Change	0.17 (0.00 to 0.57)	0.00 (-0.03 to 0.02) ²
Poststudy	0.00 (0.00 to 0.39)	0.00 (0.00 to 0.03)
γ-Tocotrienol		
Baseline	0.00 (0.00 to 0.52)	0.00 (0.00 to 0.06)
Change	0.15 (-0.29 to 0.41)	0.00 (-0.04 to 0.04) ²
Poststudy	0.02 (0.00 to 0.07)	0.00 (0.00 to 0.32)
δ-Tocotrienol		
Baseline	0.00 (0.00 to 0.09)	0.00 (0.00 to 0.47)
Change	0.00 (-0.06 to 0.08)	0.00 (-0.47 to 1.16)
Poststudy	0.00 (0.00 to 0.70)	0.00 (0.00 to 0.98)

¹Medians; range in parentheses. Twenty men received 4 capsules daily for 6 wk containing either a tocotrienol-rich palm oil concentrate or a placebo oil. For one subject from the placebo group the poststudy sample, which was taken 9 wk after the study had ended, was missing. β-Tocotrienol was not detected.

²Significantly different from the tocotrienol group, $P < 0.001$.

$P = 0.034$). Poststudy values were not different from those at the start of the study.

At the start of the study, aggregation measures were higher in the tocotrienol group than in the placebo group. Because initial values may have affected the magnitude of the changes, effects of tocotrienol supplementation were also assessed after adjustment for values on day 1 by analysis of covariance. Effects of tocotrienol supplementation on aggregation velocity ($P = 0.177$), maximum aggregation ($P = 0.569$), and TxB_2 formation ($P = 0.080$) were not significant anymore, but those on the ATP release velocity ($P = 0.042$) and the maximum ATP release ($P = 0.024$) remained significant. At a final collagen concentration of 0.60 mg/L, slightly higher responses were observed that did not differ significantly between the 2 groups.

The amount of TxB_2 in serum, after blood was allowed to clot for 1 h at 37.5°C, reflected the maximal ability of the platelets to produce TxB_2 (potency) and was not differently affected by tocotrienol supplementation (95% CI for the difference: $-55, 37$ $\mu\text{g/L}$; $P = 0.693$). Also, changes in the relative potency of TxB_2 formation, defined as the amount of TxB_2 formed in plasma by collagen-activated platelets relative to its maximum potency, were not different between groups. At a final collagen concentration of 0.45 mg/L, the 95% CI for the difference in changes between the 2 groups ranged from -1.8% to 0.0% ($P = 0.053$).



TABLE 3

Effect of tocotrienol supplementation on serum lipid and lipoprotein concentrations¹

	Tocotrienol group	Placebo group
	<i>mmol/L</i>	
Cholesterol		
Baseline	6.80 ± 0.71	6.69 ± 1.20
Change	0.16 ± 0.60	0.19 ± 0.55
Poststudy	6.84 ± 0.86	6.62 ± 1.24
LDL cholesterol		
Baseline	4.80 ± 0.84	4.70 ± 1.10
Change	-0.01 ± 0.63	0.16 ± 0.50
Poststudy	4.68 ± 0.89	4.62 ± 1.09
HDL cholesterol		
Baseline	1.12 ± 0.31	1.12 ± 0.29
Change	0.07 ± 0.12	0.04 ± 0.19
Poststudy	1.21 ± 0.31	1.16 ± 0.31
Triacylglycerol		
Baseline	1.93 ± 0.94	1.91 ± 0.91
Change	0.24 ± 0.51	-0.02 ± 0.61
Poststudy	2.07 ± 1.39	1.83 ± 0.84

¹ $\bar{x} \pm \text{SD}$. Twenty men received 4 capsules daily for 6 wk containing either a tocotrienol-rich palm oil concentrate or a placebo oil. For one subject from the placebo group the poststudy sample, which was taken 9 wk after the study had ended, was missing.

and at a collagen concentration of 0.60 mg/L from -1.9% to 0.7% ($P = 0.384$).

Urinary thromboxane A₂ metabolites

Urinary TxB₂ excretion was characterized by a large interindividual variation. At the start of the study, amounts of TxB₂ in a complete specimen of morning urine ranged between 99 and 1926 ng and amounts of 11-keto-TxB₂ between 122 and 2418 ng. After 6 wk, TxB₂ excretion in the tocotrienol group had increased slightly by 5 ± 638 ng, and had decreased by -44 ± 278 ng in the placebo group. The difference in changes, however, was not significant (95% CI for the difference: -265, 364 ng; $P = 0.753$). Amounts of 11-keto-TxB₂ in a complete specimen of morning urine had decreased by -33 ± 548 ng in the tocotrienol group but had increased by 58 ± 263 ng in the placebo group (95% CI for the difference: -366, 184 ng; $P = 0.506$). When results were expressed per mmol creatinine, effects on TxB₂ (95% CI for the difference: -71, 51 ng/mmol; $P = 0.744$) or 11-keto-TxB₂ (95% CI: -87, 20 ng/mmol; $P = 0.214$) were also not significantly different between the 2 groups.

Excretion of TxA₂ metabolites, as measured at the end of the study in one complete, 24-h urine collection, was also not significantly different between the 2 groups. At the start of the study, the correlation coefficient between the amount of TxB₂ and 11-keto-TxB₂ was 0.83 ($P < 0.001$) in a complete specimen of morning urine.

Coagulation and fibrinolysis

At the start of the study, the coagulation and fibrinolytic variables were not significantly different between the tocotrienol and placebo groups (Table 5). After the study, values had changed slightly, but the difference in changes for factor VII (95% CI for the difference: -8%, 5% of normal; $P = 0.678$), fibrinogen (95% CI for the difference: -0.34, 0.17 g/L; $P = 0.552$), modified antithrombin III (95% CI for the difference: -7.5, 5.7 μg/L;

TABLE 4

Effect of tocotrienol supplementation on collagen-induced platelet aggregation and thromboxane B₂ (TxB₂) formation in citrated whole blood¹

	Tocotrienol group	Placebo group
Aggregation		
V _a (Ω/min)		
Baseline	5.45 ± 1.96	3.85 ± 1.85 ²
Change	0.15 ± 1.44	1.68 ± 2.27 ²
Poststudy	5.37 ± 2.30	4.33 ± 1.82
I _{max} (Ω)		
Baseline	25.3 ± 9.1	19.0 ± 10.0 ²
Change	-0.5 ± 6.5	3.6 ± 8.2
Poststudy	26.3 ± 6.6	20.9 ± 7.3
ATP release		
V _r (nmol/min)		
Baseline	1.51 ± 0.90	1.17 ± 0.82
Change	0.39 ± 0.50	0.80 ± 0.67 ²
Poststudy	2.08 ± 1.22	1.38 ± 0.76
R _{max} (nmol)		
Baseline	1.58 ± 0.83	1.29 ± 0.68
Change	0.28 ± 0.35	0.64 ± 0.50 ²
Poststudy	2.04 ± 1.00	1.54 ± 0.61
TxB ₂ (μg/L)		
Baseline	37.6 ± 10.3	32.8 ± 7.4
Change	3.4 ± 8.6	8.3 ± 4.7 ²
Poststudy	43.1 ± 13.7	37.6 ± 8.5

¹ $\bar{x} \pm \text{SD}$. Final collagen concentration: 0.45 mg/L. Twenty men received 4 capsules daily for 6 wk containing either a tocotrienol-rich palm oil concentrate or a placebo oil. For 1 subject from the tocotrienol group and 3 subjects from the placebo group the poststudy samples, which were taken 9 wk after the study had ended, were missing. V_a, aggregation velocity; I_{max}, maximum aggregation; V_r, ATP release velocity; R_{max}, maximum ATP release.

²Significantly different from the tocotrienol group, $P < 0.05$.

$P = 0.786$), fragments 1+2 (95% CI for the difference: -0.07, 0.14 μmol/L; $P = 0.512$), PAI-1 (95% CI for the difference: -6.7, 9.8 μg/L; $P = 0.714$) and D-dimers (95% CI for the difference: -37, 80 μg/L; $P = 0.458$) were not statistically significant. Poststudy concentrations of both the coagulation and fibrinolytic variables were not significantly different from those at the start of the study.

DISCUSSION

In this study of mildly hypercholesterolemic men, we did not find a favorable effect of a tocotrienol-rich palm oil concentrate on serum lipids and lipoproteins. Subjects received capsules containing tocotrienols or a placebo, which both provided 20 mg α-tocopherol/d, a dose that does not affect plasma lipids and lipoproteins (20, 21). Our results seem to contrast with those of Qureshi et al (7), who showed in a 4-wk, double-blind, placebo-controlled experiment that a daily supplement of 4 capsules with a tocotrienol-enriched palm oil fraction lowered LDL cholesterol without affecting HDL cholesterol. Note, however, that the LDL-lowering effect was only significant after exclusion of 3 poor responders from the 15 subjects in the tocotrienol group. Tan et al (8) also reported that a daily supplement of only one capsule of tocotrienols effectively lowered serum LDL-cholesterol concentrations in both normal and hypercholesterolemic subjects. However, no control group was involved in that study (8), which makes it impossible to separate possible time or placebo effects



TABLE 5Effect of tocotrienol supplementation on coagulation and fibrinolytic measures¹

	Tocotrienol group	Placebo group
Factor VII (% of normal)		
Baseline	112 ± 10.7	111 ± 10.1
Change	0 ± 9.7	1 ± 11.4
Poststudy	109 ± 11.5	110 ± 7.8
Fibrinogen (g/L)		
Baseline	4.12 ± 0.54	3.81 ± 0.48
Change	0.08 ± 0.36	0.15 ± 0.50
Poststudy	4.19 ± 0.53	3.85 ± 0.52
ATM (μg/L)		
Baseline	25.4 ± 8.5	21.8 ± 9.6
Change	0.8 ± 10.8	1.6 ± 9.3
Poststudy	25.8 ± 8.7	21.3 ± 11.5
Fragment 1+2 (μmol/L)		
Baseline	0.66 ± 0.21	0.65 ± 0.19
Change	0.01 ± 0.16	-0.02 ± 0.17
Poststudy	0.66 ± 0.21	0.65 ± 0.22
PAI-1 (μg/L)		
Baseline	35.3 ± 15.2	31.9 ± 14.2
Change	4.4 ± 15.8	2.5 ± 8.4
Poststudy	37.0 ± 17.1	35.3 ± 14.4
D-Dimers (μg/L)		
Baseline	238 ± 117	200 ± 106
Change	-4 ± 84	-28 ± 93
Poststudy	217 ± 96	183 ± 105

¹ $\bar{x} \pm$ SD. Twenty men received 4 capsules daily for 6 wk containing either a tocotrienol-rich palm oil concentrate or a placebo oil. For one subject from the placebo group the poststudy sample, which was taken 9 wk after the study had ended, was missing.

from treatment effects. Our results agree with the findings of Wahlqvist et al (9). In their study, 44 hypercholesterolemic subjects received either placebo capsules or an increasing dose of tocotrienols. After 16 wk—at that time the tocotrienol group was receiving 4 capsules daily—serum lipid measures were not significantly different between the 2 experimental groups. Even consumption of 6 capsules daily did not change serum lipoprotein concentrations in patients with hyperlipidemia (11).

Recently, Qureshi et al (10) reported an LDL cholesterol–lowering effect of γ -tocotrienol (in particular) in hypercholesterolemic subjects. From additional studies with chickens (22), it was concluded that α -tocopherol suppressed the inhibiting effects of γ -tocotrienol on HMG-CoA reductase activity. It was therefore postulated that effective tocotrienol preparations should contain <15–20% (by wt) α -tocopherol and 45% γ - plus δ -tocotrienol (22), and that the variable composition of the tocotrienol supplements explained why some studies claimed effects (7, 10), whereas another study did not (9). Our supplement contained 37% α -tocopherol and 42% γ - plus δ -tocotrienol, which—according to the hypothesis of Qureshi et al (22)—may offer an explanation for the lack of effect. However, this hypothesis is not in line with the results of Atroschi et al (23), who reported no effect on serum lipoproteins when using capsules containing only 15% α -tocopherol. Therefore, the suggestion of Qureshi et al (22) awaits confirmation from studies with different population groups. In this respect, the tocotrienol-rich fraction from rice bran oil might be a more promising hypolipidemic agent because it contains a high amount of γ -tocotrienol and a low amount of α -tocopherol (2). In addition, rice bran oil

may contain 2 other tocotrienol components that have been reported to improve the serum lipoprotein profile (24).

We reported earlier that serum Lp(a) concentrations decreased when subjects switched from their habitual Western diet to a diet enriched with palm oil (12). A later study showed that this effect was at least partly due to a decreased intake of *trans* fatty acids during the palm oil–rich diet period (25), but possible effects of tocotrienols on Lp(a) could not be excluded. The present study, however, showed that tocotrienols do not change serum Lp(a) concentrations.

Several in vitro studies have shown the antioxidative capacity of both tocopherols and tocotrienols (4). Only a few studies, however, have examined the effects of vitamin E on in vivo formation of lipid peroxides. Brown et al (26) reported that a daily supplement of 280 mg α -tocopherol reduced plasma concentrations of lipid peroxides. However, we found no evidence that, at least at an additional daily intake of 140 mg, tocotrienols have a measurable effect on plasma lipid peroxide concentrations.

Concentrations of serum tocotrienols at the start of the study were low compared with those of tocopherol, which agrees with results from other studies (27–29). However, despite a substantial increase in the consumption of tocotrienols, fasting plasma concentrations increased only slightly. The changes reported, however, reflected the composition of the capsules. Interestingly, Hayes et al (27), using capsules similar to those that we did but at half the dose, reported only a slight increase in the concentration of δ -tocotrienol in plasma samples from fasting subjects but did observe an increase in α -, γ -, and δ -tocotrienol 2 h after the consumption of the capsules. However, absorption of tocotrienols was poor relative to that of tocopherols, and tocotrienols were rapidly cleared from the plasma. In addition, tocotrienols did not accumulate in tissues in hamsters. These findings may explain the difference between in vitro and in vivo findings; Hayes et al (27) therefore questioned an important physiologic role of tocotrienols in humans as hypolipidemic agents or as antioxidants. Increasing the amount of α -tocopherol in the diet decreases the concentrations of other tocopherols, as has been reported by others (30).

Wahlqvist et al (9) found that in hypercholesterolemic subjects, tocotrienols did not change platelet aggregation to collagen in whole blood, or to ADP, adrenaline, or collagen in platelet-rich plasma. Other studies, however, observed that tocotrienols reduced platelet aggregation in whole blood and in platelet-rich plasma (7). We also found that at a low collagen concentrations only, tocotrienol supplementation beneficially affected platelet aggregation velocity and ATP release. Interpretation of the results was complicated by the fact that at the start of the study platelet aggregation measures were lower in the placebo group. After adjustment for these differences in initial values, ATP release, but not platelet aggregation and TxB₂ formation after 15 min of aggregation, were still reduced in the tocotrienol group. At a higher level of collagen stimulation no differences were observed. Our results therefore suggest that tocotrienols may have a slight beneficial effect on the in vitro capacity of platelets to aggregate. Indeed, Hayes et al (27) reported that tocotrienols accumulate more readily in platelets than in plasma. No effects were observed on coagulation or fibrinolytic measures.

Urinary TxB₂ and 11-keto-TxB₂ concentrations were measured to examine the effects of tocotrienols on the in vivo formation of TxA₂. These measurements provide time-integrated information about in vivo TxA₂ biosynthesis. However, a limitation of

this method is that the location of formation is not known. Thus, urinary concentrations of TxB_2 may be confounded by renal TxA_2 production, but evidence exists that urinary 11-keto- TxB_2 is derived mainly from TxA_2 synthesized by platelets (31). However, no difference in changes was seen between the 2 groups.

Results in our study were not confounded by other factors that may change cardiovascular risk variables such as body weight or nutrient intake. We therefore conclude that in subjects at increased risk of cardiovascular disease, tocotrienol supplements do not have beneficial effects on serum lipoproteins, lipid peroxide concentrations, or hemostatic measures. 

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