Response of Hypercholesterolemic Subjects to Administration of Tocotrienols[1]

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ABSTRACT: The cholesterol-suppressive actions of Palmvitee and γ-tocotrienol were assessed in hypercholesterolemic subjects after acclimation to the American Heart Association Step 1 dietary regimen for four and eight weeks, respectively. The four-week dietary regimen alone elicited a 5% decrease ($P < 0.05$) in the cholesterol level of the 36 subjects. Subjects continuing on the dietary regimen for a second four-week period experienced an additional 2% decrease in their cholesterol levels. Dietary assessments based on unanticipated recalls of 24-h food intake records suggest that significant reductions in energy and fat, predominantly in saturated fat, intakes are responsible. The subjects experienced significant Palmvitee- and γ-tocotrienol-mediated decreases in cholesterol. The group of subjects acclimated to the dietary regimen for four weeks responded to Palmvitee (a blend of tocols providing 40 mg α-tocopherol, 48 mg α-tocotrienol, 112 mg γ-tocotrienol, and 60 mg δ-tocotrienol/day for four weeks) with a 10% decrease in cholesterol ($P < 0.05$). Dietary assessments showed no further change in energy and fat intakes. α-Tocopherol attenuates the cholesterol-suppressive action of the tocotrienols. The second group of subjects, acclimated to the dietary regimen for eight weeks, received 200 mg γ-tocotrienol/d for four weeks. The cholesterol-suppressive potency of this α-tocopherol-free preparation was calculated to be equivalent to that of the mixture of tocotrienols (220 mg) used in the prior study. Cholesterol levels of the 16 subjects in the second group decreased 13% ($P < 0.05$) during the four-week trial. Plasma apolipoprotein B and *ex vivo* generation of thromboxane B_2 were similarly responsive to the tocotrienol preparations, whereas neither preparation had an impact on high density lipoprotein cholesterol and apolipoprotein A-I levels.

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The tocotrienols initiate post-transcriptional actions which lead to the down regulation of 3-hydroxy-3-methylglutaryl–CoA (HMG–CoA) reductase activity (1). Mevalonic acid, the product of this reaction, is generally considered to be the rate-limiting substrate for the synthesis of cholesterol. A decrease in serum cholesterol, a response consistent with the tocotrienol-mediated down-regulation of HMG–CoA reductase activity, is reported from some (2,3), but not all, human studies (4). We (2) and others (3,4) have reported the failure of some individual hypercholesterolemic subjects to respond to the tocotrienols. This variation could be anticipated, as the biological action of tocotrienols may limit their impact to those subjects who have an overproduction of cholesterol.

Excessive dietary intake of cholesterol overrides the biological action of the tocotrienols, but other confounding factors may be less apparent. Subjects receiving placebos often record modest decreases in blood lipids; these changes are attributed to subtle changes in dietary patterns. We recently confirmed (5) our preliminary finding (6) that α-tocopherol attenuates the impact of the tocotrienols on HMG–CoA reductase activity. This finding addresses another confounding factor. Tocotrienol preparations available for human tests (Palmvitee; BCP, AG; PORIM, Kuala Lumpur, Malaysia) are not standardized. Our review of the tocotrienol literature found that the studies showing a cholesterol-lowering action of the tocotrienols employed either a purified tocotrienol or Palmvitee preparations containing less than 20% tocopherol (5). The following study was designed to avoid the aforementioned confounding factors.

METHODS

Study population. Subjects were recruited from a hypercholesterolemic population (serum cholesterol > 5.7 mmol/L) screened at the University of Illinois, Chicago (Chicago, IL). Volunteers were excluded on the basis of weight (>150% of Metropolitan Life ideal weight), use of cholesterol-altering medication, an elevated serum glutamate-pyruvate or glutamate-oxaloacetate transaminase activity, an elevated blood urea nitrogen or glucose level, diabetes, or a history of a liver, renal, or hypertensive disease. Subject screening was accomplished during a three-week period. A fasting blood sample

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Abbreviations: AHA Diet, American Heart Association Step 1 Diet; ApoA-I, apolipoprotein A-I; ApoB, apolipoprotein B; HDL, high density lipoprotein; HMG–CoA, 3-hydroxy-3-methylglutaryl–CoA; LDL, low density lipoprotein; TxB_2, thromboxane B_2

was collected for cholesterol determination at the initial session following the determination of eligibility. Following blocking by gender and stratification according to low and high cholesterol levels as determined at screening, the subjects in each of the four subsets were randomized into an experimental (Palmvitee) group of 20 subjects (7.02 ± 0.99 mmol cholesterol/L) and a control (Placebo) group of 16 subjects (7.32 ± 0.84 mmol cholesterol/L). The groups were uniform in male/female distribution (45:55), age (40.6 ± 12.1 y), and body mass index (28.1 ± 4.9). All subjects signed an informed consent which was approved by the University of Illinois, Chicago Medical Center Institutional Review Board. The study was conducted under IND number 30906.

Experimental design. The 16-wk study consisted sequentially of Baseline, American Heart Association Diet (7,8), Palmvitee, and γ-Tocotrienol phases.

Baseline phase. On entry, the subjects met individually and in small group sessions with counselors. The individual sessions focused on 24-h recalls of food consumption, and the group sessions provided instructions for keeping three-day records of food intake (two weekdays, one weekend day). Subjects were encouraged to follow their typical dietary pattern and were instructed to keep food records for the terminal three days of this and the following phases. Subjects also received an unanticipated telephone call for a 24-h recall of food intake.

American Heart Association Step 1 Diet (AHA Diet) phase. Subjects met in small groups for discussions of the relationship between diet and cardiovascular risk factors and for instruction on the AHA Diet. Each subject received a copy of the *1988 AHA Diet, Patient Manual*, and the telephone number of a staff contact person.

Palmvitee phase. The subjects were continued on the AHA Diet during this phase; each subject consumed four 300 mg capsules daily. Subjects in the Placebo group received capsules containing 300 mg corn oil and ~0.25 mg α-tocopherol. Palmvitee subjects received capsules containing 235 mg palm olein, 10 mg α-tocopherol, 12 mg α-tocotrienol, 28 mg γ-tocotrienol, and 15 mg δ-tocotrienol (AG; PORIM, Kuala Lumpur, Malaysia).

γ-Tocotrienol phase. The 16 Placebo subjects were continued on the AHA Diet regimen for a final four weeks, during which each received four 300 mg capsules daily (each capsule contained 250 mg palmolein and 50 mg γ-tocotrienol). γ-Tocotrienol was isolated the tocotrienol-rich fraction of palm oil (AG; PORIM).

Analyses. Initial measures include the subjects' height, weight, blood pressure, history of significant diseases, medications, and alcohol use. Weights were recorded weekly. Venous blood samples were drawn at 7:00–9:00 a.m. following an overnight fast at the Baseline phase (week three represents data obtained at screening and at week four), and at weeks seven and eight (AHA Diet), 11 and 12 (Palmvitee), and 15 and 16 (γ-Tocotrienol) of the study. Processed samples were held at −72°C until analysis.

The study design provided control and treatment cholesterol values for each subject. Automated clinical laboratory procedures were used for cholesterol determinations at weeks 4, 8, 12, and 16. We found that the standard deviations for the paired differences were on the order of 51 ± 12% of the mean. At the termination of the study, we analyzed the samples according to the manual protocol. The standard deviations for the paired differences were on the order of 15 ± 3% of the mean (from Scheme 1). We then sent random coded samples to clinical laboratories at the University of Illinois and the University of Wisconsin for analyses. The results of these analyses proved to be more consistent with those obtained by the manual protocol. The manual and automated clinical procedures yielded similar values for the subjects with the relatively lower cholesterol levels. It should be noted that the latter clinical determinations were run with instruments at each setting calibrated against a single standard, whereas the ini-

	Baseline	Δ1	AHA Diet	Δ2	AHA Diet	Δ3	γ-Tocotrienol
Group							
Placebo	7.29 ± 0.72		6.89 ± 0.82		6.69 ± 0.83		5.82 ± 0.69
Paired difference		−0.40 ± 0.07		−0.20 ± 0.03		−0.87 ± 0.10	

Δ1 > Δ2 $P < 0.025$ Δ2 < Δ3 $P < 0.001$

	Baseline	Δ1	AHA Diet	Δ2	Palmvitee
Palmvitee	7.03 ± 0.06		6.65 ± 1.04		5.97 ± 1.01
Paired difference		−0.38 ± 0.05		−0.68 ± 0.12	

Δ1 < Δ2 $P < 0.050$

SCHEME 1

TABLE 1
Impact of Four-Week American Heart Association Step 1 Diet (AHA) Regimen on Dietary Intake[a]

Dietary regimen	n	Baseline	AHA	*t*	*P*
Energy (kJ/d)	36	7498 ± 3367	5807 ± 1898	2.96	0.01
Protein (g/d)	36	71.7 ± 33.4	65.6 ± 28.5	0.83	NS
Carbohydrate (g/d)	36	221.9 ± 87.8	178.9 ± 74.1	2.23	0.05
Fat (g/d)	36	67.6 ± 46.0	47.3 ± 25.0	2.32	0.03
SFA (g/d)	36	23.6 ± 7.9	14.6 ± 8.5	4.66	0.01
MUFA (g/d)		24.9 ± 17.6	16.9 ± 16.4	1.96	0.07
PUFA (g/d)		12.6 ± 9.3	11.4 ± 5.9	0.65	NS
Cholesterol (mg/d)	36	220 ± 170	148 ± 80	2.29	0.04
Alcohol (g/d)	36	4.4 ± 11.1	0.6 ± 2.2	2.01	0.06
Fiber (g/d)	36	14.0 ± 6.6	15.2 ± 6.9	0.75	NS

[a]SFA, saturated fatty acid; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid; NS, not significant; *t*, paired two-failed *t*-test, *P*, probability.

TABLE 2
Impact of Four-Week American Heart Association Step 1 Diet (AHA) Regimen on Blood Parameters[a]

Parameter	n	Baseline	AHA	*t*	*P*
Cholesterol (mmol/L)	36	7.15 ± 0.85	6.76 ± 0.94	2.50	0.03
LDL cholesterol (mmol/L)	36	5.10 ± 0.80	4.69 ± 0.86	2.07	0.05
ApoB (g/L)	36	1.00 ± 0.12	0.91 ± 0.09	3.60	0.01
HDL cholesterol (mmol/L)	36	1.13 ± 0.20	1.15 ± 0.18	0.45	NS
ApoA-I (g/L)	36	1.12 ± 0.10	1.13 ± 0.11	0.40	NS
Triglycerides (mmol/L)	36	1.93 ± 0.44	1.86 ± 0.43	0.68	NS
Glucose (mmol/L)	36	5.97 ± 0.86	5.66 ± 0.77	1.63	NS

[a]LDL, low density lipoprotein; ApoB, apolipoprotein B; HDL, high density lipoprotein; ApoA-I, apolipoprotein A-I; NS, not significant. See Table 1 for other abbreviations.

tial analyses were conducted over a nine-month period with multiple standards. Alternatively, the extended incubation employed with the manual procedure might permit the more accurate determination of very elevated cholesterol levels.

The analyses of the coded samples were performed at Advanced Medical Research (Madison, WI). Serum cholesterol, high density lipoprotein (HDL) cholesterol, triglyceride, and glucose concentrations were estimated with reagent kits from Sigma Chemical Co. (St. Louis, MO). The incubation time for the cholesterol determinations was 10 min. Serum apoprotein A-I (ApoA-I), apolipoprotein B (ApoB), and thromboxane B_2 (TxB_2) concentrations were determined by radio immunoassay using kits from Sigma (ApoA-I, ApoB) and Chemicon International Chemicon International (El Segundo, CA) (TxB_2).

Diet records and 24-h recalls were analyzed (Nutrition Coordinating Center, University of Minnesota, Minneapolis, MN); if required, subjects were individually counseled to modify food intake to meet the goals of the AHA Diet or to maintain weight.

Statistical analyses. Treatment effects were evaluated by analysis of variance (SuperANOVA; Abacus Concepts, Berkeley, CA) and *t*-test (StatView; Abacus Concepts). *P* values shown on figures and in Tables 1 and 2 were obtained by two-tailed *t*-test of differences between means. We further evaluated the within group treatment effects on cholesterol (Fig. 7, shown later) using the paired, two-tailed *t*-test. Mean ± SD values are recorded in tables; mean ± SEM values are plotted on figures.

RESULTS

The intervention for all subjects during weeks 4–8 phase was the AHA diet. We first examined the subjects' response to the AHA diet instructions. Although somewhat limited from a quantitative standpoint, our analysis (Table 1) reveals that the subjects significantly decreased their reported intakes of energy (23%, $P < 0.01$), carbohydrate (19%, $P < 0.05$), fat and saturated fatty acids (40%, $P < 0.03$; and 38%, $P < 0.01$, respectively), and cholesterol (33%, $P < 0.04$). Concomitant with these changes, there were modest, but significant, decreases (Table 2) in cholesterol (5%, $P < 0.03$), the estimate (9) of low density lipoprotein (LDL) cholesterol (8%, $P < 0.05$), and ApoB (9%, $P < 0.01$). All subjects were continued on the AHA diet during weeks 8–12 phase of the study. Our analysis revealed no significant differences between the subjects reported intakes at weeks 4–8 and weeks 8–12. This design enabled us to evaluate the impact of an eight-week exposure to the AHA dietary regimen on blood parameters of 16 subjects (Placebo, Figs. 1–6). Our analyses of differences

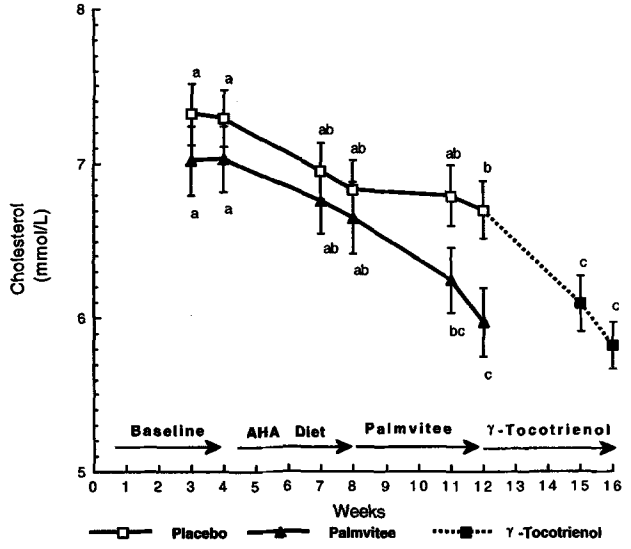

FIG. 1. Time-dependent impacts of American Heart Association Step 1 Diet (AHA) dietary regimen, Palmvitee (PORIM, Kuala Lumpur, Malaysia) and γ-Tocotrienol on serum cholesterol levels of hypercholesterolemic adult subjects. Differences between values on each plotted line which do not not share a letter are significant ($P < 0.05$).

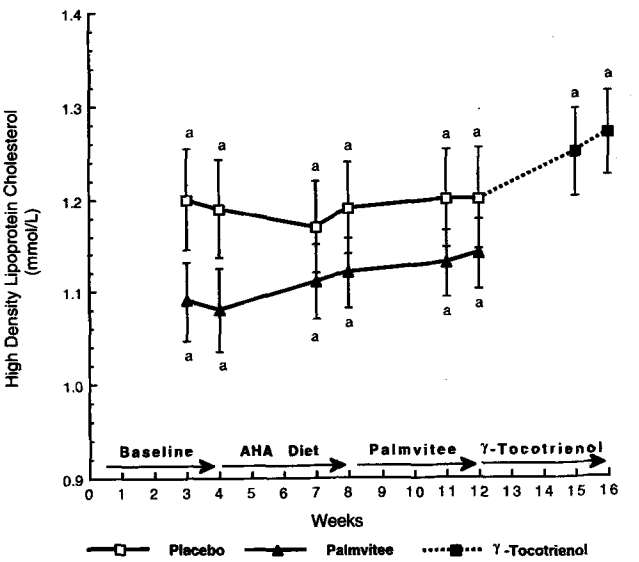

FIG. 3. Time-dependent impacts of AHA dietary regimen, Palmvitee, and γ-Tocotrienol on high density lipoprotein cholesterol levels of hypercholesterolemic adult subjects. Differences between values on each plotted line were not significant. Abbreviation and source as in Figure 1.

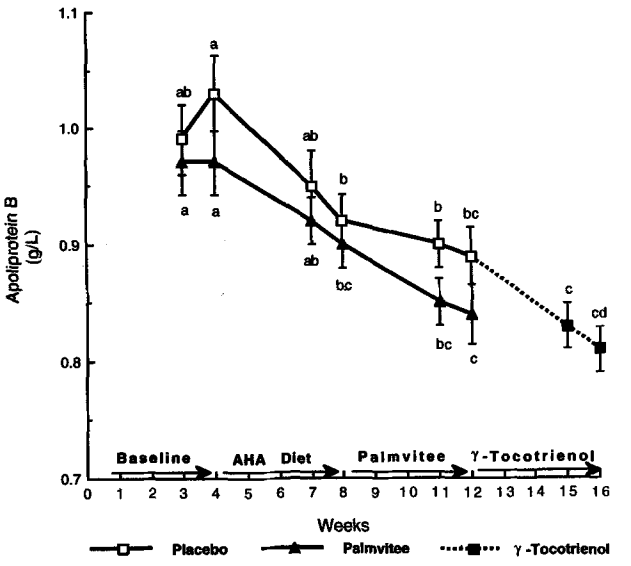

FIG. 2. Time-dependent impacts of AHA dietary regimen, Palmvitee, and γ-Tocotrienol on serum apolipoprotein B levels of hypercholesterolemic adult subjects. Differences between values on each plotted line which do not not share a letter are significant ($P < 0.05$). Abbreviation and company source as in Figure 1.

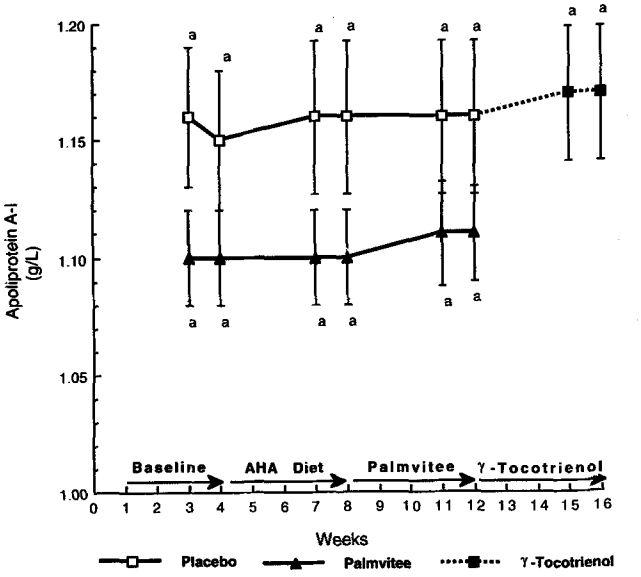

FIG. 4. Time-dependent impacts of AHA dietary regimen, Palmvitee, and γ-Tocotrienol on serum apolipoprotein A-I levels of hypercholesterolemic adult subjects. Differences between values on each plotted line were not significant. Abbreviation and company source as in Figure 1.

between Baseline (week four) and week 12 values reveal significant decreases in cholesterol (8%, $P < 0.05$, Fig. 1), LDL cholesterol (11%, $P < 0.05$), ApoB (13%, $P < 0.05$, Fig. 2), and glucose (9%, $P < 0.05$, Fig. 6). HDL cholesterol (Fig. 3), ApoA-I (Fig. 4), and triglyceride (Fig. 5) levels were not influenced by the dietary regimen. It should be noted that these subjects received 1.2 g corn oil in capsule form during weeks 8–12. In summary, the AHA diet proved to be effective in lowering the total cholesterol, LDL cholesterol (calculated, data not shown), ApoB, and glucose levels of this group of hypercholesterolemic subjects.

The cholesterol levels of the aforementioned 16 subjects decreased by 5 and 3% during the successive four-week exposures to the AHA dietary regimen. The cholesterol level of the remaining 20 subjects decreased by 5% during the first four-week exposure to the dietary regimen. These subjects re-

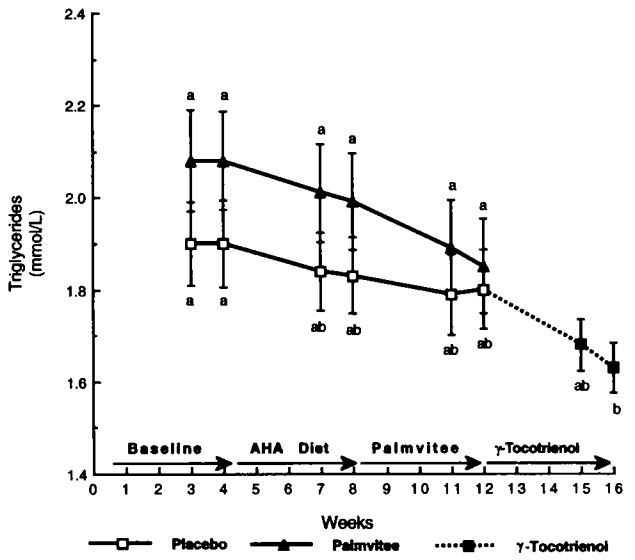

FIG. 5. Time-dependent impacts of AHA dietary regimen, Palmvitee, and γ-Tocotrienol on serum triglyceride levels of hypercholesterolemic adult subjects. Differences between values on each plotted line which do not share a letter are significant ($P < 0.05$). Abbreviation and company source as in Figure 1.

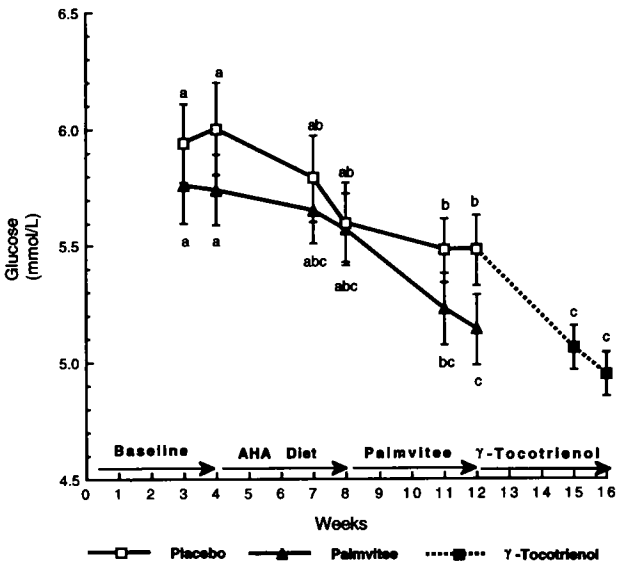

FIG. 6. Time-dependent impacts of AHA dietary regimen, Palmvitee, and γ-Tocotrienol on serum glucose levels of hypercholesterolemic adult subjects. Differences between values on each plotted line which do not share a letter are significant ($P < 0.05$). Abbreviation and company source as in Figure 1.

ceived Palmvitee, a tocotrienol-rich fraction of palm oil providing 40 mg α-tocopherol, 48 mg α-tocotrienol, 112 mg γ-tocotrienol, 60 mg δ-tocotrienol, and 1.14 g palm olein (0.6 g oleic acid, 0.15 g linoleic acid, and 0.4 g palmitic acid) during the second four-week exposure to the AHA diet. This combined regimen led to significant decreases in cholesterol (10%, $P < 0.05$), LDL cholesterol (13%, $P < 0.05$, calculated data not shown), and ApoB (7%, $P < 0.05$) levels during this four-week period (Figs. 1, 2). HDL cholesterol, ApoA-I, and triglyceride levels (Figs. 3–5) were not influenced by the combined regimen. Blood glucose at week 12 was 15% ($P < 0.05$) lower than the Baseline (week four) value (Fig. 6). In summary, the incremental response to Palmvitee likely accounted for 7% of the 10% decrease in cholesterol and associated decreases in LDL cholesterol and ApoB levels during this phase.

The tocol mixture in the Palmvitee supplement was comprised of 15% α-tocopherol and 85% tocotrienols. It has previously been noted that α-tocopherol attenuates the cholesterol-suppressive actions of the tocotrienols (5,6). Therefore, it was of interest to evaluate the impact of a tocopherol-free preparation of γ-tocotrienol on the cholesterol levels of the 16 Placebo subjects during a terminal four-week phase of the study. As shown on Figure 1, the cholesterol level of these subjects had plateaued during their second four-week exposure to AHA dietary regimen. The γ-tocotrienol supplement effected a significant 13% decrease in cholesterol (Fig. 1), with concomitant decreases in ApoB (Fig. 2) and LDL cholesterol (calculated, not shown) levels. The significant decrease in blood glucose during this period reflects that previously noted on the exposure of the other subjects to Palmvitee (Fig. 6). HDL cholesterol and ApoA-I levels remained stable (Figs. 3, 4). Finally, we note that the general decrease in triglycerides achieved statistical significance (Fig. 5). We postulate that this decrease reflects the long-range impact of the AHA dietary regimen.

We further evaluated the treatment effects on cholesterol levels using the paired *t*-test. We first calculated the difference between week 8 and week 4, week 12 and week 8, and week 16 and week 12 values for each individual (Scheme 1). The AHA diet-mediated decrease in cholesterol for the Placebo group during the first four-week exposure (–0.40 ± 0.07 mmol/L) was significantly greater than that during the second four-week exposure (–0.20 ± 0.03 mmol/L, $P < 0.005$). The decrease in cholesterol for the Palmvitee group during the four-week exposure to the AHA diet (–0.38 ± 0.05 mmol/L) equaled that observed in the Placebo group. This decrease, however, was significantly less than that observed when the subjects received Palmvitee (–0.68 ± 0.12, $P < 0.050$). This impact of tocotrienols is confirmed in the final comparison showing that the decrease in cholesterol during the γ-tocotrienol phase of the experiment (–0.87 ± 0.10 mmol/L) is significantly greater than that which occurred during the second four-week exposure to the AHA dietary regimen ($P < 0.001$).

Serum TxB_2 concentrations decreased significantly ($P < 0.05$) during the Palmvitee and γ-Tocotrienol phases of the study (Fig. 7).

DISCUSSION

The present study was designed to avoid the previously noted confounding factors which we believe underlie the failure of others (4) to demonstrate the cholesterol-suppressive action

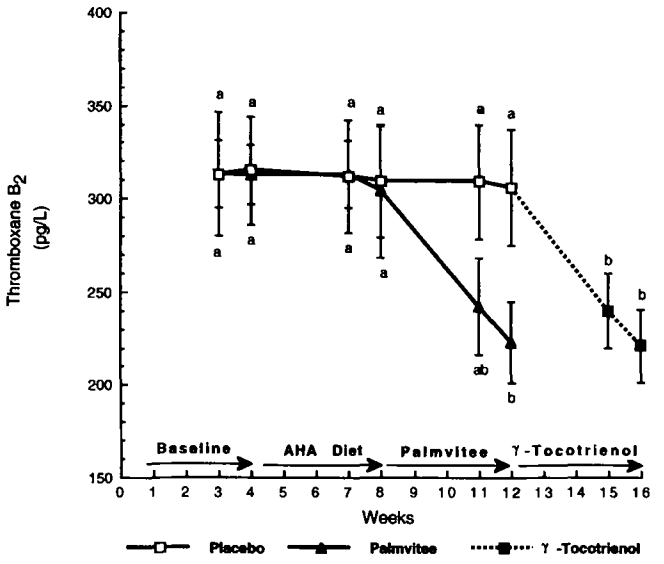

FIG. 7. Time-dependent impacts of AHA dietary regimen, Palmvitee, and γ-Tocotrienol on thromboxane B_2 concentrations in serum of hypercholesterolemic adult subjects. Differences between values on each plotted line which do not not share a letter are significant ($P < 0.05$). Abbreviation and company source as in Figure 1.

of the tocotrienols. Hypercholesterolemic subjects were first conditioned for four weeks to a dietary regimen patterned after the AHA Diet. During that acclimation period, energy intake, estimated from spontaneous diet recalls, decreased by 22%; the proportion of energy supplied by fat decreased from 34 to 30% and that provided by saturated fatty acids, from 12 to 9%. Cholesterol intake decreased from 123 to 106 mg/1000 kcal (Table 1). Four weeks' acclimation to the AHA Diet resulted in significant decreases in cholesterol (5%) and LDL cholesterol (8%) levels. The group of subjects continuing on the AHA Diet for another four weeks experienced an additional 2% decrease in these parameters. Cholesterol (Fig. 1) and LDL cholesterol after eight weeks' exposure to the AHA Diet were decreased by 7 and 10%, respectively.

As previously noted, this study was designed to segregate the impact of the dietary regimen from that of the tocotrienol supplements. Our analysis revealed no significant changes in dietary intakes during the Palmvitee phase of the study. Fat and saturated fatty acids provided 28 and 9%, respectively, of energy intake; cholesterol intake increased from 106 to 134 mg/1000 kcal. The cholesterol-suppressive action of Palmvitee achieved significance at four weeks (Fig. 1); this change is supported by concomitant and significant Palmvitee-induced changes in LDL cholesterol (not shown) and ApoB (Fig. 2).

The Palmvitee capsules provided 220 mg tocotrienols and 40 mg α-tocopherol. The *in vivo* cholesterol-lowering action of the tocotrienols is attenuated by α-tocopherol (5,6), and the tocotrienols differ significantly in their capacity to suppress cholesterol synthesis; γ-tocotrienol and δ-tocotrienol are approximately equipotent, whereas α-tocotrienol has a lower potency (10). These considerations led us to evaluate the cholesterol-suppressive action of a tocotrienol preparation free of α-tocopherol. This preparation provided 200 mg γ-tocotrienol, a quantity calculated to have the cholesterol-suppressive potency of the 220 mg mixture of tocotrienols in the Palmvitee. As shown on Figure 1, the decrease in serum cholesterol mediated by the tocopherol-free preparation reached significance at three weeks.

The significant tocotrienol-mediated decrease in serum cholesterol (Fig. 1) and ApoB (Fig. 2) (and LDL cholesterol) levels of hypercholesterolemic subjects were accomplished in the absence of a treatment-mediated impact on levels of HDL cholesterol (Fig. 3) and ApoA-I (Fig. 4). This response is at odds with that reported by by Wahlqvist *et al.* (4). They conducted a 26-week (six weeks run-in and 20 weeks experimental), rising-dose study with a Palmvitee preparation (23% α-tocotrienol, 31% γ-tocotrienol, 16% δ-tocotrienol, and 30% α-tocopherol). After the run-in period (unspecified dietary fat modification), 52 hypercholesterolemic subjects were randomly assigned to placebo (n = 26, 6.95 ± 1.58 mmol cholesterol/L) and rising-dose treatment (n = 26, 7.51 ± 1.94 mmol cholesterol/L) groups. Interpretation of the results of this study presents several problems, including that due to having a different number of subjects per group (17 to 44). The authors report that only four of the 17 subjects receiving Palmvitee from week 0 to week 16 experienced a fall in serum cholesterol. Tan *et al.* (3) reported that 14 of 15 hypercholesterolemic subjects receiving a similar preparation of Palmvitee (30% α-tocopherol) experienced a greater than 5% decrease in cholesterol. In our earlier study (2), cholesterol levels of 12–15 hypercholesterolemic subjects receiving a preparation of Palmvitee (20% α-tocopherol) decreased. As previously noted, the failure of some subjects to respond may indicate that the biological action of tocotrienols may be more complex than simply inhibiting HMG–CoA reductase.

We found significant tocotrienol-mediated decreases in serum TxB_2 (Fig. 7). This decrease, we postulate, is consistent with the finding that the tocotrienols in membrane redox systems have a major, but little appreciated, antioxidant capacity (11) that would be detected in this capacity-related *ex vivo* index of platelet cyclooxygenase activity. Whether this might reflect an additional cardiovascular benefit *in vivo* remains to be determined.

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