Supplementation with 3 compositionally different tocotrienol supplements does not improve cardiovascular disease risk factors in men and women with hypercholesterolemia[1,2]

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ABSTRACT

Background: Tocotrienols have been reported to lower LDL-cholesterol and fasting glucose concentrations and to have potent antioxidant effects, but the results are contradictory.

Objective: The objective was to study the relative effect of tocotrienol supplements of different compositions (mixed α- plus γ-, high γ-, or P25-complex tocotrienol) on blood lipids, fasting blood glucose, and the excretion of 8-*iso*-prostaglandin $F_{2\alpha}$, a measure of oxidative stress, in healthy hypercholesterolemic men and women.

Design: This was a double-blind, randomized, parallel-design study in which subjects ($n = 67$ men and women) consumed 1 of 3 commercially available tocotrienol supplements or a safflower oil placebo for 28 d. Blood and urine samples were obtained before and after the 28-d supplementation phase for analysis of fasting blood lipids, glucose, tocotrienols and tocopherols, and 8-*iso*-prostaglandin $F_{2\alpha}$.

Results: Overall, serum tocotrienols were increased in subjects who consumed tocotrienols, which showed that the putatively active components were absorbed. No significant differences in mean lipid or glucose concentrations were observed among the 4 treatment groups at the end of the 28-d supplementation phase. However, when the values were expressed as a percentage change from the concentrations during the presupplementation run-in phase, LDL cholesterol increased slightly ($7 \pm 2\%$) but significantly ($P < 0.05$) in the group consuming the mixed α- plus γ-tocotrienol supplement when compared with LDL cholesterol in the group consuming the P25-complex tocotrienol. Neither mean concentrations nor the percentage change in 8-*iso*-prostaglandin $F_{2\alpha}$ differed significantly among treatments.

Conclusion: Supplementation with 200 mg tocotrienols/d from 3 commercially available sources has no beneficial effect on key cardiovascular disease risk factors in highly compliant adults with elevated blood lipid concentrations. *Am J Clin Nutr* 2002;76:1237–43.

INTRODUCTION

Lowering elevated blood concentrations of total and LDL cholesterol is a primary goal in the prevention and treatment of cardiovascular disease (CVD) (1, 2). Traditionally, low-fat diet therapy has been promoted as the first strategy for reducing total and LDL-cholesterol concentrations, whereas cholesterol-lowering drug therapy is reserved for persons who have had a myocardial infarction or who are at high risk of a first myocardial infarction. Unfortunately, compliance with low-fat diets is generally poor, and, as a result, there is a great interest in identifying natural, non-pharmacologic alternatives to help people manage their elevated blood lipids.

A widely studied group of functional cholesterol-lowering compounds are the tocotrienols. Tocotrienols are members of the vitamin E family of compounds, and the various isomers (ie, α, γ, δ, and β) are isolated from natural oils such as those from palm, rice bran, or barley. Like tocopherols, tocotrienols have antioxidant properties in vitro (3). Studies have shown that, unlike tocopherols, tocotrienols can inhibit cholesterologenesis and lower blood LDL cholesterol (4–12). Studies in humans showed that the consumption of 130–220 mg/d of mixed or purified tocotrienols by persons with hypercholesterolemia can result in reductions in LDL cholesterol of 8–21% within 4–6 wk. These effects have been shown in addition to the dietary effects on lipid lowering in subjects consuming a low-fat National Cholesterol Education Program (NCEP) Step I diet (10, 11). These studies have also reported significant reductions in fasting glucose, serum platelet factor 4, and thromboxane β_2, and these reductions suggest that tocotrienols can provide multiple cardiovascular health benefits.

However, inconsistent effects of tocotrienols also have been reported in the literature. Results from other well-controlled studies showed no benefits of tocotrienols provided as palm oil concentrates (13–15) or tocotrienyl acetate (16) in patients with hypercholesterolemia after supplementation with 160–240 mg/d for up to 18 mo. Although there also were slight differences between the populations and designs of these studies and those of previous studies showing positive results, it is possible that the composition of the supplements used may have contributed to the negative results observed. For example, data from in vitro studies indicate

that γ- and δ-tocotrienols are more potent than α-tocotrienol in suppressing the reductase activity of 3-hydroxy-3-methylglutaryl coenzyme A (4, 5). Most recently, a novel tocotrienol [didesmethyl (P25) tocotrienol] isolated from rice bran oil was reported to have potent effects on lowering cholesterol (6, 10).

Tocotrienols may provide a natural and effective nutritional adjunct to diet therapy for reducing the risk of CVD. However, because of uncertainty surrounding the most effective tocotrienol composition for reducing LDL cholesterol, the present study was initiated to assess the relative effectiveness and potency of commercially available tocotrienol concentrates of different compositions. A secondary objective was the assessment of the effects of these tocotrienols on other key factors related to CVD risk, including fasting blood glucose concentrations and 8-*iso*-prostaglandin $F_{2\alpha}$ (8-*iso*-PGF$_{2\alpha}$), which is an in vivo marker of oxidative stress.

SUBJECTS AND METHODS

Sixty-eight healthy subjects (n = 39 M and 29 F) aged 25–65 y were enrolled in the study. Subjects were selected if their initial baseline serum total cholesterol concentrations were 5.17–7.76 mmol/L, LDL cholesterol was > 3.36 mmol/L, and triacylglycerol was ≤ 4.52 mmol/L. Subjects were considered healthy on the basis of blood chemistry analysis and as they reported on a medical history questionnaire. Subjects were excluded from the study if they currently (or within the previous 3 mo) were taking prescription medication known to have an effect on blood lipids, including but not limited to 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitors, fibric acid derivatives, thiazide diuretics and β-blockers (for hypertension), and steroid-based medications (topical or oral), as reported by the subjects on a medical history questionnaire. Women taking oral contraceptives or hormone replacement therapy before the study were admitted, provided they had been following the same regimen (ie, dosage) for ≥ 3 mo, and they were required to maintain that regimen for the duration of the study. In addition, subjects were excluded from the study if they were currently (within the past 30 d) taking bulk laxatives or dietary supplements to reduce blood cholesterol concentrations [including but not limited to garlic, phytosterol-based margarines, soy protein (> 20 g/d), red yeast rice, and soluble psyllium fiber (> 5 g/d)] as reported by subjects on the medical history questionnaire. The protocol was approved by the Institutional Review Board of Ross Products Division, and written informed consent was obtained from the subjects.

Study design

The study followed a randomized, double-blind, parallel design. All subjects were instructed to consume an American Heart Association–NCEP Step I diet for the entire 7-wk study, beginning 21 d before supplementation (the run-in phase); in the supplementation phase, subjects were randomly assigned (according to their baseline LDL-cholesterol concentrations) to receive 1 of 3 tocotrienol supplements or placebo for 28 d. Subjects agreed to follow a meal pattern including a regular daily lunch meal during the study period, and they were instructed to consume all capsules at the midday meal; this was done to enhance subject compliance and facilitate disintegration of the capsule and absorption of the lipid-soluble tocotrienols. Supplements were plainly labeled with the appropriate treatment number to ensure maintenance of the double-blind status of the study.

A trained and licensed phlebotomist performed venipuncture to obtain fasting blood samples (≈20 mL) from subjects once at

TABLE 1
Tocotrienol and tocopherol composition of study supplements[1]

Isomer	Placebo	Tocotrienol supplement		
		α- plus γ-	γ-	P25-complex
	mg/g	*mg/g*		
α-Tocotrienol	ND	113	14	7
γ-Tocotrienol	ND	91	55	8
δ-Tocotrienol	ND	36	3	1
β-Tocotrienol	ND	10	ND	ND
P25-complex	—	—	—	—[2]
α-Tocopherol	0.15	78	34	11
γ-Tocopherol	ND	ND	38	5
δ-Tocopherol	ND	ND	3	1
β-Tocopherol	ND	0.5	3	0.1

[1] Analyses of study supplements provided by Craft Technologies, Inc (Wilson, NC). Total intake of tocotrienols (α-, γ-, δ-, and β-) was 200 mg/d. ND, not determined.

[2] The novel tocotrienol [didesmethyl (P25) tocotrienol] was not analyzed, and content was based on the manufacturer's labeling.

screening, on days 19 and 21 during the Step I diet run-in phase, and on days 47 and 49 during the supplementation phase. Blood samples were analyzed for total cholesterol, LDL cholesterol, triacylglycerols, HDL cholesterol, glucose (all samples), and tocotrienols and tocopherols (run-in and supplementation phases only), and a chemistry panel that included liver enzymes, kidney, and thyroid profile was performed on screening and final supplementation phase blood samples only. Urine samples [overnight (12 h) plus morning void] were collected by subjects twice, on day 19 or 21 during the Step I diet run-in phase and on day 47 or 49 during the supplementation phase, for analysis of 8-*iso*-PGF$_{2\alpha}$ and creatinine.

Subjects were instructed to maintain their habitual physical activity and lifestyle throughout the study, and body weight was monitored at each study visit. Subjects were asked to refrain from taking drugs or dietary supplements known to affect blood lipids, as well as supplemental vitamin E or other antioxidants, during the course of the study. Case report forms were filled out every other week, and subjects maintained daily diaries for recording supplement intake and any study deviations or adverse events during the 28-d supplementation phase.

Study supplements

During the supplementation phase, participants assigned to the placebo group received capsules containing 1 g safflower oil (Solgar Laboratories, Leonia, NJ). Participants assigned to the 3 tocotrienol groups received gelatin capsules containing either a mixed α- plus γ-tocotrienol extract from palm oil (Tocovid; Hovid Sdn Bhd, Ipoh, Kuala Lumpur, Malaysia), a high γ-tocotrienol extract from rice bran oil (Nutriene; Yasoo Health, San Diego), or a novel P25-complex extract from rice bran oil (EvolvE; Bionutrics, Phoenix, AZ). The composition of these supplements is shown in **Table 1**. The total daily intake of tocotrienols was 200 mg; however, because of the inherent differences in the composition and processing of the supplements and because capsules from different manufacturers were of different doses, the number of capsules consumed differed among the treatments. Subjects were advised that they could observe differences between treatments on the basis of the number, size, and color of the supplement capsules; however, they remained completely blinded to

the identity of the treatment groups throughout the study. Study supplements were repackaged, and bottles were sealed and labeled with a masked study number. Daily α-tocopherol intake was < 1, 62, 92, and 88 mg, respectively, from the placebo, the mixed α-plus γ-tocotrienol extract, the high γ-tocotrienol extract from rice bran oil, and the P25-complex tocotrienol supplements (Table 1).

Analytic methods

Blood collection and analyses

Immediately after blood collection, serum or plasma was isolated by low-speed centrifugation at $1000 \times g$ for 10 min at $4\,°C$. The screening lipid and blood chemistry profiles were performed within 12 h on serum or plasma samples by Laboratory Corporation of America (Dublin, OH). The subsequent run-in and supplementation phase serologic analyses were performed on aliquots of serum (0.5–1.0 mL) that were quick-frozen in cryovials and stored at $-70\,°C$. Serum was analyzed enzymatically for total and HDL cholesterol and triacylglycerol by the Clinical Chemistry Laboratory at Ross Products Division (Abbott Laboratories, Columbus, OH) with the use of a validated method on a diagnostic autoanalyzer (Spectrum EPx; Abbott Laboratories, Abbott Park, IL). HDL cholesterol was measured after the precipitation of apolipoprotein B with the use of dextran sulfate (molecular weight: 50 000). The LDL-cholesterol fraction was calculated with the use of Friedwald's equation (17), where LDL cholesterol = total cholesterol − HDL cholesterol − (triacylglycerols/5) with lipid concentrations measured as mg/dL. The precision and accuracy of the cholesterol analyses met the performance criteria established by the NCEP Standardization Panel, and certification was received under the Centers for Disease Control and Prevention reference procedure. The CV for control samples was 2% for total cholesterol, 4% for HDL cholesterol, and 3% for triacylglycerol. Serum glucose was measured with an automated glucose hexokinase enzymatic assay on a diagnostic autoanalyzer (Spectrum Glucose EPx; Abbott Laboratories). The CV for control samples was 1%.

Tocotrienols and tocopherols in serum and in the study supplements were assayed by Craft Technologies, Inc (Wilson, NC). Briefly, ≈1-mL samples of blood and study supplements were extracted with hexane, dried under nitrogen gas, and resuspended in 200 μL hexane. Tocotrienols and tocopherols were analyzed by normal-phase HPLC with the use of a Diol column (LiChrosorb; Merck KgaA, Darmstadt, Germany) and hexane:dioxane (95:5) as the mobile phase (18). The retention time of individual peaks was compared with that of pure tocotrienols and tocopherols. By this method, the recovery of tocotrienols spiked into human serum was > 90%, and the calibration curves were linear from 0.2 to 20 μmol/L.

Urine collection and analysis

Urine [overnight (12 h) plus first morning void] was collected in 1000-mL polyethylene bottles. Bottles were kept cold during the collection period in insulated bags containing freezer packs. Immediately after the return of the bottles, aliquots (10 and 50 mL) of each urine sample were sampled and stored at $-70\,°C$ before analysis of $8\text{-}iso\text{-}PGF_{2\alpha}$ and creatinine. The $8\text{-}iso\text{-}PGF_{2\alpha}$ was measured by radioimmunoassay of urine samples after alkaline hydrolysis (GenOx Corp, Baltimore). The average CV for this assay was 6.2%. Urinary creatinine was determined by a validated automated colorimetric assay on a diagnostic autoanalyzer (Spectrum EPx; Abbott Laboratories). Values are expressed as pmol $8\text{-}iso\text{-}PGF_{2\alpha}$/mmol creatinine.

Step I diet modification and assessment

All subjects received dietary instruction for lowering blood cholesterol according to the NCEP (1) Step I diet guidelines that restricted total fat intake to ≤30% of energy, saturated fat to ≤10% of energy, and cholesterol to ≤300 mg/d. Instruction included a 1-h group education session during which subjects 1) received verbal instruction from a registered, licensed dietitian; 2) watched a 15-min video entitled "Heart-Healthy Eating" (Patient Information Video Series; Dallas: American Medical Communications, 1994); and 3) received diet guides, recipes, and booklets (eg, *Step by Step*, NIH publication #94-2920; Bethesda, MD: National Institutes of Health, 1994) incorporating and supporting these guidelines. Subjects were instructed on how to complete a food-frequency questionnaire [MEDFICTS (meat, eggs, dairy, fried foods, in-baked goods, convenience foods, table fats, and snacks); from *Step by Step*], which was designed to assess adherence to NCEP Step I and Step II (in which total and saturated fat and dietary cholesterol are further restricted) diets (1). The frequency of intake and portion sizes of common foods that are major contributors of fat, saturated fat, and cholesterol were recorded; a numerical value was assigned on the basis of the frequency of consumption and the serving sizes of foods within each category; and a total score was calculated. The total achievable score was 190 points; a score ≥70 indicated the need to make some dietary changes, a score of 40–70 indicated compliance with a Step I diet, and a score of < 40 indicated compliance with a Step II diet. Targets for dietary change could be identified within each category, and subjects not complying with Step I diet recommendations were counseled about specific dietary changes. Additional diet questionnaires were completed by subjects throughout the 7-wk study for assessment of diet compliance as described below.

Compliance

Case-report forms were completed biweekly to ensure compliance with study instructions. To be considered in compliance with the supplementation regimen, subjects were required to consume ≥90% of the capsules, as assessed by capsule counts and the maintenance of daily diaries. Subjects completed diet questionnaires between days 5 and 10 of the diet run-in (mid-run-in phase), again between days 18 and 21 during the run-in phase, and finally between days 24 and 28 during the supplementation phase. In addition, all 5 fasting blood samples, all 2 urine samples, and all paperwork (including diaries and diet assessment questionnaire) had to be returned to the study monitor and verified for a subject to be considered compliant with the study protocol.

Statistical and analytic plans

It was calculated that a sample size of 17/group was needed to detect a 10% change in total cholesterol of 0.52 mmol/L with a two-sided test at $\alpha = 0.05$ and statistical power of 80%. The eligible subjects were ranked in descending order, blocked by 4, and randomly assigned to 4 groups according to their baseline LDL-cholesterol concentrations. The data collected at baseline and during the run-in phase (average from study days 17 and 21) and the supplementation phase (average from study days 47 and 49) were examined to test the assumption of normally distributed residuals with the use of the Shapiro-Wilk test. Comparison

TABLE 2
Characteristics of study subjects at baseline[1]

| | | Tocotrienol supplement | | |
	Placebo (n = 11 M, 6 F)	α- plus γ- (n = 9 M, 8 F)	γ- (n = 10 M, 7 F)	P25-complex (n = 8 M, 8 F)
Age (y)	41 ± 1	44 ± 2	42 ± 2	45 ± 2
BMI (kg/m^2)	28 ± 0.7	26 ± 0.8	26 ± 1	27 ± 0.7
Total cholesterol (mmol/L)	5.92 ± 0.13	6.02 ± 0.10	5.92 ± 0.13	5.99 ± 0.16
LDL cholesterol (mmol/L)	3.82 ± 0.13	3.82 ± 0.16	3.85 ± 0.13	3.90 ± 0.16
HDL cholesterol (mmol/L)	1.37 ± 0.08	1.42 ± 0.13	1.37 ± 0.10	1.45 ± 0.10
Triacylglycerol (mmol/L)	1.57 ± 0.17	1.65 ± 0.18	1.46 ± 0.14	1.41 ± 0.23

[1] $\overline{x}$ ± SEM. Lipid values at baseline were assessed by LabCorp of America (Dublin, OH). To convert cholesterol values from mmol/L to mg/dL, divide by 0.02586; to convert triacylglycerol values from mmol/L to mg/dL, divide by 0.0113. There were no significant differences between groups in baseline values.

of the groups for values obtained during the run-in and supplementation phases and the calculated percentage change from the run-in phase to the supplementation phase was first carried out with two-way repeated-measures analysis of variance (ANOVA). Because an interaction (group-by-time) was significant, one-way ANOVA was used to analyze the run-in and supplementation phases and the percentage change data, if the assumption of non-normally distributed residuals was met at each time point. Those variables for which the residuals showed evidence of being non-normally distributed (with $P < 0.05$ used for the Shapiro-Wilk test) at ≥ 1 time point were ranked, and one-way ANOVA was carried out on the rank data (Kruskal-Wallis one-way layout test). When significant group effects were detected, a post hoc Tukey's honestly significant difference test was used to identify the pair that caused the significant difference. In addition, Spearman's rank correlation coefficients were calculated to compare blood lipid and tocotrienol data and the correlation between blood lipid and urine data. Demographic variables were summarized with frequencies and percentages. All results were considered to be significant if P was < 0.05.

RESULTS

Of the 68 subjects enrolled into the study, 67 completed the entire 49-d course. One subject voluntarily dropped out of the study for personal reasons at 19 d, before providing any blood samples during the run-in phase and before supplement distribution, and therefore that person's data were not included in the analyses. Characteristics of the study subjects at baseline are listed in **Table 2**. Compliance with the study protocol and supplementation regimen by the remaining subjects was excellent, as determined by the maintenance of body weight ± 1.5 kg throughout the 7-wk study and by subject diaries, pill counts, and the return of all empty clinical product capsule bottles. Compliance with the Step I diet was excellent, as shown by maintenance of a diet score < 70 (**Table 3**).

Recorded adverse events during the study were minimal, and the frequency and severity did not differ among treatments. Common adverse events during the study included cold and flu symptoms, stomach pains, gas, and loose stools. Most of these events were described as mild (n = 19) or moderate (n = 8) in intensity. One subject in the placebo group experienced an adverse event, attributed to a 24-h intestinal flu, that was severe in intensity. No abnormal laboratory values resulted from the study treatments.

Overall, the concentrations of tocotrienols were higher in the serum of subjects consuming the active tocotrienol supplements than in the serum of those who did not (**Figure 1**), which indicates that the putatively active components were absorbed. The groups consuming the mixed α- plus γ-tocotrienol and P25-complex tocotrienol supplements had 20–27% higher serum concentrations of α-tocotrienol than did the placebo group, whereas the group consuming the high γ-tocotrienol supplement had a 9-fold elevation in the plasma concentrations of this isomer. However, because of the large variability in the serum concentrations of tocotrienols detected, only serum concentrations of γ-tocotrienol were significantly higher than those in the placebo group ($P < 0.05$). Tocotrienol supplements also contained α-tocopherol that was 25–50% higher in all active supplement groups than in the placebo group ($P < 0.05$; Figure 1). Differences were also observed among groups in γ-tocopherol concentrations (lower in the α-tocotrienol group than in the placebo group, $P < 0.05$) and β-tocopherol (higher in both rice bran oil tocotrienol groups than in the placebo group, $P < 0.05$).

Mean (± SEM) concentrations of fasting serum lipids and glucose during the run-in phase and at the end of the 28-d supplementation phase are shown in **Table 4**. No significant effects of tocotrienol supplementation on fasting total or LDL-cholesterol or glucose concentrations were observed among the treatment groups at the end of the 28-d supplementation phase. When the values were expressed as a percentage change from the concentration during the run-in phase, however, the increases in total and LDL cholesterol were significantly ($P < 0.05$) greater in the group consuming the α- plus γ-tocotrienol supplement than those in the group consuming the P25-complex tocotrienol supplement. With the exception of a small positive correlation between the serum concentration of β-tocotrienol and total cholesterol concentrations at the end of the supplementation phase ($r = 0.260$, $P < 0.049$), no

TABLE 3
Diet assessment scores throughout the study period[1]

| | | Tocotrienol supplement | | |
	Placebo (n = 17)	α- plus γ- (n = 17)	γ- (n = 17)	P25-complex (n = 17)
Baseline (screening)	85 ± 8	75 ± 6	61 ± 6	78 ± 6
Mid run-in phase	40 ± 5	38 ± 4	35 ± 4	38 ± 4
Run-in phase	44 ± 4[a]	38 ± 4[a,b]	30 ± 3[b]	34 ± 3[a,b]
Supplementation phase	40 ± 4	38 ± 4	30 ± 4	37 ± 3

[1] $\overline{x}$ ± SEM. A score of ≤70 indicates Step I diet compliance. Values in the same row with different superscript letters are significantly different, $P < 0.05$.
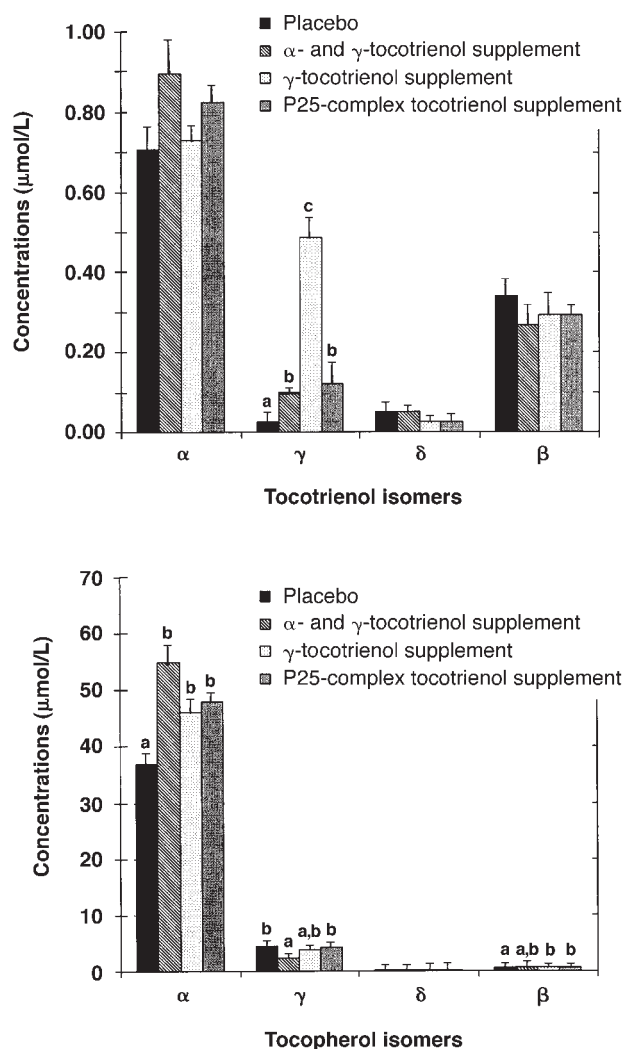
FIGURE 1. Mean (± SEM) serum concentrations of tocotrienol and tocopherol isomers after 28 d of daily supplementation with safflower oil placebo (*n* = 17) or mixed α- plus γ- (*n* = 17), high γ- (*n* = 17), or P25-complex (*n* = 16) tocotrienol. Bars with different superscript letters are significantly different, *P* < 0.05.

significant correlations were observed between the serum tocotrienol concentrations and changes in fasting serum lipids or glucose (data not shown).

No significant differences were observed among groups in the mean 12-h excretion of 8-*epi*-PGF$_{2\alpha}$ after tocotrienol supplementation, expressed per mmol urinary creatinine or as a percentage change from the run-in phase (Table 4). No relation was observed between serum tocotrienols and urinary 8-*epi*-PGF$_{2\alpha}$ (data not shown).

DISCUSSION

The results of this study show that daily supplementation with commercially available tocotrienol supplements at a level of 200 mg total tocotrienols/d has no measurable beneficial effect on key CVD risk factors in highly compliant adults with elevated blood cholesterol concentrations. These results suggest that the

mixed tocotrienol supplement from palm oil may even have a negative effect by increasing LDL-cholesterol concentrations.

These results were unexpected, given that our study design, subject population, and dosage and composition of tocotrienols were nearly identical to those in 3 double-blind, placebo-controlled studies by Qureshi et al (10–12) that showed substantial cholesterol reduction. In contrast, the results from the current study did not show any blood cholesterol- or glucose-lowering response, even in those subjects who had the highest serum concentrations of tocotrienols and, thus, greater absorption. Our study data are confirmatory, however, of those from other controlled studies reporting no effects on blood lipids (13–16). These studies used supplements in compositions or doses similar to those in the studies described above but had slightly different populations and designs. For example, Mensink et al (14) conducted a study with men having serum total cholesterol between 6.46 /L and 7.98 mmol/L who were advised to maintain their typical diet while consuming 160 mg tocotrienols/d for 6 wk. Wahlqvist et al (16) reported no change in blood lipids in subjects who consumed from one 60-mg palm oil tocotrienol capsule/d to 4 such capsules, providing 240 mg/d, despite significant increases in serum total tocotrienols. In the study by Tomeo et al (13), subjects with coronary artery disease started at a dose of 160 mg tocotrienol/d, but the dosage was increased after 3 mo in an (unsuccessful) attempt to lower serum lipids. Despite the ineffectiveness of the tocotrienol supplements in this latter study, however, ultrasonography scans of the patients' carotid arteries revealed apparent atherosclerotic lesion regression in 7 of the 25 patients taking the active supplement, whereas none of the control group exhibited such regression and 10 of the 25 patients showed carotid artery lesion progression. Consequently, the authors concluded that the antioxidant properties of the supplements were beneficial in this patient population, despite having no measurable effects on blood lipids.

Although tocotrienols have been shown to be antioxidants in vitro, the results of this current study showed no effects on the excretion of 8-*iso*-PGF$_{2\alpha}$. Isoprostanes are formed as products of noncyclooxygenase oxidative modification of arachidonic acid that results from free radical attack of cell membrane phospholipids or circulating LDLs (19, 20). They are released in response to cellular activation, they circulate in plasma, and they are excreted in the urine; thus, their formation has been described as a noninvasive marker of oxidation and is therefore of potential relevance to human vascular disease (21–23). Furthermore, elevated excretion has been shown to be "normalized" to within "healthy" ranges after supplementation with high concentrations of antioxidants, primarily α-tocopherol (24). It is possible that effects on other measures of oxidation (eg, LDL oxidation) that were not assessed in this study could have been observed (16).

We also considered that a tocotrienol intake > 200 mg/d might be needed for a significant effect on the endpoints of interest. We chose to study an intake of 200 mg/d, which represented the midrange of the concentrations of total tocotrienol supplementation reported in the literature and accordingly was a level at which we strongly expected to find significant cholesterol-lowering effects, if there were any. The fact that we observed significant elevations in the serum concentrations of tocotrienol with no cholesterol-lowering trends in those subjects who had the highest serum tocotrienol concentrations suggests that the amount of supplementation was not a limiting factor. Furthermore, subjects were instructed to consume all capsules at the midday meal instead of

TABLE 4
Fasting serum lipids and glucose and 12-h overnight urinary isoprostane concentrations at run-in phase and after 28 d of supplementation[1]

	Placebo (n = 17)		Tocotrienol supplement					
			α- plus γ- (n = 17)		γ- (n = 17)		P25-complex (n = 16)	
	Run-in	Supplementation[2]	Run-in	Supplementation[2]	Run-in	Supplementation[2]	Run-in	Supplementation[2]
Total cholesterol (mmol/L)	5.86 ± 0.13	5.89 ± 0.18 (0.1 ± 2)[a,b]	5.89 ± 0.16	6.15 ± 0.16 (5 ± 2)[a]	5.79 ± 0.13	5.97 ± 0.13 (3.5 ± 2)[a,b]	6.07 ± 0.21	5.99 ± 0.21 (−1 ± 2)[b]
LDL cholesterol (mmol/L)	3.88 ± 0.13	3.88 ± 0.16 (0.1 ± 2)[a,b]	3.85 ± 0.13	4.13 ± 0.13 (7 ± 2)[a]	3.72 ± 0.16	3.85 ± 0.13 (5 ± 3)[a,b]	4.0 ± 0.18	3.93 ± 0.21 (−1 ± 2)[b]
HDL cholesterol (mmol/L)	1.19 ± 0.08	1.24 ± 0.08 (4 ± 2)	1.19 ± 0.08	1.19 ± 0.10 (0.5 ± 2)	1.24 ± 0.08	1.29 ± 0.10 (4 ± 2)	1.21 ± 0.08	1.21 ± 0.08 (−0.1 ± 2)
Triacylglycerol (mmol/L)	1.81 ± 0.17	1.68 ± 0.20 (−7 ± 5)	1.83 ± 0.19	1.85 ± 0.20 (5 ± 6)	1.76 ± 0.23	1.77 ± 0.22 (5 ± 6)	1.97 ± 0.29	1.84 ± 0.22 (−1 ± 6)
Glucose (mmol/L)	4.61 ± 0.11	4.61 ± 0.06 (−0.4 ± 1)	4.66 ± 0.11	4.66 ± 0.11 (0.5 ± 2)	4.61 ± 0.11	4.66 ± 0.11 (2 ± 1)	4.61 ± 0.11	4.66 ± 0.11 (1 ± 1)
8-epi-PGF$_{2\alpha}$ (pmol/mmol creatinine)	293 ± 37	342 ± 37 (12 ± 6)	399 ± 52	442 ± 52 (5 ± 5)	304 ± 27	299 ± 34 (−0.6 ± 6)	394 ± 55	444 ± 73 (4 ± 7)

[1] $\overline{x}$ ± SEM. Run-in, average of analyses of values obtained from fasting blood samples drawn on days 17 and 19 of low-fat diet run-in phase; supplementation, average of analyses of values obtained from fasting blood samples drawn on days 47 and 49 of low-fat diet plus supplementation phase; 8-epi-PGF$_{2\alpha}$, 8-epi-prostaglandin F$_{2\alpha}$. To convert cholesterol values from mmol/L to mg/dL, divide by 0.02586; to convert triacylglycerol values from mmol/L to mg/dL, divide by 0.0113; to convert glucose values from mmol/L to mg/dL, divide by 0.0555. Values in the same row with different superscript letters are significantly different, $P < 0.05$.
[2] Percentage change from run-in in parentheses.

throughout the day as was the protocol in most other studies. Although this was primarily done to enhance subject compliance with a multiple-capsule supplementation scheme, we felt that it should also have facilitated the absorption of the lipid-soluble tocotrienols and the maintenance of adequate plasma concentrations throughout the study. A recent study in healthy adult volunteers confirms that the absorption of mixed tocotrienols is greater when they are given with a meal than when they are given to subjects in a fasted state (25). Although concentrations of tocotrienols were increased after the 28-d supplementation phase, especially after administration of the γ-tocotrienol-rich supplement, overall plasma concentrations of tocotrienols were lower than those of vitamin E, as has been reported in other studies (14, 16, 26). Results from recent studies suggest that this may not be the result of impaired bioavailability of tocotrienols; instead, the maintenance of a low plasma concentration of tocotrienols after supplementation more likely reflects alternative (unknown) pathways of their metabolism and excretion (27).

In conclusion, the results of this study do not support a beneficial effect of commercially available tocotrienol supplements on CVD risk factors in men and women with hypercholesterolemia. Dietary methods with proven cholesterol-lowering benefits (eg, reducing saturated fat and cholesterol and adding soluble fiber and soy protein) should not be overlooked as the first-step dietary approach to reducing CVD risks.

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