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DIFFERENTIAL SERUM RESPONSES OF TOCOPHEROLS AND TOCOTRIENOLS DURING VITAMIN SUPPLEMENTATION IN HYPERCHOLESTEROLAEMIC INDIVIDUALS WITHOUT CHANGE IN CORONARY RISK FACTORS

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ABSTRACT

The effects of tocotrienol-rich vitamin E from palm oil on serum vitamin E concentrations, serum lipids, plasma prostaglandins and platelet function (assessed by bleeding time and aggregation) were investigated in 44 subjects (23 males, 21 females) with hypercholesterolaemia. Following a 6-week run-in period, subjects were randomized for 20 weeks to receive either tocotrienol or placebo (superolein from palm oil), in increasing dosages. By the 4th week of supplementation, and at lowest dosages, there were significant increases in serum concentrations of total vitamin E, total tocopherol, and total tocotrienol in the active, but not placebo group, and these persisted in the highest dosage group for 16 weeks. At no stage during the study were there any significant changes in serum lipids (total cholesterol, HDL cholesterol, LDL cholesterol or triglycerides), or any changes in lipids predictable by serum vitamin E status itself, even when body composition was taken into account. There were also no changes in plasma prostaglandins or in platelet function. Evidence was obtained that the serum responses to ingested tocotrienols and tocopherols favoured tocopherol over tocotrienol, and alpha tocopherol over gamma tocopherol.

KEY WORDS: Palm oil, Serum vitamin E, Serum lipids, Thromboxane-B2, Prostaglandin F1-alpha, Platelet aggregation.

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INTRODUCTION

Dietary studies based on corn oil, coconut oil, and soya bean oil have demonstrated that saturated fatty acids excluding stearic acid raise serum cholesterol levels. Since the fatty acid composition of palm oil is approximately 50% saturated fatty acid (palmitic) with 40% oleic and 10% linoleic acid, it has been assumed that palm oil consumption would increase serum cholesterol concentration (1). However, there are not enough experimental data to either support or refute this hypothesis. According to the Keys-Hegsted equation (2, 3), a replacement of half of the dietary fat by palm oil could increase serum cholesterol by up to 0.5 mmol/L (4). Some data, however, suggest that a palm oil fraction which is vitamin E rich, reduces serum cholesterol to 20% of the basal levels (5). It is possible that a discrepancy between effects of palm oil predicted from equations derived from metabolic studies like those of Keys and Hegsted, and observations where palm oil itself or a palm oil fraction are ingested, may be attributable to components other than fatty acids. One of the active components of palm oil is tocotrienol (6) which has several forms.

Qureshi et al. (7, 8) have demonstrated that barley can lower serum cholesterol. This has been confirmed by McIntosh et al (9). One of the components of barley is tocotrienol. The question is, therefore, whether barley supplementation studies in poultry, where hepatic HMG CoA reductase inhibitory activity has been demonstrated (10), may be indicative of a therapeutic role for tocotrienols in humans with hypercholesterolaemia.

In a short term double blind randomised study, we have examined the effects of administering capsules of tocotrienol rich vitamin E from palm oil (Palm vitee) and a placebo of vitamin E-depleted palm oil (Superolein).

SUBJECTS AND METHODS

Following a 6-week run-in period, during which dietary fat modification was reinforced, capsules of Palm vitee or placebo (with identical appearance to Palm vitee) were given daily, increasing the dosage at four weekly intervals from 1 through to 4 (1 to 2, then 3 and, subsequently 4) capsules (Fig. 1, where T3 refers to tocotrienol).

Fifty-two subjects, an equal number of males and females, between the ages of 25 and 75 were recruited for the study. On referral, they all had a total cholesterol greater than 6.5 mmol/L and triglycerides less than 2.5 mmol/L. During the study, 8 subjects dropped out leaving a total of 44 subjects comprising 21 males and 23 females. Subjects were not regularly on aspirin. Their informed consent was obtained and the study protocol was approved by the Ethics Committee of Prince Henry's Hospital, Monash Medical Centre, Melbourne, Australia.

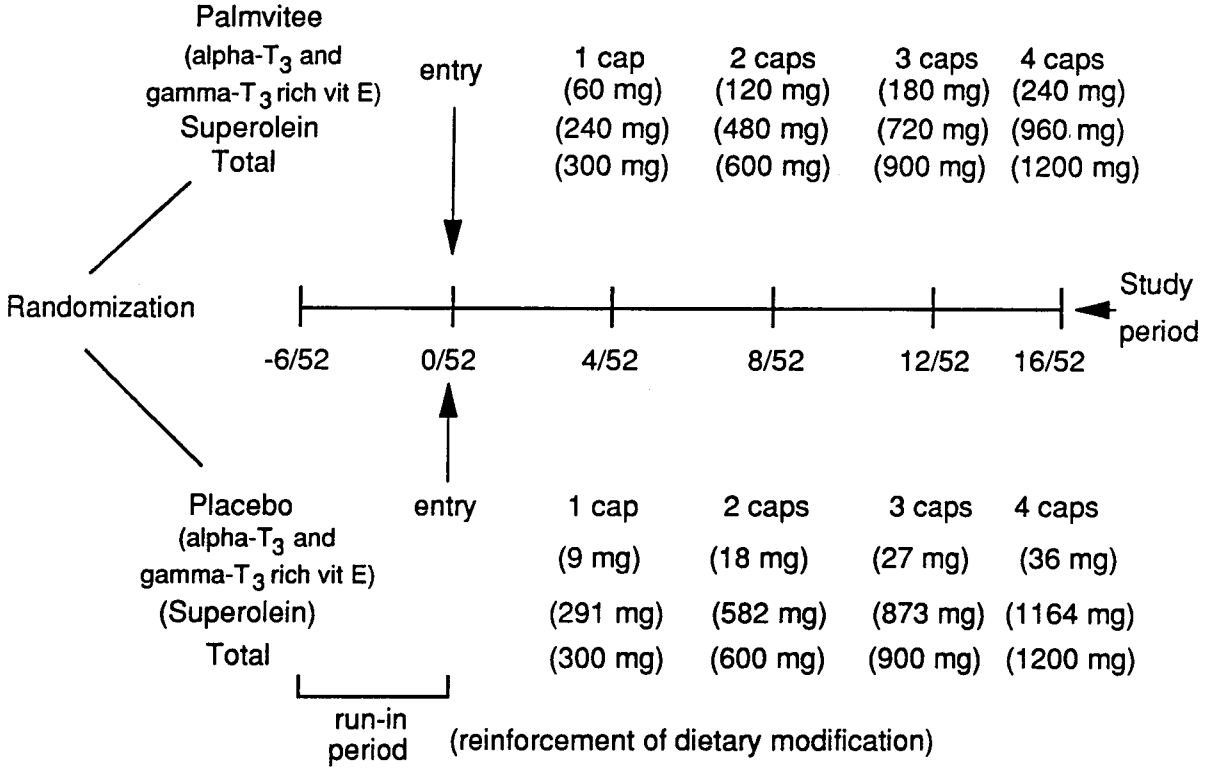

Figure 1: STUDY DESIGN

Dietary supplements

The vitamin E supplements were provided by the Palm Oil Research Institute of Malaysia (Kuala Lumpur) as Palm vitee. The capsules used in this study contained 70% of the vitamin E as a concentrated fraction of tocotrienols (T3)(23% alphatocotrienol, 31% gamma-tocotrienol, and 16% delta-tocotrienol) and a 30% fraction of alpha tocopherol. The capsules contained either 60 mg tocotrienol rich vitamin E (TRVE) in 240mg palm superolein (Palm vitee) or 9 mg TRVE in 291mg palm superolein (placebo), confirmed by independent analysis. Compliance was assessed by counting the number of capsules that were left-over at the end of each 4 week period.

Measurements

Initial measurements included weight, height, body composition (anthropometry and bioelectrical impedance), blood pressure, body circumference and bleeding time. A one-week dietary record, history of medication use, alcohol consumption and smoking habits were recorded. In order to exclude secondary hypercholesterolaemia, all subjects were screened for fasting glucose, serum creatinine, serum uric acid, and liver and thyroid function at entry.

We took 60ml of fasting venous blood at the beginning of each four week period. Serum samples for vitamin E and prostaglandin assays were kept frozen at -70°C and analysed in a batch for each subject at exit from the study. Blood was analysed for total serum cholesterol (TC), high-density lipoprotein cholesterol (HDLC) and triglycerides (TG). Low-density lipoprotein cholesterol (LDL) was calculated by the Friedewald equation (11). Serum vitamin E fractions, plasma thromboxaneB2 (TxB2), plasma 6-keto prostaglandin Fl-alpha (6-keto PGF1-alpha) were also measured. Platelet aggregation was assessed in platelet rich plasma (PRP) against adenosine diphosphate (ADP), collagen, and adrenaline.

SERUM ASSAYS

Tocopherol and Tocotrienol Assays

Tocopherol and tocotrienol fractions were estimated using an HPLC method in a darkroom with red filtered light (12). 300μl serum was precipitated with an equal volume of methanol and the vitamin E extracted with 1.2 ml hexane. Samples were then vortexed and centrifuged and 800μl of supernatant pipetted into a 3 ml brown bottle. Samples were dried under nitrogen and redissolved in 50μl hexane. Duplicate 20μl samples were applied through the 6UK injector to the HPLC column. The HPLC column was microporasil, 10μm (30 cm x 0.46 cm I.D.); the eluent was hexane-isopropanol; the flow rate was 2.2 ml/minute and detection with a fluorescence detector at EX 298 nm, EM 325 nm with attenuation 8; ambient temperature was 20-25°C. Tocopheryl acetate was used as an internal standard.

Lipid assays

Serum cholesterol concentrations were measured by standard enzymatic methods based on cholesterol esterase/cholesterol oxidase using TRACE Liquid Cholesterol reagent Cat No:13225 (TRACE Scientific P/L Melbourne 3168, Australia). The assay was calibrated with Australian Lipid Calibrator Lot 33C & CSC Melbourne, Australia) with assigned values traceable to the Lipid Standardization Laboratory at Centers for Disease Control, Atlanta, USA (13).

Serum triglycerides, as total glycerol, were measured by a standard enzymatic coloromethc technique based on Glycerol Phosphate Oxidase/Peroxidase using American Monitor Triglycedde reagent, Cat No 4185 (American Monitor Corp. Indianapolis USA 46268). The assay was calibrated against a primary glycerol standard (Boehringer Mannheim, Preciset 2.29mmol/L) and showed good agreement with target values in External Quality Assurance Programmes (RCPA-AACB QAP, Australia).

HDL cholesterol assays were performed by Polyethylene Glycol 6000 precipitation of Apo B containing lipoproteins (14, 15), and analysis of the supernatant using the same enzymatic cholesterol reagent as for total cholesterol. The assay was carried out using a ROCHE "Cobas-Bio" Centrifugal Analyzer (ROCHE Products, DEE WHY, Australia), calibrated with an aqueous cholesterol standard (0.75mmol/L) (16).

Prostaglandin assays

Plasma (500μl) was acidified to pH 3.0 with 1 N HCL. Three volumes of ethyl acetate were added and mixed well. The organic and aqueous phases were separated by centrifugation. The upper organic phase containing the prostaglandins was removed and the ethyl acetate was evaporated. The residue was dissolved in buffer. The recovery was calculated by adding 100μl of ^{3}H-labelled TxB2 or 6-keto-PGF1-alpha respectively. The total number of counts added was 70,000 counts per minute. The recovery for TxB2 was 77.4% and for 6-keto PGF1-alpha 67.7%. The radioimmunoassay used a 6 keto PGF1-alpha [^{125}I] and TxB2 [^{125}I] kit supplied by Advanced Magnetic Inc with Biomag Magnetic Superation (17).

Platelet aggregation

Platelet aggregation was measured by the method of Born (18) in PRP and whole blood (WB). Aggregation to ADP and adrenaline was measured at final concentration of 6.25 and 5 umol/L for PRP and aggregation to collagen, at 1.0 and 0.5umol/L in PRP and WB. All aggregation studies were completed within 3 hours of blood collection. Aggregation responses were quantified by measuring the increase or decrease of aggregation at 5 minutes, with the platelet-poor-plasma (PPP) and PRP readings as 100% and 0% aggregation, respectively. Platelets were counted by using a standard counting chamber in a 1:100 dilution of PRP.

Statistical methods

We used the SAS (Statistical Analysis System) for personal computers to test the study hypothesis. The univariate analysis of variance was used to test whether the active group differed from the placebo for serum vitamin E fractions, serum lipids, and platelet aggregation. Total vitamin E was calculated as the sum of all serum vitamin E fractions. Repeated measures analysis of variance was used to test whether changes in serum vitamin E, serum lipids and platelet aggregation occur in the course of supplementation, and whether there were between- and within-subjects differences; because all observations were required, available degrees of freedom were reduced by this statistical approach. The statistical significance level was set at 5%. Multivariate analysis were used to consider factors additional to vitamin E which might have acted in concert with it, to determine lipid status.

RESULTS

Age, weight, height, and body mass index (BMI) at entry are shown in Table 1. 35 subjects (17 in the active and 18 in the placebo group) were included in the repeated measures analysis, since these had no missing values over the 16 weeks.

The supplemented and placebo groups did not differ in age, weight, height, or BMI. There were no differences between the at entry group and the repeated measures group.

The treated and placebo groups were also similar in serum lipids and total vitamin E at entry (Table 2). Serum lipids and vitamin E status for subjects included in repeated measures and multivariate analyses are also shown in Table 2; again, there were no differences between the at entry group and the repeated measures group.

The relationships between age, fatness, lipids, vitamin E, and its major fractions are shown in Tables 3 and 4. The only relationship significant at the 1% level was that between beta tocopherol and age, which was positive.

For the 26 subjects who had lipid measurements at each time point, there were no significant differences in serum TC, TG, HDLC and LDLC values between baseline and intervention periods, or between intervention and non-intervention groups (Table 5). For 35 individuals, differences from 0 to 16 weeks in these variables, for active and placebo groups, are shown in figure 2, with confidence limits.

A rise in serum total vitamin E occurred with the lowest dose, at 4 weeks, after the introduction of the vitamin E supplement but not with placebo (Fig. 3a). Serum total tocopherol and of serum total tocotrienol, responded similarly over the 16 week period (Fig. 3b, 3c).

Figure 4 shows the effects of successive increase in dose of the vitamin E supplement over 16 weeks on the individual tocopherol and tocotrienol fractions. The vitamin E supplement

contained exclusively alpha tocopherol rather than other tocopherols. Serum alpha tocopherol, by trend analysis, rose progressively during the study in the active group. As far as tocotrienol fractions are concerned, the supplement contained dominantly alpha and gamma tocotrienols, but

TABLE 1
Characteristics of Study Subjects

		At entry			Subjects included in the repeated measures analysis		
	Gender	N	Mean	SEM	N	Mean	SEM
Age (year)							
TRVE	F	11	51.9	2.3	9	50.4	2.1
	M	11	51.4	3.2	8	50.9	3.9
Placebo	F	12	55.5	4.2	10	50.7	6.7
	M	10	52.8	3.4	8	49.6	3.5
Weight (Kg)							
TRVE	F	11	74.7	4.2	9	72.5	4.6
	M	11	73.6	2.4	8	73.8	2.9
Placebo	F	12	77.7	5.0	10	77.2	5.4
	M	10	78.2	4.6	8	77.3	4.9
Height (cm)							
TRVE	F	11	161.0	2.1	9	159.7	2.2
	M	11	171.9	1.7	8	173.6	1.9
Placebo	F	12	156.7	2.3	10	140.0	14.3
	M	10	170.9	1.9	8	171.0	1.9
BMI(kg.m-2)							
TRVE	F	11	29.4	1.9	9	28.7	4.9
	M	11	25.0	0.8	8	24.3	0.8
Placebo	F	12	31.1	1.7	10	32.3	4.7
	M	10	27.0	1.6	8	26.5	1.7
WHR							
TRVE	F	11	0.87	0.03	9	0.87	0.03
	M	11	0.92	0.02	8	0.92	0.02
Placebo	F	12	0.91	0.02	10	0.91	0.02
	M	10	0.93	0.02	8	0.93	0.02

N is the number of subjects in each group.

BMI is Body Mass Index in $kg.m^{-2}$.

WHR is the "waist:hip ratio" measured as the mean of abdomimal circumference 12 cm below the xiphisternal notch, divided by hip circumference at the greatest gluteal protuberance.

TRVE refers to the tocotrienol rich vitamin E supplement used in the study.

TABLE 2
Baseline Measurements For Serum Lipids, and Serum vitamin E

	Gender	At entry N	Mean	SEM	Subjects included in the repeated measures analyses N	Mean	SEM
Serum total cholesterol (mmol/L)							
TRVE	F	11	7.24	0.40	9	7.2	0.40
	M	11	7.30	0.27	8	7.3	0.27
Placebo	F	12	6.81	0.44	10	6.8	0.44
	M	10	6.89	0.36	8	6.89	0.36
Triglycerides (mmol/L)							
TRVE	F	11	1.74	0.46	9	1.74	0.46
	M	11	1.33	0.17	8	1.33	0.17
Placebo	F	12	1.64	0.43	10	1.64	0.43
	M	10	1.96	0.31	8	1.96	0.31
HDLC (mmol/L)							
TRVE	F	11	1.37	0.17	9	1.37	0.17
	M	11	1.14	0.05	8	1.14	0.05
Placebo	F	12	1.23	0.18	10	1.23	0.18
	M	10	1.14	0.11	8	1.14	0.11
LDLC (mmol/L)							
TRVE	F	11	5.01	0.40	9	5.01	0.40
	M	11	5.57	0.26	8	5.57	0.26
Placebo	F	12	4.73	0.40	10	4.73	0.40
	M	10	5.41	0.66	8	4.51	0.66
Serum total vitamin E (μmol/L)							
TRVE	F	11	3.89	0.33	9	3.76	0.33
	M	11	3.85	0.29	8	3.85	0.29
Placebo	F	12	4.04	0.36	10	3.94	0.36
	M	10	4.72	0.34	8	4.60	0.34
Serum total tocopherol (μmol/L)							
TRVE	F	11	3.85	0.33	9	3.72	0.34
	M	11	3.79	0.27	8	3.79	0.27
Placebo	F	12	3.99	0.36	10	3.89	0.38
	M	10	4.68	0.33	8	4.56	0.35
Serum total tocotrienol (μmol/L)							
TRVE	F	11	0.04	0.009	9	0.04	0.01
	M	11	0.06	0.027	8	0.06	0.03
Placebo	F	12	0.05	0.010	10	0.05	0.01
	M	10	0.03	0.006	8	0.03	0.016

Abbreviations as in Table 1.
HDLC-high density lipoprotein cholesterol
LDLC-low density lipoprotein cholesterol

TABLE 3
Correlations (r) Between Serum Total vitamin E, Tocopherols, Tocotrienols, Lipids and AGE, BMI and WHR at Entry.

	AGE	BMI	WHR
Vitamin E -total	0.32*	-0.12	0.26
Tocopherol-total	0.31	-0.12	0.26
-alpha	0.24	-0.17	0.22
-beta	0.49**	0.12	0.29
-gamma	0.29	0.18	0.20
-delta	0.34*	0.02	0.10
Tocotrienol-total	0.37	0.06	0.19
-alpha	0.10	-0.10	-0.38*
-gamma	0.34*	0.09	0.29
-delta	0.10	0.06	0.20
Lipids			
-CHOL	0.21	-0.25	0.21
-TRIG	0.07	0.06	0.20
-HDLC	0.33*	0.06	-0.17
-LDLC	0.12	-0.33	0.15

CHOL: cholesterol.
TRIG: triglyceride.
HDLC: high density lipoprotein cholesterol.
LDLC: low density lipoprotein cholesterol.
* $p < 0.05$
** $P < 0.01$.

the only significant increase in serum tocotrienol was in the alpha fraction, whilst the gamma and delta did not change significantly. When change with time in serum gamma tocotrienol was adjusted by age, BMI and WHR, there was still no significant difference ($+ 0.020 \pm 0.012$, mean change $\pm$ SEM).

We did not observe any significant changes in TxB2 or 6-keto-PGF1-alpha with increased dose, or with time, or between active and placebo groups (Table 6). The skin bleeding time remained unchanged (Table 7). There were no significant differences between men and women. Platelet aggregation studies with ADP, collagen and adrenaline were all negative (Table 8).

TABLE 4
Correlations (r) Between Serum vitamin E, Tocopherols, Tocotrienols and Lipids.

	CHOL	TRIG	HDLC	LDLC
Vitamin E -total	0.19	0.01	-0.10	-0.22
Tocopherol-total	0.20	0.01	-0.11	0.22
-alpha	-0.19	0.01	-0.11	0.22
-beta	0.19	0.30	0.02	0.08
-gamma	-0.02	0.37*	-0.09	-0.18
-delta	0.25	-0.01	0.40*	0.11
Tocotrienol-total	0.02	-0.06	0.25	-0.04
-alpha	-0.07	-0.17	0.22	-0.03
-gamma	0.02	0.02	0.10	-0.05
-delta	0.15	-0.14	0.40*	0.08

Abbreviations used as in Table 3.
* $p < 0.05$

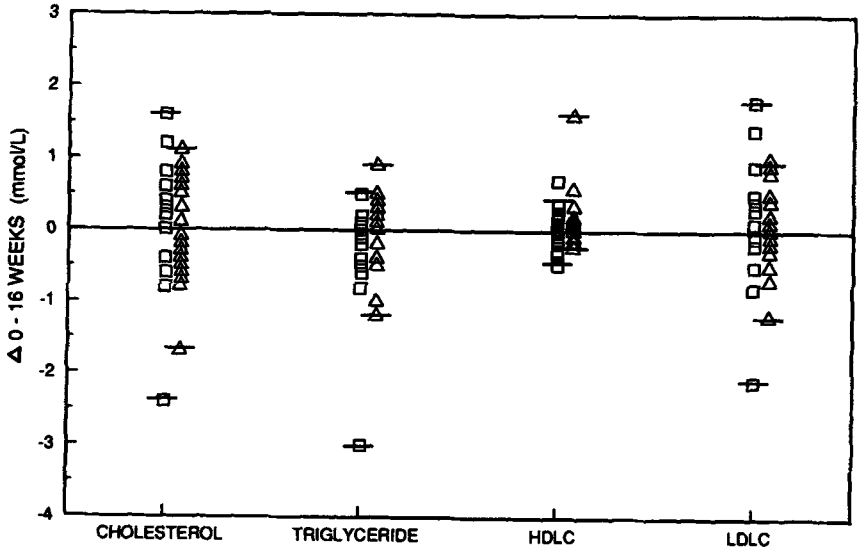

Fig. 2. Change (△) in serum lipids during 16 weeks of TRVE supplementation. (□) TRVE (N=17); (△) Placebo (N=18); -- with overlaping points. 95% Confidence limits are shown.

TABLE 5
Serum Lipids During TRVE Supplementation

	PERIOD				
	0/52	4/52	8/52	12/52	16/52
Cholesterol (mmol/L)					
TRVE	7.51	7.52	7.56	7.53	7.74
	(0.26)	(0.38)	(0.42)	(0.24)	(0.31)
Placebo	6.95	7.00	6.85	6.82	6.75
	(0.31)	(0.34)	(0.33)	(0.32)	(0.33)
Triglyceride (mmol/L)					
TRVE	1.76	1.69	1.59	1.52	1.42
	(0.35)	(0.35)	(0.26)	(0.29)	(0.16)
Placebo	2.08	2.05	1.95	2.09	1.92
	(0.38)	(0.37)	(0.29)	(0.35)	(0.31)
HDLC (mmol/L)					
TRVE	1.32	1.30	1.25	1.33	1.26
	(0.09)	(0.08)	(0.11)	(0.09)	(0.09)
Placebo	1.28	1.28	1.33	1.26	1.44
	(0.12)	(0.12)	(0.13)	(0.13)	(0.17)
LDLC (mmol/L)					
TRVE	5.44	5.48	5.51	5.50	5.70
	(0.27)	(0.28)	(0.33)	(0.24)	(0.35)
Placebo	4.87	4.83	4.64	4.69	4.58
	(0.27)	(0.27)	(0.27)	(0.31)	(0.27)

The number of subjects were 26 when data were available on all occasions.
Abbreviations as in Table 2.
Mean (SEM) are shown.

DISCUSSION

Serum vitamin E responses to supplementay tocotrienol

Serum total vitamin E had risen maximally with the lowest dose of TRVE by 4 weeks and remained elevated at similar levels thereafter; the increment exceeded 1 μmol/L. Insofar as responses to tocotrienol supplementation were concerned, with the lowest dose at 4 weeks, there was a doubling in serum tocotrienol concentration. This was not different at 16 weeks. It is

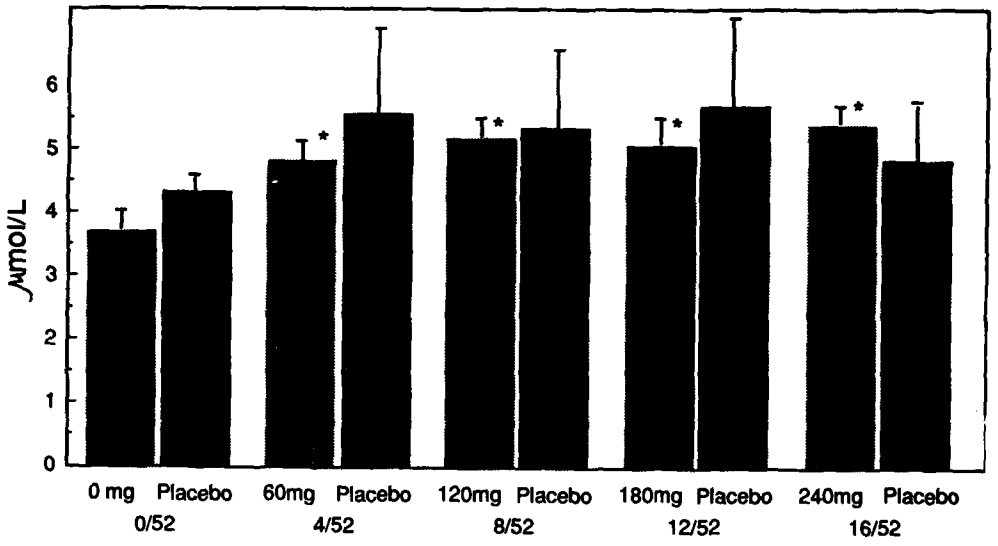

Fig. 3a. Serum total vitamin E during TR vitamin E supplementation - mean (SEM). N=35; * $p < 0.05$.

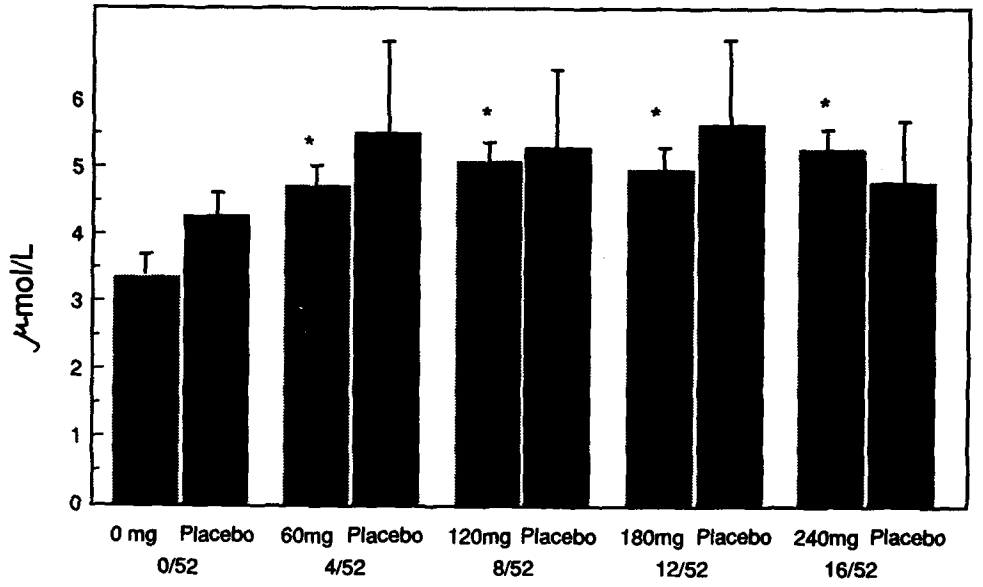

Fig. 3b. Serum total tocopherol during TR vitamin E supplementation - mean (SEM). N=35; * $p < 0.05$.

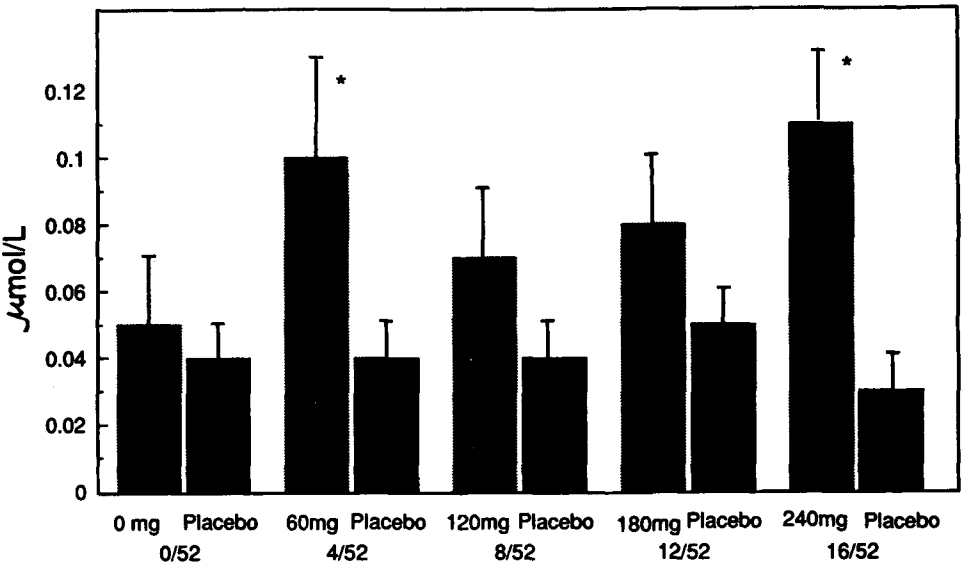

Fig. 3c. Serum total tocotrienol during TR vitamin E supplementation - mean (SEM).
N = 35 * $p < 0.05$.

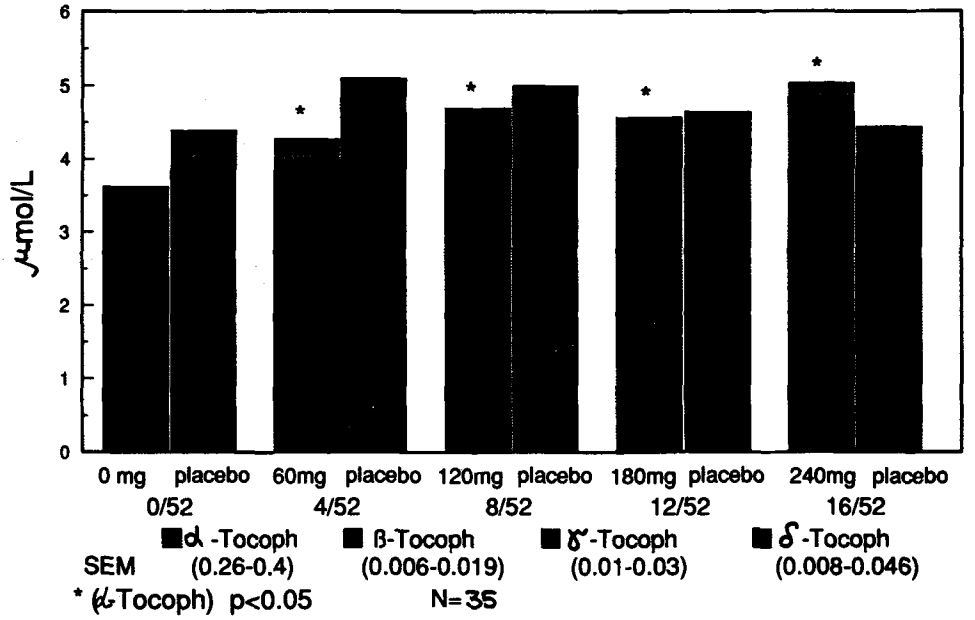

Fig. 4a. Serum tocopherol fractions during TR vitamin E supplementation.

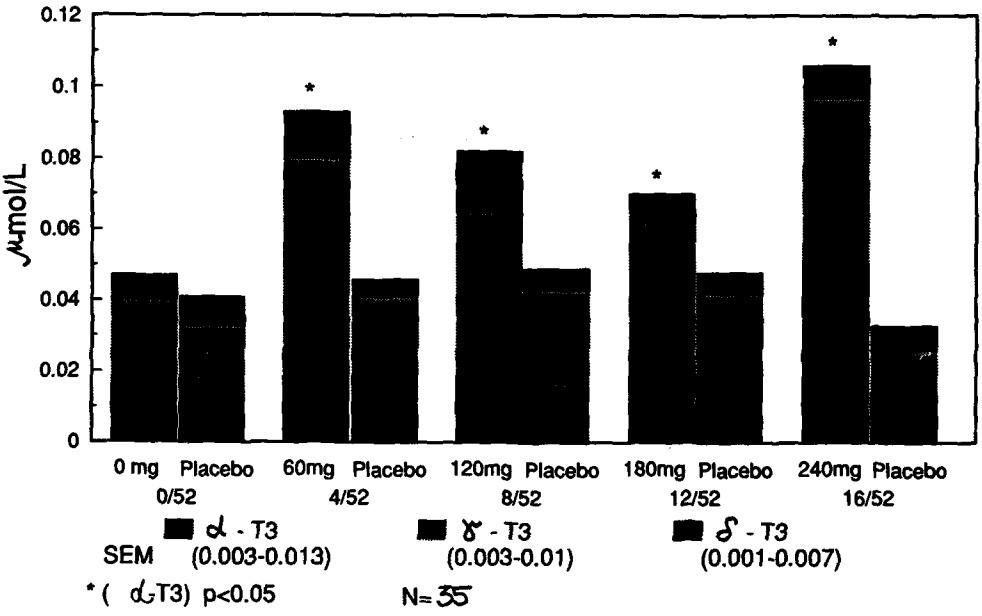

Fig. 4b. Serum tocotrienol fractions during TR vitamin E supplemention.

of interest that only 0.05 of the 1μmol/l vitamin E increase was attributable to tocotrienol; the rest was due to tocopherol. The background diet during these studies was not changed and therefore the changes in tocopherols, with an increase in alpha tocopherol, presumably simply reflect alpha tocopherol supplementation. However, the work of Traber and Kayden (19) documented that alpha tocopherol was preferentially incorporated over gamma tocopherol into lipoprotein. This is of interest in terms of the physiological interaction between different forms of vitamin E during removal from the circulation and it appears that there is no significant reduction of other serum tocopherols (beta, gamma and delta) as a consequence of alpha tocopherol ingestion.

Even though the ingested molar ratio of tocotrienol to tocopherol was 2.3, the incremental molar ratio in serum at 16 weeks was 0.04 in the active group, and with no change in the placebo. This has implications for the differential handling of these 2 forms of vitamin E during absorption or clearance from the serum. Likewise, although mainly alpha and gamma tocotrienols were similarly supplemented, only serum alpha tocotrienol responded significantly to supplementation. Any biological effects of vitamin E supplementation or serum lipoproteins, are likely to depend not only on the vitamin E profile in the supplement, but also that in the serum following ingestion.

TABLE 6
Plasma Thromboxane B2 and 6-keto-PGF1-alpha During TRVE Supplementation

	PERIOD				
	0/52	4/52	8/52	12/52	16/52
Thromboxane B2 (log moles10^{-9}/L)					
TRVE	2.62	2.75	2.69	2.80	2.82
	(0.29)	(0.22)	(0.28)	(0.27)	(0.32)
Placebo	3.16	2.54	3.00	2.71	2.67
	(0.33)	(0.35)	(0.30)	(0.37)	(0.41)
6-keto-PGF1-alpha (log moles10^{-9}/L)					
TRVE	1.11	1.11	0.81	0.71	1.16
	(0.16)	(0.17)	(0.12)	(0.12)	(0.20)
Placebo	1.00	0.94	0.89	0.82	0.99
	(0.24)	(0.15)	(0.16)	(0.15)	(0.17)

The number of subjects were 34 when data were available on all occasions.
TRVE refers to tocortrienol-rich vitamin E supplement used in the study.
Mean (SEM) are shown.

TABLE 7
Skin Bleeding Time During Administration of TRVE

		PERIOD				
		0/52	4/52	8/52	12/52	16/52
TRVE	F	4.78	4.33	4.33	4.11	4.44
		(0.55)	(0.33)	(0.44)	(0.48)	(0.24)
	M	3.22	3.22	3.22	3.11	3.00
		(0.22)	(0.34)	(0.45)	(0.26)	(0.23)
Placebo	F	3.92	3.91	4.09	3.54	3.10
		(0.36)	(0.46)	(0.46)	(0.28)	(0.18)
	M	2.86	2.86	2.62	3.25	3.57
		(0.26)	(0.26)	(0.32)	(0.59)	(0.50)

The number of subjects were 44 when data were available on all occasions.
Skin bleeding time measurements are in minutes.
Mean (SEM) are shown.

Serum lipid responses to supplementary tocotrienol

With the error observed in cholesterol measurement in the study (inclusive of analytical and biological errors), and the t values, at the 5% level of significance (two-tailed)with 25 degrees of freedom (as used in trend analysis) or with 51 degrees of freedom (maximum possible with subject recruitment), we would have been able to observe a significant difference in serum total cholesterol of 0.84 or 0.79 mmol/L, respectively. For HDL cholesterol, the differences observable would have been 0.26 or 0.25 respectively. This, of course, would require that the placebo group did not change significantly. Arithmetically, we observed a change in total cholesterol of +0.23 mmol/l and HDL cholesterol of -0.04 mmol/l from the beginning to the end of the study in the active group.

By contrast, in a responsive subset on tocotrienol for 4 weeks, Qureshi et al (5) reported a lower total (biological and analytical) error; the reported reduction in serum cholesterol was 2.41 mmol/L. We would certainly have been able to observe this order of magnitude of fall in serum total cholesterol, but we could not. Moreover, we found only 4 out of 17 subjects in our active group to have a fall in serum total cholesterol from 0 to 16 weeks at treatment. It should be noted that Qureshi et al. (5) did not report a significant effect of TRVE supplementation on serum lipids for their total study population, but only for "responders".

One possible explanation for our negative finding is that, in our study, tocotrienol concentrations did not reach the necessary level to inhibit cholesterol synthesis. The concentrations achieved in the Qureshi study at 4 weeks with 4 capsules of TRVE from palm oil were higher than ours in a subset of 7 out of 15. A non controlled study has shown that palm-oil-vitamin E concentrate has hypocholesterolaemic effects (20), possibly through its HMG-CoA reductase activity (21, 8), which might be expected to be dose-dependent. In our study, however, supplementation with the same preparation, with increasing dosage did not result in reduced TC or LDL cholesterol concentrations at any dosage.

Even though the group means for lipids did not change (and the changes did not reach 95% confidence limits), the question remains as to whether or not an individual's baseline vitamin E status, or change in vitamin E status during the study, might have accounted for the individual's change in lipid status. We have, therefore, analysed this possibility by repeated measure analysis of variance and found no evidence that this is the case. Moreover, we have taken into account body composition at baseline (fatness as body mass index and distribution of fatness by waist-hip ratio) to assess the possibility that vitamin E might have been interactive with body composition, to allow the determination of lipid status. Whether at baseline (cross-sectionally), or during the study, the multivariate analysis of these several factors which may have been collectively contributory to lipid serum status, has been unrewarding.

TABLE 8
Platelet Aggregation During TRVE Supplementation

	PERIOD				
	0/52	4/52	8/52	12/52	16/52
TRVE					
(ADP 1 x 10^{-5} M)					
1° & 2° phase	100	100	100	100	100
1° phase only	0	0	0	0	0
Absent AGG'N	0	0	0	0	0
(ADP 5 x 10^{-6} M)					
1° & 2° phase	100	100	100	100	100
1° phase only	0	0	0	0	0
Absent AGG'N	0	0	0	0	0
(ADP 2.5 x 10^{-6} M)					
1° & 2° phase	77	86	82	88	85
1° phase only	18	14	18	6	15
Absent AGG'N	5	0	0	6	0
(ADP 1.25 x 10^{-6} M)					
1° & 2° phase	27	32	27	44	46
1° phase only	68	64	64	50	46
Absent AGG'N	5	4	9	6	8
(ADP 6.25 x 10^{-7} M)					
1° & 2° phase	5	5	5	6	15
1° phase only	62	68	63	63	54
Absent AGG'N	33	27	32	31	31
(COLL 2mg/ml)					
1° & 2° phase	100	100	95	82	92
Reduced AGG'N	0	0	5	12-	8
Absent AGG'N	0	0	0	6	0
(COLL 1 mg/ml)					
1° & 2° phase	86	82	90	76	92
Reduced AGG'N	9	9	5	12-	0-
Absent AGG'N	5	9	5	12	8

(COLL 0.5mg/ml)					
1° & 2° phase	48	55	50	65	46
Reduced AGG'N	0-	5	0	0-	15
Absent AGG'N	52	40	50	35	39
(ADR 1 x 10^{-5} M)					
Normal AGG-N	68	82	77	71	62
Reduced AGG'N	18	5	9	18	31
Absent AGG'N	14	13	14	11	7
(ADR 5 x 10^{-5} M)					
Normal AGG-N	68	86	72	71	54
Reduced AGG-N	18	4	14	18	38
Absent AGG'N	14	10	14	11	8
PLACEBO					
(ADP 1 x 10^{-5} M)					
1° & 2° phase	100	100	100	100	100
1° phase only	0-	0-	0-	0-	0-
Absent AGG'N	0-	0-	0-	0-	0-
(ADP 5 x 10^{-6} M)					
1° & 2° phase	100	100	100	100	100
1° phase only	0-	0-	0-	0-	0-
Absent AGG'N	0-	0-	0-	0-	0-
(ADP 2.5 x 10^{-6} M)					
1° & 2° phase	86	95	90	88	100
1° phase only	14	5	10	12	0-
Absent AGG'N	0-	0-	0-	0-	0-
(ADP 1.25 x 10^{-6} M)					
1° & 2° phase	45	32	48	35	38
1° phase only	45	59	52	59	62
Absent AGG'N	10	9	0-	6	0-
(ADP 6.25 x 10^{-7}M)					
1° & 2° phase	5	5	0-	6	0-
1° phase only	71	68	71	76	88
Absent AGG'N	24	27	29	18	12

(COLL 2mg/ml)					
1° & 2° phase	77	90	86	83	88
Reduced AGG'N	9	5	14	6	0-
Absent AGG'N	14	5	0-	11	12
(COLL 1 mg/ml)					
1° & 2° phase	71	73	76	78	74
Reduced AGG'N	10	18	5	5	13
Absent AGG'N	19	9	19	17	13
(COLL 0.5mg/ml)					
1° & 2° phase	40	36	48	47	38
Reduced AGG'N	10	0-	4	6	12
Absent AGG'N	50	64	48	47	50
(ADR 1 x 10^{-5} M)					
Normal AGG'N	59	63	62	72	50
Reduced AGG'N	18	32	19	17	38
Absent AGG'N	22	5	19	11	12
(ADR 5 x 10^{-5}M)					
Normal AGG'N	59	64	62	72	50
Reduced AGG'N	18	31	19	11	38
Absent AGG'N	23	5	19	17	12

The number of subjects were 40, when data were available on all occasions.
AGG'N refers to platelet aggregation.
ADP: adenosine 5-diphosphate.
COLL: collagen.
ADR: adrenaline.

Prostaglandins and platelet function response to tocotrienol

We used the prostaglandin catabolites, TxB2, as an indicator of thromboxane and 6-keto PgFl alpha, as indicator of prostacyclin production. Our findings were negative and did not support previous observations (5) that tocotrienol might alter the production of prostanoids and reduce platelet aggregation.

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